Human Embryonic Development

Effects of Physical Activity and Sleep in Physiological Pregnancies

Alexander Vietheer

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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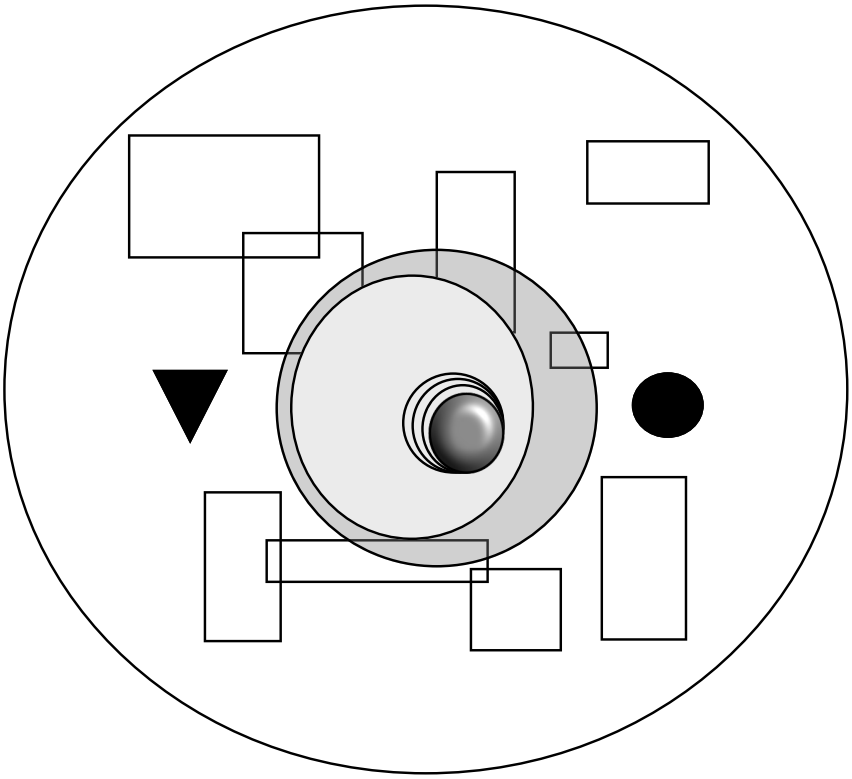


"Do not go where the path may lead, go instead where there is no path and leave a trail."

(Ralph Waldo Emerson)

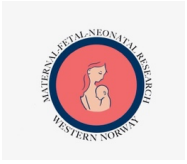
Scientific environment

This project was planned and conducted within the environment of the CONIMPREG group, which is affiliated with the Department of Obstetrics and Gynecology at Haukeland University Hospital (HUS) in Bergen, Norway, and the Maternal Fetal and Neonatal Research Group of Western Norway, a part of the Department of Clinical Science (K2) at the University of Bergen (UIB), Norway.

The practical research work took place at the Fetal Medicine Unit at HUS. Statistical analysis was facilitated and validated by Rolv Terje Lie and Øystein Ariansen Haaland. Rolv Terje Lie is a professor in the Department of Global Public Health and Primary Care at UIB and is also affiliated with the Centre for Fertility and Health at the Norwegian Institute of Public Health in Oslo, Norway. Øystein Ariansen Haaland is a professor of medical statistics in the Department of Global Public Health and Primary Care at UIB.

Validation of sleep data was supported by the National Center for Sleep Medicine (SOV.no at HUS) and the Bergen Sleep Chronology Network (BeSCN at UIB).

Dr. Roberto Romero, M.D., D.Med.Sci., Chief of the Perinatology Research Branch at the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)/National Institutes of Health (NIH), located at the Wayne State University School of Medicine in Bethesda, MD, USA, contributed to the interpretation and editing of Paper III.



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Similarly, Professor Roberto Romero, head of the Perinatology Research Branch at the Eunice Kennedy Shriver National Institute of Child Health and Human Development/National Institutes of Health, not only co-authored the third paper but also played a pivotal role in securing financial support in the project's concluding years.
Professor Bjørn Bjorvatn from the Department of Global Public Health and Primary Care at UIB, and Professor Anette Harris from the Research Center for Sleep, Work, and Health, as well as the Bergen Sleep Chronology Network (BeSCN) at UIB, assisted me with the sleep data validation and warmly welcomed me into their respective research groups. Their collaborative and professional feedback, along with their valuable advice on interpreting sleep data, was of significant value to my work.

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Abbreviations

AFP	α-fetoprotein
AGM	Aorta-gonadmesonephros
AIC	Akaike information criterion
ANS	Autonomous nervous system
BIA	Bioelectric impedance analysis
CNS	Central nervous system
CS	Carnegie stage
CONIMPREG	Conception to implantation in pregnancy
DOHaD	Developmental origin of health and disease
DNA	Deoksyribonukleinsyre
E	Embryonic age in days
EEM	Extraembryonic mesoderm
EPO	Erythropoietin
EMP	Erythro-myeloid progenitor
GA	Gestational age
G6PD	Glukose-6-phosphate dehydrogenase
HPRT1	Hypoxanthine phosphoribosyl transferase 1
HSCs	Hematopoietic stem cells
HSPCs	Hematopoietic stem and progenitor cells
IVS	Intervillous space
LIF	Leukemia inhibiting factor
LMP	Last menstrual period
MA	Menstrual age (from the first day of the last menstrual period)
MAOA	Monoamine oxidase A
MKs	Megakaryocytes
MPPs	Multipotent hematopoietic progenitors
mya	Million years ago
Mo	Mode: The most frequent value in a sample

NCD	Noncommunicable disease
PAD	Physical activity duration
PGC's	Premordial germ cells
PGK	Phosphoglycerate kinase
PSCs	Pluripotent stem cells
PSG	Polysomnography
Q1, Q2, Q3	First, second, and third quartile (i.e., 25^{th}, 50^{th} and 75^{th} percentile)
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SE	Sleep efficiency
SEM	Standard error of the mean
SD	Standard deviation
SOL	Sleep onset latency
SRY	Sex-determining region Y protein
TST	Total sleep time
TVS	Transvaginal ultrasound
US	Ultrasound
WASO	Wakefulness after sleep onset
WHO	World Health Organization
XIAP	X chromosome-linked inhibitor of apoptosis protein
XIST	X-inactive specific transcript
XCI	X chromosome inactivation
YS	Yolk sac

Glossary

Aorta-gonad-mesonephros region	Region of the first adult definitive hematopoietic stem cells, located in the embryonic mesoderm that develops from the para-aortic splanchnopleure[1].
Allele	The inherited maternal or paternal version of a DNA-sequence (gene)[2].
Alpha-fetoprotein (AFP)	Polypeptide chain that consists of 30 peptides, predominantly glutamic acid, alanine, leucine, aspartic acid, and cystine. The 4% carbohydrate content include *N*-acetylglucosamine, mannose, galactose, fructose, and sialic acid[3].
Amniotic cavity	Fluid-filled space initially between the amniotic membrane and epiblast. After embryonic folding, it surrounds the embryo and later the fetus[4].
Amniotic membrane	Composed of delaminated cells from the epiblast surrounding the embryo and forming the amniotic sac. The inner layer (amniotic ectoderm) is delineated by amniotic cuboidal cells. Outside is a thin mesodermal layer adjacent to the chorion[5].
Embryonic coelom	Emerges from the extra-embryonic coelom and becomes part of the embryo when the lateral plate mesoderm splits into the somatopleure and splanchnopleure. Over time, this space is segmented into the peritoneal, pleural, and pericardial cavities[6].
Embryonic genome activation	During reprogramming the zygotic genome remains silent (without transcription). Through maternal-to-zygotic transition the transcriptional control is passed to the zygote and the zygotic genome can be expressed[7].

Fate mapping	Determination of tissue origin during development of the embryo using genetic mark inserted into a cell (e.g., fluorescent protein or molecular barcodes)[8].
Apocrine	Apocrine glands develop from protrusions of the membrane that detach and enter the duct, losing a portion of the cellular membrane during this process[9].
Cytotrophoblast	Undifferentiated cells that are sustained through the fusion of the cytotrophoblast. They are beneath the completely differentiated, non-proliferating syncytiotrophoblast[10].
Extraembryonic coelomic cavity	Space that is bounded by extraembryonic mesoderm, also called chorionic cavity[11].
Genetic imprinting	Genomic imprinting is a process of silencing genes, e.g., through DNA methylation[12].
Gestational age (LMP)	Gestational age or menstrual age, calculated as the number of days from the first day of the last menstrual period.
Glucose-6-phosphate dehydrogenase (G6PD)	Cytoplasmic enzyme of all cells, essential for protecting against cellular damage caused by reactive oxygen species; accomplished by supplying substrates to prevent oxidative harm[13].
Head fold	Cranial or mesencephalic flexure: Ventral bending of the embryo's cranial region. Likely driven by rapid forebrain growth and notochord stiffness. Results in ventral positioning of the mouth and heart[14].
Hemothrophic nutrition	Transfer of substances between mother and fetus carried by blood through the placenta[15].

Histotroph	Originating from the endometrium and uterine glands and accumulating in the area between maternal and fetal tissues. Initially, the blastocyst's trophectoderm phagocytoses this material, and subsequently, the process is continued by the placental trophoblast or the yolk sac's endoderm[15].
Histotrophic nutrition	Absorption of secretions from the oviduct and uterus, enhanced by the breakdown products of endometrial cells[16].
Hypoxanthine phosphoribosyltransferase 1 (HPRT1)	Catalyzes the transformation of hypoxanthine into inosine monophosphate and guanine into guanosine monophosphate. This enzyme is pivotal in the production of purine nucleotides via the purine salvage pathway. Alterations in this gene can lead to conditions like Lesch-Nyhan syndrome or gout[17].
Laminin 111	Essential protein in early embryonic development. The "111" identifies the protein isoform's chain composition of $\alpha1\beta1\gamma1$[18]. When various parts of the trimer chains missing (e.g., if the $\beta1$ and $\gamma1$ chains) the basement membrane fails to form, and epithelial formation cannot longer occur[19].
Lateral folding (or flexion)	Lateral edges of the embryonic disc undergo sharp ventral flexion. Contact made by each germ layer's edges at head and tail regions, closing towards the umbilicus. Finally, ectoderm covers the entire body surface, excluding the umbilical region where the connecting stalk and yolk sac converge[14].
Linage tracing	Cell lineage experiments show the relationships between cells marking them at one time point and detecting the progenies derived from these marked cells at a later time point[8].

MAOA	The monoamine oxidase A (MAOA) gene is on the X chromosome. The protein product breaks down monoamine neurotransmitters in the central nervous system, including the catecholamines dopamine, adrenaline, and noradrenaline, histamine, and serotonin (5-HT)[20]. The gene has also been associated with mental development of premature children[21]. It plays also a role in the trajectories of attentional development[22].
Merocrine	Secretions via exocytosis but without cell damage[9].
Multipotent stem cells	Differentiate into a limited range of cell types (e.g., hematopoietic stem cells, neural stem cells, mesenchymal stem cells)[23].
Myeloid-bias	A particular form of linage-bias of stem cells that produce skewed ratios of lymphoid to myeloid cells precursors. The myeloid-biased cells produce reduced levels for the T- and B- lymphocyte lineages but normal levels of myeloid precursors[24].
Pluripotent stem cells	Pluripotent stem cells have the capacity to develop into every cell type within the body, excluding those that form the placenta[23].
Phosphoglycerate kinase (PGK)	This glycolytic enzyme not only catalyzes an ATP-producing step in glycolysis and the reverse reaction in gluconeogenesis but also has diverse roles in pathogenesis, nucleic acid interactions, cancer progression, cell death, and viral replication[25].
Polyembryoma	Mixed germ cell tumor characterized by embryoid bodies, consisting of a central core of embryonal carcinoma cells, an amnion-like cavity, and a yolk sac tumor component[26].

Polysomnography (PSD)	Several concurrent but independent tests that monitor and record different body functions during sleep in different channels. Modern PSD includes: Electroencephalogram (EEG); electrooculogram (EOG); electromyogram (EMG); electrocardiogram (ECG); pulse oximetry; respiratory monitor; capnography; transcutaneous monitors; microphone; video camera; thermometer; light intensity tolerance test; nocturnal penile tumescence test; esophageal tests; nasal and oral airflow sensor; gastroesophageal monitor; blood pressure monitor[27].
Somatopleure	Lateral plate mesoderm of the body wall[28].
Sleep health dimensions[29]	Sleep satisfaction*, Alertness, Timing, Efficiency, Duration
***Sleep satisfaction**	Feeling sufficiently rested, awake and alert[29]
Splanchnopleure	Lateral plate mesoderm connected with the gut tube as well as with the heart including the cardiac muscle[28].
SRY	Product of the SRY gene, also known as the testis-determining factor (DTF); a protein crucial for the formation of testes due to its DNA-binding activity. It encodes the sex-determining region Y protein.[30].
Syncytio-trophoblast	Syncytialized trophoblasts (without cell boundaries) that orchestrate the complex biomolecular interactions between the fetus and mother[10]
Totipotent stem cells	Omnipotent cells that have the capacity to develop into any of the 220 cell types present in an embryo, including cells that form extra-embryonic structures like the placenta[23].
Trophoblast	Cells forming the outer layer of a blastocyst providing nutrients to the embryo and developing into a large part of the placenta[10]

Tail (or caudal) fold	Ventral bending of the embryo's caudal region to reorient the cloaca and allantois. Subdivision of the cloaca into urinary and anal openings[14].
XIAP	XIAP, an X chromosome-linked inhibitor of apoptosis protein, suppresses caspases and prevents apoptosis. XIAP can act as a proapoptotic protein by enhancing mitochondrial membrane permeability, offering potential cancer therapy benefits[31].
XIST	Xist RNA is non-coding RNA. Serves as the primary regulator of X chromosome inactivation (XCI). Facilitates an epigenetic mechanism that balances X-linked gene expression between females (XX) and males (XY) in mammals[32].

Preface

Today, it is widely accepted that environmental and maternal factors have potential to influence early fetal development[33]. Hypertension, overweight, and hyperglycemia are the key metabolic risk factors, along with tobacco, alcohol, sodium-intake, and physical inactivity as the leading behavioral risk factors[34]. The new WHO (World Health Organization) sustainable development goals emphasize the significance of earliness in disease prevention. Accordingly, they urged, to initiate preventive measures already prior to conception and during pregnancy[35].

Regarding the continual rise of obesity and metabolic disease globally including the high-income countries[36], we need further insight and strategies. Identifying early mechanisms and mediators of vulnerability for future development of disease, such as developmental programming in pregnancy, has been one of the main focuses in the scientific field of developmental origin of health and disease (DOHaD)[33]. However, to distinguish between physiology and pathophysiology starts with the knowledge of physiological ranges and adaption mechanisms.

What can we, in fact, expect during healthy pregnancy?

By a prospective longitudinal study design starting from before conception and careful choice of non-invasive measurement methods, the present work contributes to new insights on the influence of maternal and environmental factors on early human development and possible mechanisms.

Abstract in English

Background: The field of developmental origins of health and disease (DOHaD) has spurred research into prenatal health determinants. Although nutrition and physical activity are well recognized for their importance, maternal sleep in early pregnancy, especially before conception, has received less attention despite its crucial physiological role and significant interplay with physical activity, nutrition, and body composition.

Animal studies have demonstrated the role of the yolk sac in transferring nutrients and providing early blood and immune cells during early pregnancy. The latter, for example, has been traced to adulthood as a tissue macrophage. However, analogous evidence in human development was scant until recent findings demonstrated the vital functions of the yolk sac in human pregnancy.

Aim: This study aimed to determine the normal variation in sleep and physical activity before and during pregnancy and to examine their effects, in conjunction with maternal body composition, on embryonic development, specifically the size of the yolk sac and crown-rump length (CRL).

Methods: A prospective study was conducted on 190 healthy women intending to conceive naturally. Sleep and physical activity patterns were assessed before pregnancy and in each trimester. Actigraphy recordings from the preconception and first trimester were analyzed along with serial transvaginal ultrasound measurements of the yolk sac and embryo throughout the first trimester.

Statistics: Cohort and measurement summary statistics were calculated. Associations between sleep, physical activity, and embryonic measurements were analyzed—stratified and unstratified by embryonic sex and gestational age—using linear and quantile regression analyses, controlling for maternal age, parity, and body composition. Repeated measurements were modeled with linear mixed-effects regression.

Main results: The study revealed large individual variation in maternal sleep and physical activity patterns starting from preconception (± 2 SD range: 307–523 minutes and 120–608 minutes, respectively). Sleep duration increased notably in the

first trimester from 415 minutes to 458 minutes, whereas physical activity levels, including light and moderate-to-vigorous activity, decreased progressively, reaching the lowest levels in the last trimester (for example, average daily physical activity duration that was 362 minutes before conception, 262 minutes at 13 weeks, 251 minutes at 24 weeks, and 215 minutes at 36 weeks of gestation). Specifically, preconception sleep duration and physical activity had distinct correlations with yolk sac size and growth dynamics, as well as CRL between six and 11 weeks of gestation—a critical period for embryonic development. These associations were independent of maternal body composition and specific to narrow time frames, with factor-specific interactions observed between gestational age and embryonic sex. Discussion: Maternal sleep and physical activity patterns demonstrate significant individual variations and undergo considerable changes from preconception to the first trimester. These changes, which are intricately linked to hormonal, circulatory, and metabolic regulation, can influence the menstrual cycle and potentially affect the early embryonic nutrition provided by the endometrium and its glands. The observed variations in yolk sac size could represent a physiological adaptation of the membrane surface area, optimizing embryonic nourishment and growth. Implications: Time- and sex-specific variations in the yolk sac due to sleep and physical activity occur during a critical developmental phase marked by significant organ development and shifts in organ function allocation. These observations not only necessitate new considerations for future research designs but might also have implications for pre-pregnancy care, pregnancy management, and lifelong health outcomes.

Abstract in Norwegian

Bakgrunn: Forskningsfeltet "Developmental Origin of Health and Disease" (DOHaD) har vist hvor betydningsfull fosterutviklingen er for individets fremtidige helse og sykdomsrisiko. I senere år har den tidligste perioden av svangerskapet fått økt oppmerksomhet. Viktigheten av sunn ernæring og fysisk aktivitet er anerkjent, mens søvnens rolle i tidlig svangerskap, og spesielt før svangerskapet, har vært lite i fokus. Dette til tross for søvnens velkjente fysiologisk betydning for fysisk aktivitet, ernæring og kroppssammensetning i sin alminnelighet.
Dyrestudier har vist at plommesekken er essensiell for å overføre næringsstoffer og danne de første blod- og immunforsvarscellene til embryo i utvikling. Noen celler kan spores helt til voksen alder, for eksempel vevsspesifikke makrofager. Humane studier er imidlertid få og sammenlignbare konklusjoner usikre. Det er først i senere tid at plommesekkens kritiske funksjoner hos mennesket er blitt dokumentert med sikkerhet.
Mål: Å fastslå den normale variasjonen i maternelle søvn- og fysisk aktivitetsmønstre før og under graviditet. Dernest, å undersøke hvordan disse to faktorene påvirker embryonal utvikling, det vil si størrelsen på plommesekken og embryolengde, målt fra isse til sete, på engelsk "crown-rump length" (CRL). Disse forholdene ble analysert i sammenheng med andre relevante maternelle faktorer som kroppssammensetning.
Materiale og Metode: I denne prospektive studien ble 190 friske kvinner som planla en naturlig unnfangelse inkludert. Søvn- og aktivitetsmønstre ble registrert med aktigrafi før svangerskapet, og registreringene ble gjentatt for hvert trimester. Kroppssammensetning ble også målt før graviditet ved hjelp av bioelektrisk impedansanalyse. For å fastslå påvirkningen av disse faktorene på embryonal utvikling, ble størrelsen på plommesekken og CRL (isse-sete lengde) målt gjentatte ganger med transvaginal ultralyd i første trimester.
Statistikk: Deskriptiv statistikk for kohorten og målingene ble utført. Sammenhengene mellom søvn, fysisk aktivitet og embryonale målinger ble analysert ved hjelp av lineær og kvantil regresjon, både med og uten stratifisering etter embryonalt kjønn og

gestasjonsalder, og i subanalyser justert for mors alder, paritet og kroppssammensetning. Serielle målinger ble modellert med lineære blandet-effekt modeller (eng. linear mixed-effects models).

Hovedfunn: Studien avdekket allerede før svangerskapet en betydelig variasjonsbredde blant deltakerne i søvn- (± 2 SD: 307–523 minutter) og fysisk aktivitetsmengde (± 2 SD: 120–608 minutter). I løpet av det første trimesteret økte gjennomsnittlig søvnmengde betydelig fra 415 til 458 minutter, mens den fysiske aktiviteten, inkludert subgruppene lett og moderat til kraftig aktivitet, gradvis minket gjennom hele svangerskapet (for eksempel ble gjennomsnittlig daglig fysisk aktivitet målt til 362 minutter før svangerskapet, 262 minutter ved 13 uker, 251 minutter ved 24 uker, og 215 minutter ved 36 uker i svangerskapet).

Særlig før konsepsjon viste søvn og fysisk aktivitet en klar sammenheng med plommesekkens størrelse i den kritiske utviklingsperioden mellom den 6. og 11. svangerskapsuken, samt med dens vekstdynamikk og embryonets størrelse (CRL). Disse forbindelsene var spesifikke for korte tidsvinduer og avdekket også faktorspesifikke interaksjoner mellom svangerskapsalder og embryonalt kjønn.

Fortolkning: Maternelle søvn- og aktivitetsmønstre viste tydelig individuell variasjon og endret seg betydelig fra før konsepsjon til slutten av første trimester. Endringer i søvn og aktivitet er knyttet til hormonelle, sirkulatoriske og metabolske reguleringsmekanismer, og kan dermed påvirke menstruasjonssyklusen og mulig tidlig embryonal ernæring som leveres av endometriet og dets kjertler. Derfor kan de observerte variasjonene i plommesekkens størrelse reflektere en fysiologisk tilpasning av membranens overflateareal for å optimalisere ernæring og vekst av embryoet.

Implikasjoner: Tids- og kjønnsspesifikke variasjoner i plommesekken, påvirket av søvn og fysisk aktivitet, oppstår i en kritisk fase av utviklingen som er kjennetegnet ved hurtig organ-utvikling og skiftende funksjoner. Disse funnene stiller ikke bare nye krav til utforming av fremtidige studier, men kan også ha implikasjoner for omsorgen i tiden både før og under svangerskapet, samt for barnets langsiktige helse.

What is known	What this study shows
▪ **In general, sleep disturbances are more common during pregnancy, a time when sleep duration is higher and physical activity is lower.** ▪ **Animal studies and epidemiology show that fetal development is sensitive to environmental factors.** ▪ **Generally, embryos develop rapidly through many stages in a short time.** ▪ **Sex differences are known throughout biology, also in humans.** ▪ **Sleep is important for a healthy life in the general population.** ▪ **Physical activity is part of daily life involving metabolism and general health.** ▪ **Sex differences are known throughout biology, also in humans.**	▪ **Robust references ranges quantify the development of sleep and physical activity development from preconception to the end of pregnancy in healthy women (Paper I).** ▪ **In healthy human pregnancies, maternal factors influence embryonic development (Paper II–III).** ▪ **Using ultrasound, we demonstrate that the embryo is sensitive to specific cues in only short time windows in healthy pregnancies (Paper II-III).** ▪ **Sleep duration in healthy women influences the embryo (yolk sac size) but in short time windows of their pregnancy (Paper II).** ▪ **Physical activity duration and intensity influences yolk sac in embryos of healthy pregnancies (Paper III).** ▪ **The effects of maternal sleep and physical activity affect the human yolk sac in a sex-specific fashion, both in timing and responses (Paper II–III).**

List of Publications

I. **Vietheer A**, Kiserud T, Lie RT, Haaland ØA, Kessler J. Sleep and physical activity from before conception to the end of pregnancy in healthy women: a longitudinal actigraphy study. *Elsevier,* Sleep Medicine (2021) Jul;83:89-98. DOI: 10.1016/j.sleep.2021.04.028. PMID: 33991895.

II. **Vietheer A**, Kiserud T, Haaland ØA, Lie RT, Kessler J. Effect of maternal sleep on embryonic development. *Nature,* Scientific Reports (2022) Oct 12;12(1):17099. DOI: 10.1038/s41598-022-21516-6. PMID: 36224237.

III. **Vietheer A**, Kiserud T, Ebbing C, Lie RT, Haaland AØ, Romero R, Rajkumar H, Kessler J (2023): "Maternal Physical Activity affects Yolk Sac Size and Growth in Early Pregnancy, but Girls and Boys Use Different Strategies", *Nature*, Scientific Reports (2023) Nov 13:20246. DOI: 10.1038/s41598-023-47536-4.

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1. Introduction

Timing and gestational age (GA) play pivotal roles in the development of embryos and fetuses. Embryonic development typically commences with conception, i.e., within 24 hours following ovulation[37,38]. However, the precise timing of ovulation can vary among women during a menstrual cycle, depending on the length of the current follicular phase (Mo = 13 days ± 4 days in 75%)[39].

Thus, determining gestational age by estimating menstrual age (MA), based on the number of days since the first day of the last menstrual period (LMP), raises two significant implications: 1- the absolute value of GA calculated by LMP (menstrual age) does not align with the embryonic age (E), which is determined by the number of days since conception/ovulation, and 2- the correlation between embryonic age (E) and menstrual age (MA) is limited as it fails to account for variations in time of ovulation. Consequently, the "true" GA can be either overestimated or underestimated by several days, potentially introducing unrecognized bias. Note, that even a minor difference of few days can have significant impact during early development. Unfortunately, precise data on the exact timing of conception is often unavailable.

Alternative methods, such as calculating gestational age based on sonographic measurements of fetal biometry, including the fetal crown-rump length (CRL), have been proposed. However, these methods also do not adequately address ovulatory variations, because the biometric reference curves for gestational age are still based on the LMP. Furthermore, biometric age determination commonly relies on mean estimates, which truncate early age and growth variations. Although the LMP method and/or sonographic age estimation using biometric measurements are commonly used—due to the unknown time of conception—it is crucial to be aware of specific limitations and potential pitfalls when studying effects and mechanisms during early pregnancy development.

1.1 The first days of human development and implantation

Fertilization (conception) occurs usually in the ampulla of the fallopian tube[40]. The contact between a sperm and the oocyte induces a complex sequence of molecular and physical events resulting in the formation of a zygote, which is a zona pellucida surrounded one-cell embryo. Here, the maternal and paternal pronuclei with their haploid sets of 23 chromosomes unite to a nucleus with a new unique diploid set of 46 chromosomes. Shortly after conception (30 hours), the zygote passage through the fallopian tube and undergoes a serious of mitotic cell divisions into smaller cells (blastomeres)[40] *(Fig. 1).* When conceptus reaches the eight-cell stadium, the human embryonic genome is activated[41,42]. Further cell divisions result approximately three days after conception in a compact ball of 16–32 cells (morula) that enters the uterine cavity; this process is called compaction[40].

The morula develops then to a blastocyst (64-cell stadium)[43] through fluid separation of the blastomeres. The fluid originates from the uterine cavity and penetrates the zona pellucida. It also forms the cystic cavity separating an inner layer of centrally located blastomeres (i.e., the inner cell mass or embryoblast, which gives rise to the embryo) and a thin outer cell mass—the peripheral layer called trophoblast which gives rise to the embryonic placenta part[40,44].

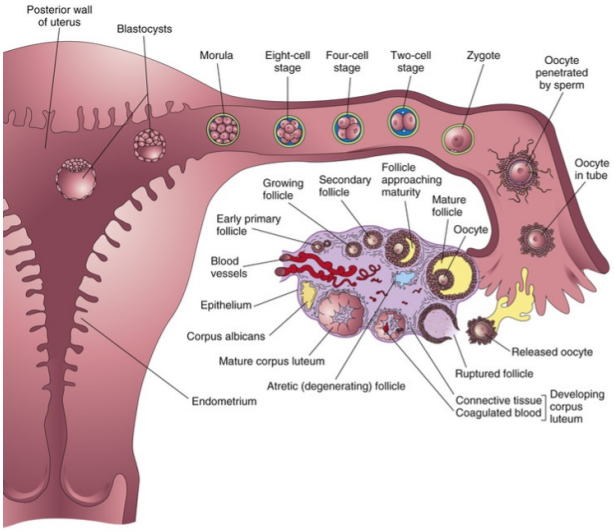


Fig. 1 "Summary of the ovarian cycle, fertilization, and human development during the first week." *Figure reprinted from Moore et al. (2020)[40], The Human Development with permission. © 2020, Elsevier Inc. All rights reserved.*

Implantation

Human embryos, like the embryos of great apes, encounter interstitial implantation, i.e., the late blastocyst penetrates through the endometrial epithelium and invades the connective tissue[43]. As soon as the blastocyst attaches to the endometrium the trophoblast or outer cell mass differentiate in two layers—an inner layer of cytotrophoblast and an outer layer of highly invasive syncytiotrophoblast. The syncytiotrophoblast is a multinucleated mass without cell boundaries that in finger-like extensions penetrates the endometrium (E 7) as it invades the connective tissue below and allows the conceptus to be embedded in the endometrium during the next days (E 8–12)[40] (*Fig. 2).*

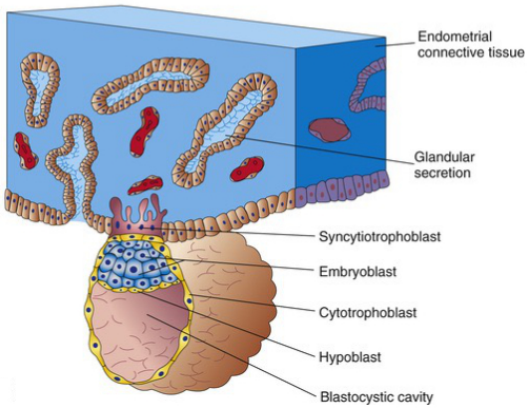


Fig. 2 Attachment of the blastocyst to the endometrial epithelium during early stage of implantation. At (E 7), the syncytiotrophoblast penetrated the epithelium and started to invade the endometrial connective tissue. *Figure adapted from Moore et al. 2020[40], The Human Development with permission. © 2020, Elsevier Inc. All rights reserved.*

Implantation is a sensitive process that depends on synchronization of blastocyst and receptive endometrium that only in a relatively brief period (2–3 days) expresses essential proteins, such as bone morphogenetic protein[45]. During this process, surrounding connective tissue cells accumulate glycogen and lipids—some of them degenerating adjacent to the syncytiotrophoblast and thereby providing the embryo with nutrition[45].

The former blastocyst is progressively enclosed by syncytiotrophoblast, and the development of lacunae can be seen (E 9). These contain a nutrient-rich fluid, derived from disrupted endometrial capillaries and eroded uterine glands, that can reach the embryonic disc through diffusion[45].

1.2 Formation Primary and Secondary Human Yolk sac

The yolk sac (YS) evolved in our aquatic ancestors more than 500 million years ago, serving primarily to absorb and deposit nutrients[43]. However, with the emergence of lactation during phylogenesis and successful adaptation for in utero development with placentation, there was a diminished reliance on the YS for nutrition[46]. In turn, repurposing of the extraembryonic membranes was required[43], and the human YS, in fact, never contains any yolk. Consequently, some authors have preferred to refer to it as the primary or secondary umbilical vesicle[47]. Additionally, the primary YS or umbilical vesicle has been referred to as Heuser's membrane and the exocoelomic membrane[44].

Although eggs of placental mammals does not contain any yolk, the YS has remained an fundamental part of embryonic development[43] *(Fig. 3)*.

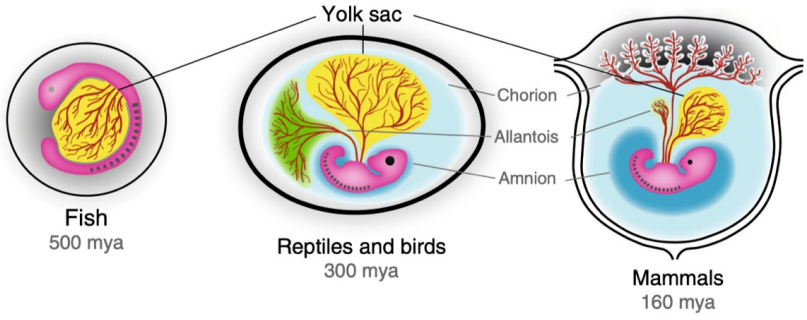


Fig. 3 "Extraembryonic membranes in fish, birds, and mammals"[43]. The yolk sac was already present in our aquatic ancestors a million years ago (mya). Phylogenetically, the oldest extraembryonic tissue. The amnion, chorion, and allantois originate from the amniotic egg, subsequently adapted in mammals, to support embryonic development inside the uterus. *Figure from "Origin and function of the yolk sac in primate embryogenesis", Ross C & Boroviak TE, Nature Communications © 2020, Crown; reused with permission under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International (CC B Y-NC-ND 4.0).*

Primary Yolk sac Formation, Embryonic Disc, and Amniotic Cavity

The YS formation starts already during implantation[48]. Approximately at the fifth day post fertilization (E 5), the inner cell mass or embryoblast gives apparently rise to a thin cell layer lining the trophoblast within the blastocyst. This layer is a first form of endoderm, also referred to as primary endoderm, primitive endoderm, or hypoblast[49]. Together with the trophoblast it constitutes a bilaminar omphalopleure called the primary YS[50]. The endodermal part adjacent to the embryonic disc is called visceral endoderm, while the parietal endoderm is the peripheral remainder that is adjacent to the trophoblast[44].

At (E 8–11), a small space emerges in the embryoblast, which is the "primordium of the amniotic cavity", and the embryoblast differentiates into epiblast and hypoblast forming two layers within the embryonic disc. While the hypoblast faces towards the YS and becomes a more robust part of the primitive visceral endoderm, the epiblast is directed towards the amniotic cavity[45] *(Fig. 4).* Its pluripotent stem cells (PSC's) give not only rise to amnioblasts enclosing the amniotic cavity[45], but differentiate later during gastrulation also into mesoderm and definitive endoderm[43,51].

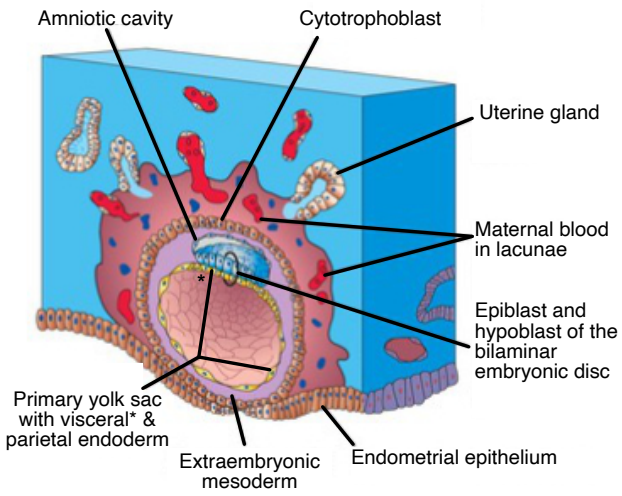


Fig. 4 Section through a blastocyst at implantation partially embedded in the uterine endometrium (≈ E 8). The actual size of the conceptus is 0.1 mm. Note the slit-like amniotic cavity. *Figure adapted from Moore et al. 2020 [45], The Human Development with permission. © 2020, Elsevier Inc. All rights reserved. Original annotations have been removed using the Affinity Designer 2, v.2.0.3 Brush Healing Tool.*

Secondary Yolk sac

At (E 8–9), the secondary YS or secondary umbilical vesicle emerges from the primary YS through accumulation of spindle-shaped cells that probably delaminate from primary visceral and parietal endoderm[43]. Thereby, it also originates from the extraembryonic cell linage[51]. Then, at (E 9–12), these cells form a reticular pattern called extraembryonic mesoderm (EEM) between the endoderm and the basal lamina of the trophoblast[44]. Additionally, it seems to be "supplemented" with mesodermal cells from the embryonic disc[43,50]. This early formation is characteristic of primate embryogenesis[43] *(Fig. 5)*.

During implantation, the blastocyst size must be small to penetrate the endometrial epithelium. However, after successful interstitial implantation it may re-expand through early and extensive growth of EEM which, in turn, provides necessary space for the embryo to grow[50]. At the end of that process, the whole amnion, the primary YS, and trophoblast are lined by the EEM, which, through matrix production (i.e., fibronectin, collagen, and laminin 111)[52], subsequently develops coelomic spaces within its reticular meshwork. These spaces constitute the extraembryonic coelom, which conflates during further development to form the extraembryonic coelomic cavity or chorionic cavity, finally enclosing the amnion, embryo, and secondary YS[44,45] *(Fig. 5)*.

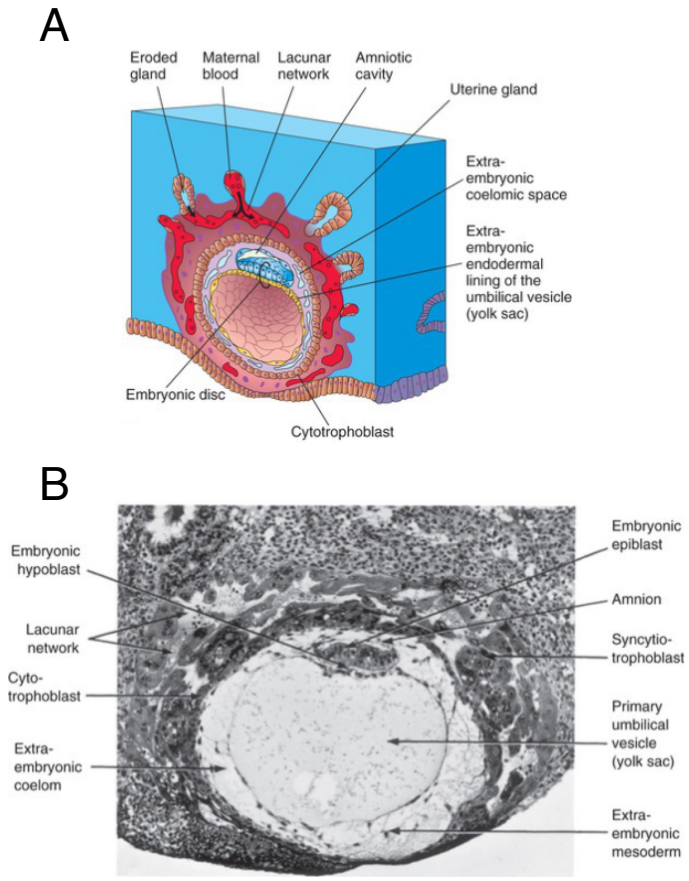


Fig. 5 Embedded blastocyst day 12. Coelomic spaces appeared in the extraembryonic mesoderm, forming the beginning of the coelomic cavity. (A) Schematic representation of the section underneath (B) Section through the implantation site at E 12 with the surrounding uterine endometrium (x100). *Figures adapted from Moore et al. 2020* [45]*, The Human Development with permission. © 2020, Elsevier Inc. All rights reserved. Source Fig. B: Hertig AT, Rock J: Two human ova of the pre-villous stage, having an ovulation age about 11 and 12 days respectively, Contrib Embryol Carnegie Inst 29:127, 1941. Courtesy, Carnegie Institute of Washington, DC.*

During coelomic cavity formation, the primary YS is assumed to undergo periodic collapse and re-expansion, eventually resulting in its rupture[44]—similar fluctuations have been observed for primate's blastocyst size in *in vitro* experiments[44]. Simultaneously, a more robust visceral endoderm proliferates beneath the epiblast of the bilaminar embryonic disc[44]. This endoderm extends from the embryonic margins, referred to as the extraembryonic endoderm, initiating the formation of the secondary YS. Upon disruption of the primary YS, the initially smaller secondary YS emerges within the coelomic cavity, consisting of visceral endoderm (derived from the former

primary YS), extraembryonic endoderm, and a few parietal endoderm cells with residual mesoblasts from the primary YS (E 12–15)[44]. The mesoblast cells of the adjacent extraembryonic mesoderm organize into a mesothelial layer of the YS, while peripheral cells beneath the trophoblast form a mesothelial layer lining the coelomic cavity[44].

The connecting stalk is the embryonic attachment to the trophoblast which connects the embryo with the chorion. It also originates from the extraembryonic mesoderm and its matrix proteins that accumulate around that region at (CS 6)[53]. When the coelomic cavity is formed, the connecting stalk persists as the origin for the umbilical vessels to the placenta. It derives from local mesoderm lined trophoblast[54].

In secondary YS development, morphologically, it may seem that the distal part of the primary YS is "pinched off" from the proximal part, thereby forming the secondary YS as ventral protrusion of the primitive gut[50] *(Fig. 6)*.

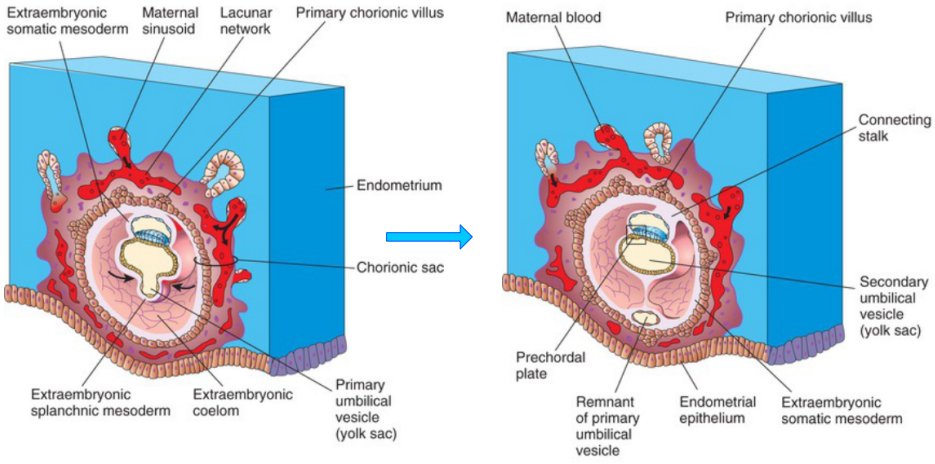


Fig. 6 "Pinched-off" secondary yolk sac (E 12–15). Note that the coelomic cavity developed from fused matrix vesicles of the extraembryonic mesoderm (E 12–15). *Figure adapted from Moore et al. 2020[45], The Human Development with permission. © 2020, Elsevier Inc. All rights reserved. Figures merged to a figure panel and modified using Affinity Designer 2, v.2.0.3 Brush Healing Tool.*

Nevertheless, the precise cellular developmental origin of the secondary YS and the molecular mechanisms are still not clear[43].

Successive Development of the Secondary Yolk sac

The secondary yolk sac is initially a small structure positioned under the embryonic disk, occurring alongside numerous vesicles found throughout the blastocyst cavity. These vesicles are predominantly located in areas away from the embryo[43] *(Fig. 7)*.

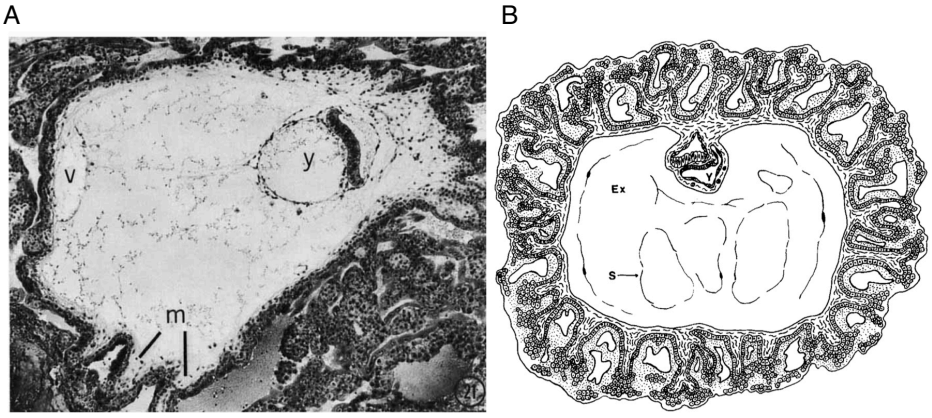


Fig. 7 An early (E 14) villous human blastocyst[50]. (A) Mesodermal villi (m) developed over large parts of the chorionic surface with peripheral spread of the extraembryonic mesoderm. Most epithelial remnants of the primary yolk sac have degenerated, although a vesicular remnant (v) persists abembryonically. Secondary yolk sac (y). Carnegie No. 7801, Section 12-1-4. X 120. **(B)** Diagram of a human embryo (E 16). Within the exocoelomic cavity (Ex), the definitive secondary yolk sac (y) begins to extend over the embryonic disc and consists of three layers: endoderm, mesoderm with some blood islands, and the outer mesothelium). The remnants of the primary yolk sac can be seen as acidophilic strands (S). *Figures adapted from WISTAR INSTITUTE OF ANATOMY AND BIOLOGY., ASSOCIATION OF AMERICAN ANATOMISTS with permission. © John Wiley & Sons – Books.*

The YS then rapidly extends beyond the embryonic margins during CS 7 and CS 8 and reaches a two millimetre diameter (E 16–19)[55] consisting of three layers: 1- The inner endodermal cell layer which is in contact with the serous fluid of the lumen of the YS and has features of a synthesizing and secretory tissue[56] consisting of three layers: 1- The inner endodermal cell layer which is in contact with the serous fluid of the lumen of the yolk sac and has features of a synthesizing and secretory tissue[56], 2- The outer mesothelial cell layer representing the boundary to the exocoelomic cavity, and 3- the scant intermediate mesenchymal tissue layer in between.

The membrane with its layers increases in thickness within a few days, hematopoietic foci appear (≈E 16) and later the YS membrane contains size-variating vitelline vessels (5 to 110 µm) with a continuous endothelial cell lining without a basal lamina

(Fig. 8). These endothelial vessel cells are similar to the surrounding mesenchymal cells, except for the presence of coated vesicles and absence of glycogen granules[57].

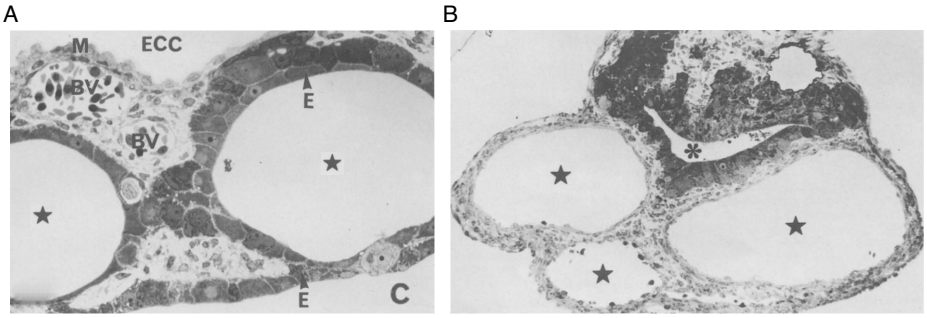


Fig. 8 Semi-thin (0.5 µm) yolk sac sections stained with toluidine blue of the human yolk sac wall (Fig. 8-A) and vitelline duct (Fig. 8-B) at eight weeks (MA)[58].

Fig. 8A: Flattened mesothelial cells (**M**) facing the exocoelomic cavity (**ECC**), while the endoderm (**E**) lines the yolk sac cavity (**C**) and contains large ducts(★). Small blood vessels (**BV**) are located within the mesenchyme, separating the two layers. **Fig. 8-B:** Section of the vitelline duct proper (*), the contents of which drain into the embryo. Large blood vessels (★) adhere to the duct. *Figure adapted from Jones and Jeauniaux (1995) modified using the Affinity Designer 2, v.2.0.3 Brush Healing Tool with permission. © Elsevier Science & Technology Journals.*

The vessels in the lumen are filled with various types of erythroblasts, as well as megakaryoblasts, megakaryocytes, platelets, macrophages, mononuclear cells, and rarely, monocyte-like cells[57]. Goh et al. (2023)[59] found that in human YSs, the extravascular mesenchymal tissue contains collagen fibrils, a sublayer of smooth muscle cells, macrophages, and a small number of dendritic cells. The smooth muscle cells formed between the mesoderm and the endoderm.

Notably, it has been previously reported that the YS endoderm at CS10 (approximately 6 weeks MA) is comparatively thick, and that the most prevalent hematopoietic cell types are HSPCs, erythroid cells, macrophages, and megakaryocytes[44]. However, at later stages, Goh et al. (2023) described a shift to a predominance of erythroid cells and macrophages, which were the only cells that were sustained, whereas both HSPCs and megakaryocytes were proportionately diminished[59]. As such, the ratio of hematopoietic to nonhematopoietic cells was approximately 3:1 in the early YS, while this ratio approached 1:3 by 10 gestational weeks owing fibroblast expansion and the formation of more non-hematopoietic cells than hematopoietic cells. These were then predominantly mesenchymal cells, now forming a thicker loose cellular network[59].

The circulation development of early human embryos begins at stage 7 (E 16) and is complete and functioning from about (E 24)[57]. Note, that this is not equivalent to an established intra-placental circulation with hemotrophic nutrition.

Anatomically the umbilical arteries and vein develop in the connecting stalk, while the complex vascular system of the YS wall is connected to the embryonic circulation via the vitelline artery and vein[60] that forms part of the vitelline duct, also called YS stalk *(*Fig. *9).* This embryonic structure is originally part of the endodermal protrusion (primitive gut) that has a wide connection to the embryonic midgut and connects the embryo with the secondary YS.

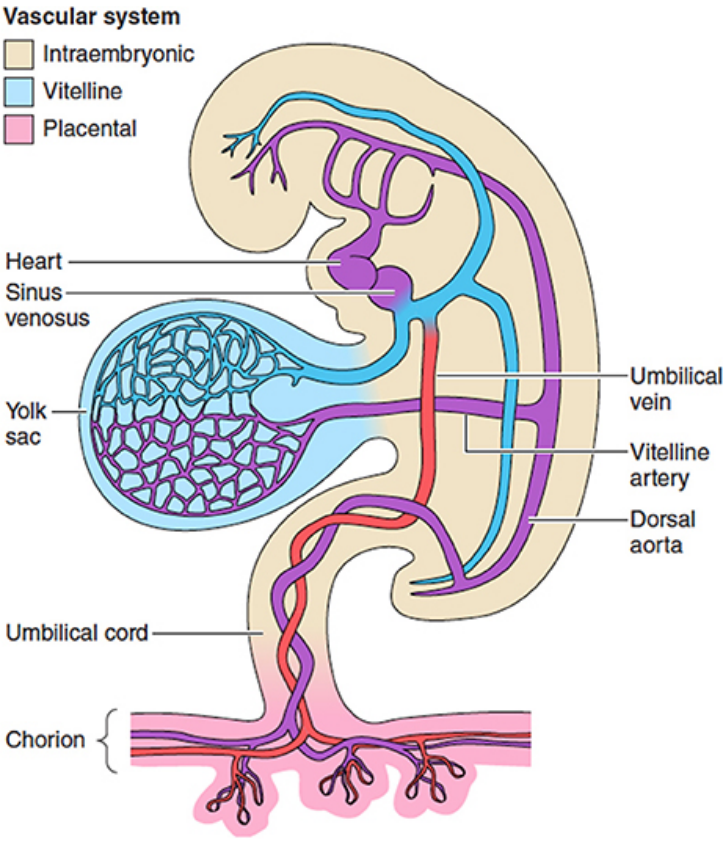


Fig. 9 Circulation of the early human embryo – connection of the intraembryonic vessels with the vitelline vessels of the secondary yolk sac and the vessels of the connecting stalk (later umbilical vessels). *Reused with permission under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International (CC BY-NC-ND 4.0). © 2018 Burton and Jauniaux*[61].

Later during gastrulation (E 21–28)[50] (*Fig. 10),* the embryonic cephalocaudal and lateral flexion/folding of the embryo results in the narrowing of the proximal YS, the cavity of which remains continuous with the developing gut tube and forms a narrow vitelline duct[28]. The vitelline duct is, like the YS, covered by a mesothelial layer. However, in contrast to the YS wall, the mesenchymal layer between the endoderm and mesothelium, is barely apparent at any stage[44].

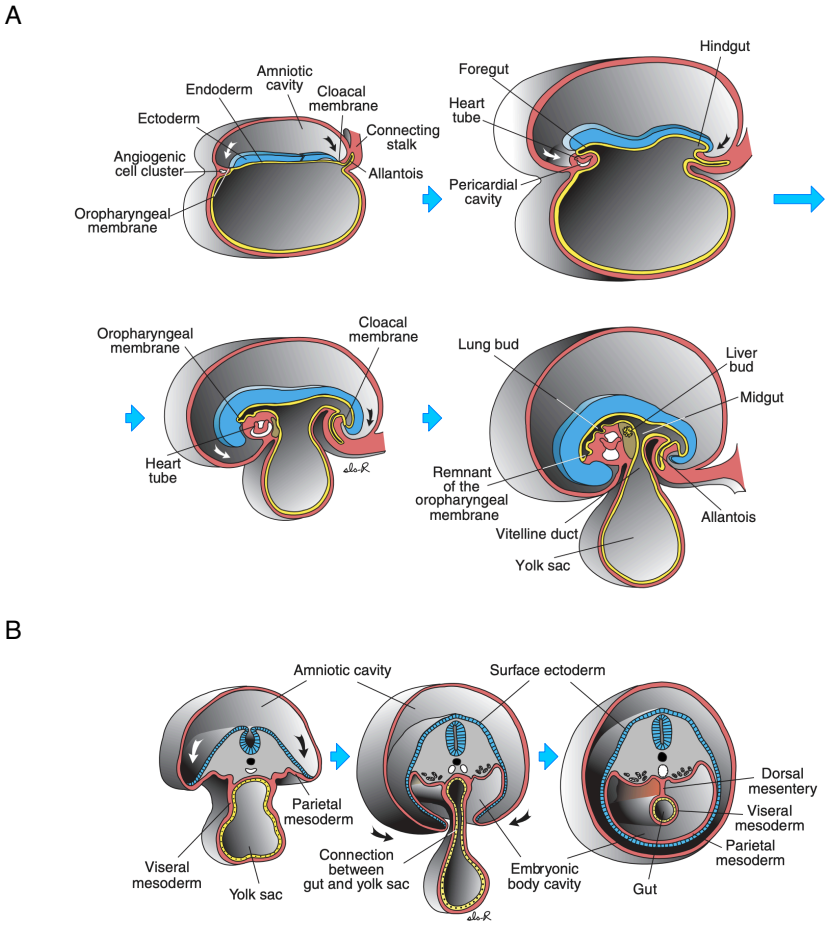


Fig. 10 (A) Sagittal midline sections of embryos through development demonstrating cephalocaudal folding and its effect on the position of the endoderm-lined cavity at 17, 22, 24, and 28 days. The arrows specify the order of head- and tail- folding events. **(B) Cross sections through embryos showing the effect of lateral folding;** the endoderm-lined cavity is narrowed to the vitelline duct (connection midgut-secondary yolk sac) until the ventral abdominal wall is finally closed. *Figure adapted from Sadler, TW in Langman's medical embryology, ed.12 with permission. © 2012 Lippincott Williams & Wilkins, a Wolters Kluwer business. Figures have been merged to a panel (A & B) and blue arrows have been added using Affiniy Designer 2, v.2.0.3.*

The folding process, especially the tail fold, also brings the connecting stalk (body stalk) and the YS closer to each other forming the umbilical root. After amniotic expansion, the amnion incorporates the whole embryo, including connecting stalk and YS, and takes origin at the umbilical root. Further expansion forms the umbilical cord as a tube of an amnion covering both the vitelline duct and the connecting stalk. The cord successively elongates, and the vitelline duct continues to narrow while the YS remains in the umbilical sheath[6] *(Fig. 11)*.

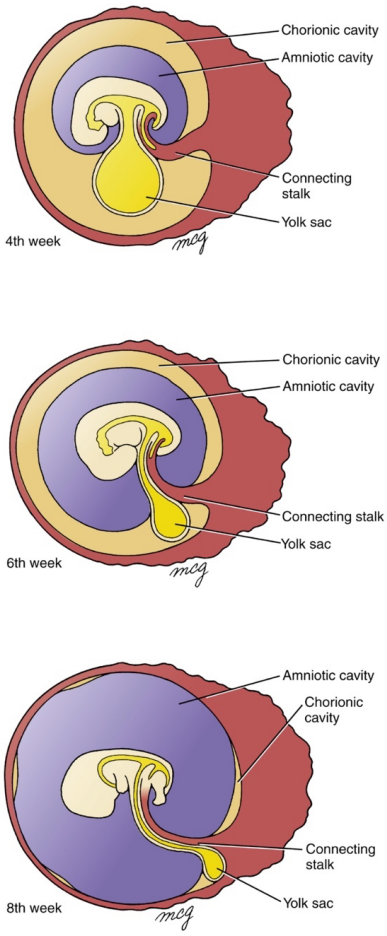


Fig. 11 Development of the umbilical cord[28]**.** Fusion of the vitelline duct with the body stalk into the umbilical cord. Umbilical cord elongation due to expansion of the amniotic cavity leads to further narrowing of the vitelline duct. Close to the placental cord insertion, the YS remnant can often be found as a flat, gold nodule. *Figure reused from Larsen's Human Embryology with permission. © 2021, Elsevier Inc. All rights reserved.*

Regression of the secondary YS starts about (E 45–50)[44], by the end of the first trimester, when the exoceolomic fluid is absorbed and the amnion has fused with the chorion[60]. After its degeneration, the secondary YS may occasionally be seen as a flat, gold, nodular remnant between amnion and chorion of the fetal surface[60] *(Fig. 12)*.

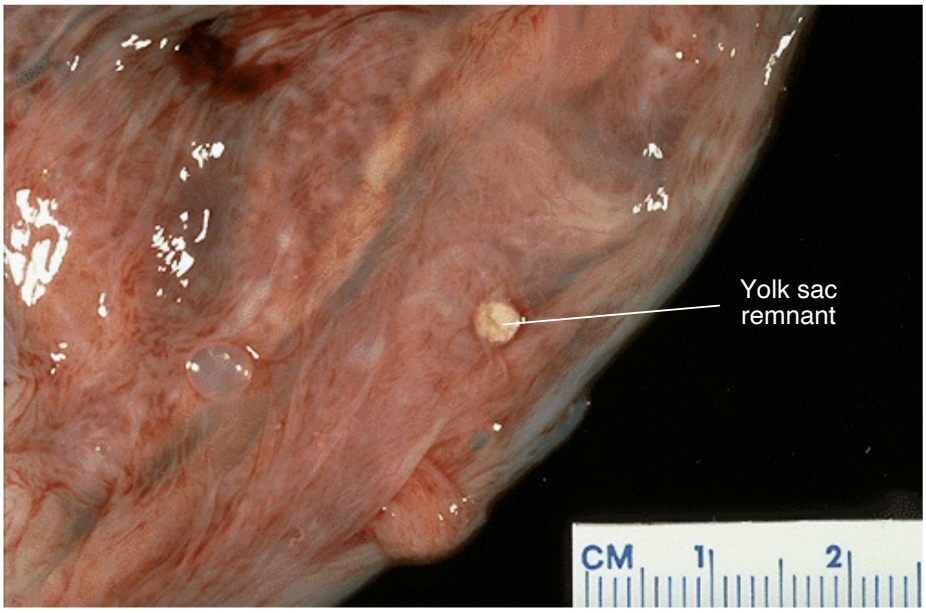


Fig. 12 Yolk sac remnant on the fetal surface of a term placenta. *Picture adapted with permission. ©2002-2023, PathologyOutlines.com, Inc[62]. Original annotations were removed using Affinity Designer 2, v.2.0.3 Brush Healing Tool.*

1.3 Function of the secondary yolk sac

The secondary human YS has a crucial role during embryonic development. It is the first hematopoietic organ[1,63] and can be considered as upgraded extension of the embryonic gut, evolved to take on nutritional, endocrine, and liver-specific functions during organogenesis[43].

Hematopoesis and stemcells

Human hematopoiesis occurs in several waves[43]. During the first wave, at 5–6 weeks (MA) or CS 7 onward, large and nucleated erythrocytes, macrophages, and

megakaryocytes form in the blood islands of the YS mesoderm[1,43,64]. Both, the surrounding vascular endothelium and these hematopoietic cells likely develop from a transient bipotent precursor cell (angioblast or hemangioblast) with either embryonic or extraembryonic origin[43,63]. The blood islands of the YS wall rapidly form an extensive vascular plexus that envelopes the whole YS[43] and is founding the basis for the embryo-vitelline circulation. After initiation of cardiac activity, erythroblasts can be found in the circulation, including the cardiac cavity[1,64].

All stages of human YS macrophages, immature to mature, are in the early human YS[57]. Their ultrastructure is very similar to the macrophages in the hepatic sinusoids of the embryo[57], and it has been suggested that, analogous to mice, human macrophages migrate to the embryo via blood circulation through the YS stalk during a limited time window[65]—the second wave of hematopoiesis . Here erythro-myeloid progenitors[66] and lymphoid progenitors [67,68] was long thought to function transiently, only to support the developing embryo before generation of definitive hematopoietic stem and progenitor cells (HSPCs) in the aorta-gonadmesonephros region (AGM) during a third hematopoietic wave (i.e., after 7 weeks)[1,63,69]. Earlier, it has been suggested that hematopoietic cells, derived from human YS, lack lympho-hematopoietic stem cell potential[1,63,70].

However, lineage tracing experiments in mice and fate mapping could show that certain adult tissue-resident macrophages, such as microglia, Kupffer cells, and alveolar macrophages, do not solely develop from hematopoietic stem and progenitor cells (HSPCs)[69]. Furthermore, these experiments have demonstrated that not only populations of macrophages[68,71–74] but also mast cells[75] and lymphocytes[76] develop from HSC-independent YS-derived progenitors. These are thought to seed developing organs and to generate tissue-resident populations maintaining their selves throughout adulthood[59,69].

Due to the limited access to early-stage human tissue, the human embryonic hematopoiesis system has for a long time been less understood than that of mice. Despite this, erythro-myeloid progenitors have been found within human YSs[66,77,78]. In addition, as early as (E 19), *in vitro* colonization of both, human embryonic YS tissue and tissue from the whole paraaortic splanchnopleure (P-Sp), including the

dorsal aorta of the embryo proper, showed separately potential to generate hematopoietic colonies[70]. Later, mRNA sequencing of hematopoietic cells from human embryos at (CS 11–23), as well as the functional assessment of myeloid-biased progenitors derived from YSs, showed similarities with their tissue-resident adult counterparts, indicating comparable mechanisms in human embryos[79]. Moreover, it is possible to identify a particular type of T-cells (Vδ2+) during embryonic development as early as 5 gestational weeks (MA) in the liver and thymus, which is mainly present before 12 gestational weeks (MA). It is believed that these cells could have a distinct origin from HSC-independent progenitors within the YS, like the (Vγ3+) T-lymphoid lineage observed in mice[69]. Atkins and colleagues[69]suggested the existence of a program for multipotent hematopoietic progenitors (MPPs) in the YS. They arrived at this conclusion after demonstrating *in vitro* that mesoderm generated from pluripotent human stem cells (PSCs) had the ability to produce various blood cell lineages, including erythro-myeloid and lymphoid YS progenitors. More recently, Goh et al.[59], arrived at the conclusion that initial erythropoiesis is YS restricted, based on their finding that the human embryonic liver remained macroscopically pale before CS 14, a finding that was supported by hemoglobin (Hb) subtype tracking.

At later gestational age (7–8 weeks), the liver replaces the YS as the main hematopoietic site and the bone marrow becomes a hematopoietic organ thereafter—the end of the 3rd month[64].

In summary the process and molecular mechanisms of primary hematopoiesis and hematopoietic reallocation are complex processes; cellular response, cell counts, and cell characteristics are involved in changes of the local microenvironment, growth factors, and signaling that depend on the location and stage of development. In addition, the vascular system of the YS plays an indispensable role in nutrient transport throughout early gestation[43]. Recent evidence from research on mice, human tissue, and stem cell studies indicates that embryonic YS progenitor cells play a significant role in the development and maintenance of immune cell populations in adult tissues[69].

Nutrition

The maternal circulation to the human placenta is limited during the initial 10-12 weeks of pregnancy[80]. As such, the intra-placental circulation is not fully established and functional until the end of the first trimester[15,81–84], and the embryo primarily depends on uterine secretions, known as histotrophic nutrition, for nourishment[15]. The absence of intra-placental circulation in the early stages of the first trimester cannot only be demonstrated through ultrasound Doppler imaging[61] *(Fig. 13)*, but also intra-arterial measurements of placental oxygen concentrations that does not increase before 10–12 weeks of gestation (MA)[82].

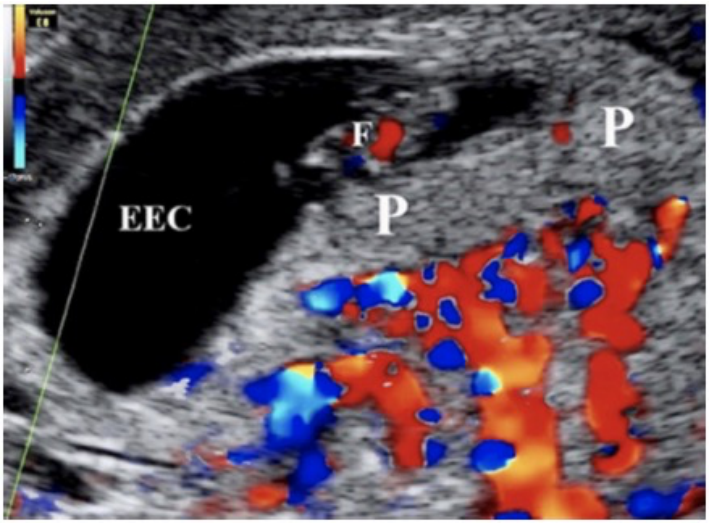


Fig. 13 Ultrasound of the extraembryonic coelomic cavity (EEC) with placenta (P) and fetus (F). The Color-Doppler flow illustrates the lack of circulation within the early placenta, while fetal blood flow could be demonstrated. *Reused with permission under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International (CC BY-NC-ND 4.0). © 2018 Burton and Jauniaux*[61].

Additionally, the umbilical circulation does not establish a connection between the fetal and placental systems until approximately four weeks post-conception[85]. Consequently, despite the invasive nature of human implantation[15], and although maternal erythrocytes have been detected within the lacunar spaces from (E 12–13)[86], a sufficient bloodborne hemotrophic nutrition seems unlikely during this early stage. Another consideration is the initial composition of the intervillous space, which is

exclusively filled with a clear fluid[87] and remains unconnected to the spiral arteries[86,88]. These arteries, still occluded by trophoblast cells[89,90], have not undergone remodeling and present high resistance[90]. It is only at a later stage that the intercellular spaces within the trophoblast begin to coalesce, forming an expansive network of channels that eventually permits maternal arterial blood to flow into the intervillous space[87,89,90].

The human endometrial surface contains about 15 gland openings per square milimeter[16]. The significance of these glands was evident in sheep, as the inhibition of their secretions during early pregnancy was closely associated with miscarriage and restricted growth[80,91]. The glandular epithelium's apical surface shows microvilli that exhibit signs of both apocrine and merocrine secretory mechanisms and can produce the histotroph or "uterine milk"[92] that contains lipids, mucopolysaccharides, glycogens, and glycoproteins, such as glycodelin and MUC-1[15,93]. These products could be found within the intervillous space (IVS) and the cytoplasm of the villous syncytiotrophoblast[93], indicating that they provide the trophoblast with substrates for organogenesis and glycolysis[94]. Moreover, tracer studies have demonstrated that syncytiotrophoblast absorbed proteins pass into multivesicular bodies, and it has been proposed, that they are degraded to their constituent amino acids serving as source to the fetus[95].
The plasma filtrate that is penetrating through the trophoblastic plugs on the tips of the spiral arteries[15] could be another source of nutrients in the intervillous fluid. However, although the maternal glycoproteins may be degraded within the syncytiotrophoblast, the resulting amino acids and sugars still need to be conveyed to the fetus[15]. The detection of glycodelin in amniotic fluid suggests that certain maternal substances traverse the trophoblast into the core of the villi. From there, they can move through the stromal channels into the coelomic fluid and the amniotic space[15]. During that passage they get also in contact with the secondary YS, that floats within the exocoelomic cavity[96] *(Fig. 14)*.

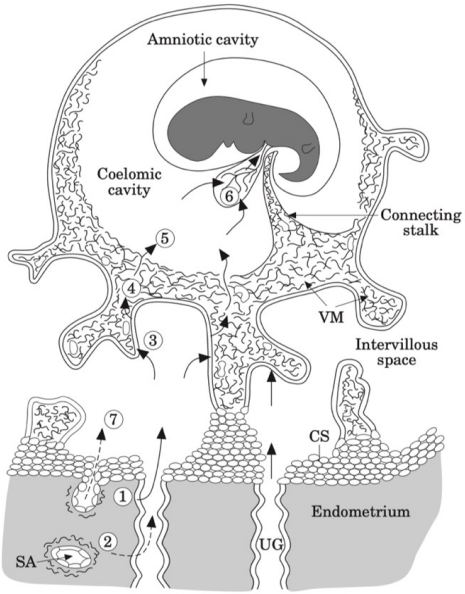


Fig. 14 Schematic representation of the steps along the "histotrophic pathway" during early human pregnancy[97]. (1) Secretion from uterine glands into the intervillous space. **(2)** Maternal serum transudate originating from the spiral arteries (SA) forms another part of the fluid in the intervillous space. **(3)** Trophoblasts absorb maternal secretions, which can either be broken down and used in local synthesis processes, or **(4)** transferred unaltered into the villous mesenchymal core (VM). From there, the substances migrate through stromal pathways into the coelomic fluid. **(5)** The products diffuse along stromal channels into the coelomic fluid. **(6)** Absorption by the epithelia of the yolk sac with transport to the fetus through the vitelline duct and vitelline circulation. **(7)** The plugs of cytotrophoblast cells occluding the mouths of the spiral arteries loosen towards the end of the first trimester, allowing first maternal serum, and later whole blood, to enter the intervillous space directly w*ith permission. © Elsevier Science & Technology Journals, published by W.B./SAUNDERS CO. LTD.*

The ultrastructure of the outer mesothelial layer of the human YS has large similarity with that of an absorptive epithelium with characteristic villi on the surface[58,98] *(Fig. 15 A).* As such, Burton et al.[15] could demonstrate, for example, the uptake of maternal glycodelin. Transport from the YS membrane to the embryo is either possible through the vitelline vascular plexus, that develops between E 16 and E 24 or through the vitelline duct. Note, that increasing vascularisation of the YS falls together with the successive closure of the vitelline duct, suggesting a redistribution process of the supplying route[99]. This is in line with results from Doppler studies of the vitelline circulation[100], where the detection rate of arterial blood flow increased significantly from five to seven gestational weeks (MA). After 10 weeks, no arterial blood flow was detected, while the umbilical circulation increased. This falls together with increased placental circulation[61], an established vascular fetomaternal connection, and the suggested debut of hemotrophic nutrition.

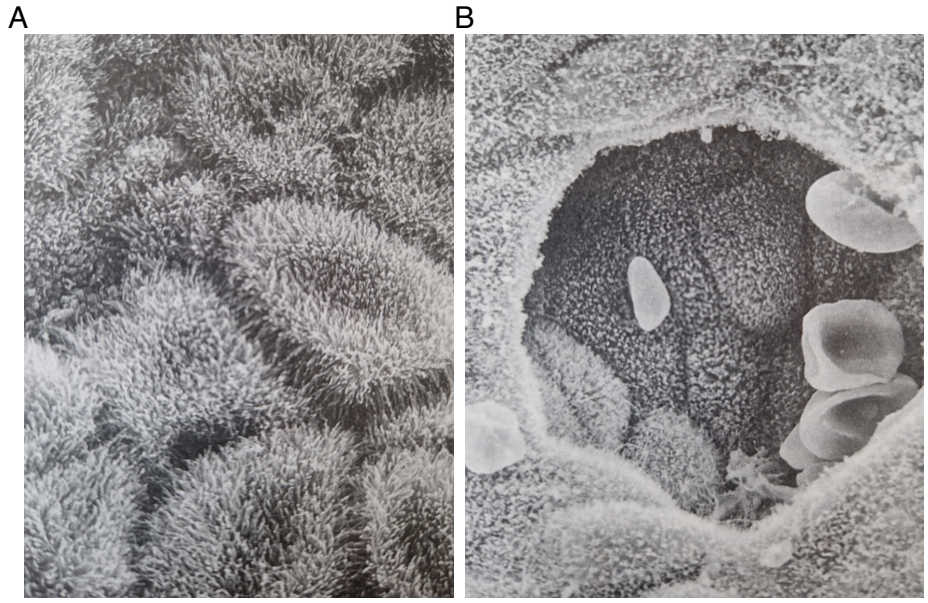


Fig. 15 (A) Surface ultrastructure of the mesothelium consisting of mesothelial cells covered only with microvilli. The cell boundaries were relatively evident. Scanning Electron Microscope x 900; **(B) Orifice of an endodermal yolk sac wall tubule surrounded by endodermal cells.** The surface of cells forming the endodermal tubule has shorter villi than absorptive mesothelial cells. Blood cells are present in the tubule; 6th week of pregnancy; Scanning Electron Microscope x 900. *Picture adapted from Nogales FF, The human yolk sac and yolk sac tumors*[101]*, © Springer.*

However, the inner endodermal layer of the YS membrane also has a dense apical microvillous border facing the YS cavity *(Fig. 15 B)* and numerous endocytic vesicles[58,102,103]. Their presence indicates an active endocytic machinery[99]. Nutrient-filled vesicles are transferred from the endosome to the lysosome, where the contents are processed, for example, proteins by lysosomal cathepsins[104], transcripts of which indicate proteolytic activity in the human YS[105]. In addition, various other lysosomal enzymes involved in digestion and energy metabolism (e.g., acid phosphatase, galactosidase, lactate dehydrogenase, γ-glutamyl-transferase, and choline phosphotransferase) have been found in the cytoplasm of apical endodermal YS cells[3]. During gastrulation, spatially resolved single-cell transcriptional profiling revealed that cell states in both the human liver and YS possess gene clusters associated with coagulation, as well as lipid and glucose metabolic processes[59]—similar transcripts and mechanisms of transport and nutrition have been reported in other species, such as mice, rabbits, and rats[59,105]. Notably, it has been reported in rats

that the visceral YS accounts for up to 95% of the amino acid uptake during organ formation[106]. After being processed the products can be transferred directly or resynthesized to new proteins from the YS to the developing embryo[43,99]. Again, the middle mesenchymal layer provides blood vessels to access the fetal circulation *(Fig. 16)*.

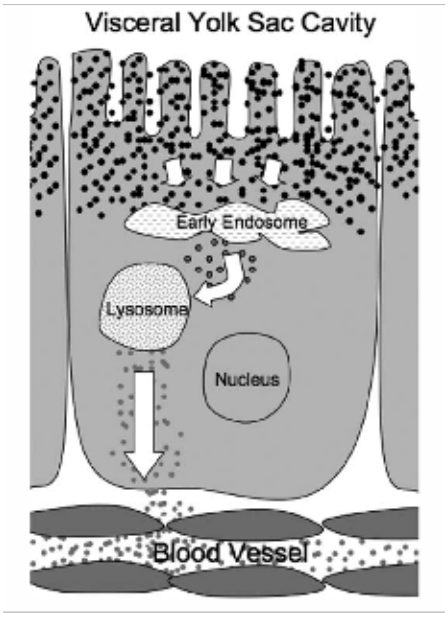


Fig. 16 Absorption of nutrients in the endoderm of the human yolk sac. Nutrients are endocytosed by microvilli on the apical surface of the endoderm and enter the early endosome for transfer to the lysosome for digestion. After that, breakdown products are secreted into the mesenchymal cell layer, where they have access to the vitelline circulation and embryo. *Reused from Zohn and Sarkar (2010)[99] with permission. © John Wiley & Sons – Books.*

The interface between the extra-embryonic coelomic cavity and the embryonic compartments has been further elucidated by detecting glycoproteins in the fluid of the human YS, which are not synthesized by the cells of the YS membrane; for example, human choriogonadotropin[107].

It is noteworthy that the orientation of human YS membranes is inverted compared to that of rodents. In humans, the absorptive endodermal cell layer is internal rather than external. Nutrients must first traverse the YS lumen before encountering the apical endoderm[99]. However, the compositional similarities between the coelomic and YS fluids suggest a free exchange of proteins and other nutrients across these two compartments[108].

Finally, mRNA transcripts encoding a wide array of solute carrier proteins, which facilitate the transport of amino acids, glucose, vitamins, nucleoside sugars, and ions

from the coelom into the cavity of the secondary YS, suggest the preservation of essential transport functions in the YS membrane[105].

In summary, the human YS plays a crucial role in embryonic nutrition prior to the full development of the placenta. *De novo* synthesis of various molecules and enzymatic digestion are primarily functions of the inner endodermal cell layer of the secondary YS. Conversely, the outer mesothelial layer likely supports embryonic nutrition by conveying nutritive fluids from the trophoblast to the embryo[3]. This transfer occurs via two principal pathways: through the blood vessels in the YS wall and through the YS cavity, which essentially extends the primitive gut[43].

Cholesterole

The YS is suggested to be involved in embryonic cholesterol supply and metabolism, especially before the start of liver cholesterol synthesis and before the vascularization of chorionic villi is established[109,110]. Cholesterol is a lipid that is primarily transported by the lipoproteins HDL and LDL[110]. In embryonic development, it plays a critical role in cell membrane formation, production of bile acids, and synthesis of steroid hormones[109]. As such, maternal cholesterol is also involved in progesterone synthesis by the corpus luteum, suggesting its involvement in early development even prior to implantation[111].

Additionally, cholesterol participates in signaling pathways, such as the modulation of targeted gene activation or repression. These pathways influence the development of various organ systems, including the neural tube, brain, limbs, and heart[109].

Studies in humans support the concept of cholesterol transport through the YS, evidenced by the expression of receptors for lipoproteins on the visceral endoderm[109]. These studies also document the secretion of lipoprotein particles, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL)[56,112]. Moreover, a more recent investigation has identified mRNA molecules that encode for various apolipoproteins, the cholesterol efflux transporter ABCA1, and lipoprotein receptors[105]. These findings collectively underscore the YS's role in establishing a maternal connection to essential pregestational mechanisms,

with potentially significant implications for the embryo during critical stages of development.

Antioxidants

Free radicals, as part of reactive oxygen species (ROS) and reactive nitrogen species (RNS), are highly reactive by-products, ubiquitously produced during normal cellular processes[113,114]. Their accumulation can inflict damage on DNA, proteins, and lipids[113]. Hence, their detoxification with antioxidants is crucial, particularly during development.

The YS is involved in the transport and biosynthesis of antioxidants, including α-tocopherol (vitamin E)[115], ascorbic acid (vitamin C)[115], and uric acid[114]. It also synthesizes glutathione, utilizing enzymes such as γ-glutamyl cysteine synthetase and glutathione synthetase. Superoxide dismutase and glutathione peroxidase enhance the antioxidant defence[115]. First-trimester analyses of maternal and fetal serum, along with fluid from the amnion and coelomic cavity, have revealed a subsidiary role for the glutathione-related detoxification system[114]. Conversely, embryonic compartments exhibit elevated levels of vitamin C and uric acid[114]. The presence of vitamin E in the uterine glands, coelomic fluid, embryo, and secondary YS mesothelium[114] suggests an YS-mediated antioxidant transport mechanism.

It is noteworthy that diminished activity or availability of antioxidant enzymes or their substrates may result in embryopathy[115]. For instance, animal studies under diabetic conditions have demonstrated a link between glucose metabolism and oxidative phosphorylation; elevated glucose levels can increase phosphorylation rates and consequently raise the likelihood of reactive oxygen species production[115]. In YSs of embryos exposed to diabetic environments, there is a reduction in superoxide dismutase activity, growth, and protein content, coinciding with an increased incidence of congenital anomalies[116]. Generally, diabetes affects the development of human offspring[117,118], with epidemiological studies further substantiating a significant association with congenital malformations[119].

More recently, the antioxidative capacity of melatonin and its role in reproductive processes have garnered increasing attention[120,121]. This hormone has demonstrated

greater antioxidant efficiency than vitamins C and E[122], actively scavenging ROS, upregulating the expression of genes for superoxide dismutase and glutathione, and inhibiting pro-oxidative enzymes[121]. Melatonin's signaling effects, mediated through MT1 and MT2 receptors found in ovarian follicles, the endometrium, trophoblast, and blastocyst with inner cell mass, appear to be beneficial during implantation and early development[120,123]. It is noteworthy that the inner cell mass is also the origin for cells of the primary YS. Since melatonin levels are closely linked to sleep and circadian rhythms[124], melatonin represents a maternal link between sleep, circadian regulation, and early embryonic development.

However, ROS and RNS are not solely deleterious; they have also positive effects on cellular immune responses[125] and signalling functions[126]. At physiological levels, free radicals regulate a variety of cellular functions, such as gene expression by redox sensitive transcription factors and post-transcriptional regulation through changes in mRNA stability[115].

Vitamins and trace elements

Several micronutrients have been detected directly within the secondary YS[114,127], while others have been indicated indirectly both in human and animals, through the transcripts of soluble carrier proteins, suggesting their transport[105]. Micronutrients, essential for normal physiological functions, include vitamins that act as antioxidants, prohormones, or cofactors in metabolic reactions, and trace elements that function as catalytic or structural components within organic molecules[128]. Trace elements may also contribute to immune responses[129].

Recent studies in mice and humans have shown that the YS expresses high levels of metal transporters for zinc and iron, as well as for vitamins, such as B12, C, E, and folate[105]. In rodents, it has been demonstrated that vitamin B12 forms a complex with intrinsic factor/transcobalamin in plasma, which is endocytosed by the YS endoderm[130]. Vitamin B12, as a cofactor in folate metabolism and amino acid synthesis, plays a significant role in development[131]. Plasma retinol (vitamin A), bound to retinol-binding protein, has been detected in rat embryo cultures radio-labelling studies[127]. This protein is likely transported into the YS endoderm via

retinol-binding protein-receptor mediation and secreted into embryonic circulation[127]. Elevated levels of the retinol transporter transthyretin in rodents supports the notion of active retinol transport, crucial for eye development, retinoic acid synthesis, and signaling throughout embryogenesis[43].

Protein synthesis

The secondary YS endoderm has been suggested to be actively involved in protein synthesis, as evidenced by its structural characteristics[102] and the presence of proteins identified in animal and human immunohistochemistry studies. For example AFP, the first embryo-specific protein of YS origin identified in humans[132,133], along with other proteins such as albumin[134,135] and α_1-antitrypsin[134,135]. Ferritin[134,135], transferrin[134,135], and α_2-H-globulin[3], have been detected in the cytoplasm of the large endodermal cells located in the inner portion of the YS wall in embryos between six to eight weeks of age. Albumin acts as a carrier for fatty acids, dyes, metal ions, and hormones[3], and is important for maintaining osmotic pressure. Studies have involved not only in vitro cultivation of human YS tissue fragments[56,134], but also YS tumors and polyembryomas[3,112]. The protective role of AFP in initial development, postulated due to its estrogen-binding capacity across various species, remains speculative in humans[3]. However, human AFP has shown affinity for other hydrophobic molecules, including fatty acids, and it binds to metal ions, bilirubin, deoxycholate, and retinoic acid, suggesting a role in modulating the local microenvironment and cellular function[137].

By approximately 60 days post-conception, the fetal liver also begins to synthesize AFP, albeit with structural differences from the YS form. The source of AFP, whether vitelline or hepatic, has been discerned via electrophoresis[138].

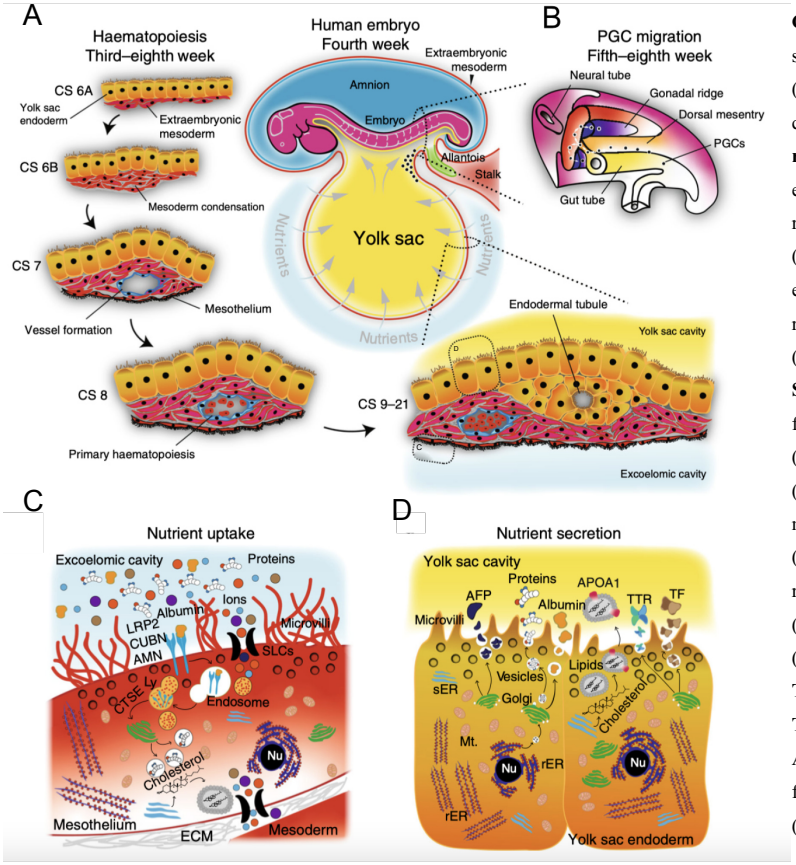
More recently, the expression of transport proteins such as AFP and albumin, in addition to alpha-1-antitrypsin, EPO, and coagulation proteins (including thrombin, prothrombin, and fibrin), has been verified through spatially resolved single-cell transcriptional profiling in the human YS endoderm and embryonic liver hepatocytes[59]. The pivotal role of these proteins, expressed by the YS, is underscored

by *in vivo* experiments showing embryonic lethality in homozygous null mice lacking coagulation factors prior to establishing liver synthetic functions[139].
Serum proteins including transferrin, AFP, prealbumin, albumin, and alpha-1-antitrypsin were primarily observed in early preparations from human YS tissue fragments[134]. In later stages—specifically, eight weeks and 11 ½ weeks gestation—levels of these proteins either declined or were not detected, implying that protein synthesis in the endoderm is most active during the early stages of YS development and decreases as gestation advances[59,134]. Further ultrastructural analysis indicated that protein synthesis in the YS endoderm ceases around the ninth gestational week (MA)[58], which is in line with transcriptional profiling data[59]. This data also demonstrates a shift in expression patterns between the early and late YS endoderm stages, showing a subsequent decrease in the synthesis of EPO and enzymes by the YS endoderm, with embryonic hepatocytes taking over these functions[59].
Additionally, an increase in genes associated with cell stress and death post-nine weeks gestation (MA) has been observed[59], corroborating the presence of both time-specific and organ-specific expression patterns during embryonic development.

Primordial germ cells (PGC's)

Primordial germ cells (PGCs) are haploid and serve as the progenitors of sperm and egg cells. They were first identified in the YS as early as 1911[140]. *In vitro* models have posited the epiblast as the likely origin of human PGCs[141,142]. During the fourth and fifth week of gestation (MA), these cells migrate from the embryo and enter the YS during gastrulation[43]. Subsequently, as the YS's endoderm undergoes lateral folding, the PGCs re-enter the embryo[43]. Following their translocation from the hindgut into the dorsal mesentery, PGCs migrate toward the gonadal ridges[43]. It has been hypothesized that the posterior YS serves as a resting place, safeguarding the developing PGCs from the signalling cascades within the embryo proper. This region offers a niche for the formation and consolidation of the germ cell gene regulatory network, while also providing a suitable environment for epigenetic reprogramming, which enables PGCs to develop into any type of cell (totipotency)[43].

1.4 Synopsis of the yolk sac functions



CS (Carnegie stage), **PGC** (primordial germ cells), **Nu** (nucleus), **rER** (rough endoplasmic reticulum), **sER** (smooth endoplasmic reticulum), **Mt** (Mitochondrion), **SLC** (solute carrier family), **Ly** (lysosome), **CTSE** (Cathepsin family member E), **LRP2** (LDL related receptor 2), **CUBN** (Cubulin), **ApoA** (apolipoprotein A), **TTR** (transthyretin), **TF** (transferrin), **AFP** (alpha-fetoprotein), **AMN** (amnionless).

Fig. 17 Overview of the functions of the primate secondary yolk sac (YS). (A) Hematopoiesis and vasculogenesis with the formation of a complex network of blood vessels starting at (CS 6A). Reciprocal signaling between the extraembryonic endodermal inner layer and mesodermal mid-layer leads to the formation of primitive blood islands. These processes prepare for embryonic circulation, facilitated by embryonic heart formation, during subsequent development. **(B)** Primordial germ cells (PGCs) are specified in the embryo and migrate through the YS to the hindgut and then towards the genital ridges. **(C)** The outer mesothelium exhibits characteristics of absorption, degradation, and synthesis, as indicated by the presence of LRP2-CUBN-AMN endovesicular complexes in the plasma membrane. After nutrient transfer from the exocoelomic fluid to the intracellular compartment through endosomal inclusion, transport continues to lysosomes that contain hydrolytic enzymes such as cathepsins to degrade maternal proteins and complex molecules. Finally, nutrients are either transported or synthesized and subsequently exocytosed into the surrounding blood vessels or directed through the extracellular environment towards the endodermal tubules and the YS cavity. **(D)** The inner layer (endoderm) contains an abundant rough and smooth endoplasmic reticulum, glycogen vesicles, and exocytotic vesicles. Its cells produce and release carrier proteins (e.g., AFP, transthyretin, albumin, and transferrin) into the YS cavity. They can be absorbed by the endoderm of the primitive gut and enable as such embryonic nutrition via the vitelline duct. *Figure reused from Ross & Boroviak, (2020)*[43]*. With permission under a Creative Commons Attribution 4.0 International License.*

1.5 Yolk sac size

The first static ultrasound analysis of the human yolk sac size was in 1979[143] and only two years later, the first growth curve could be reported[144] ranging between seven and 11 gestational weeks (MA). Before transvaginal ultrasound was introduced in the late 80's[145], measurements of yolk sac size demonstrated large variation when correlated with CRL or menstrual age[144,146]. This was changed by employing the transvaginal approach, making it possible to visualize the secondary yolk sac earlier (≈1week) and with higher precision[136].

There are different methods for determining the yolk sac size using ultrasound. One common method is to calculate the arithmetic mean of the largest diameters of the two orthogonal measurements. Here, different caliper placements are possible (i.e., inner-to-inner[147], center-to-center[136,148,149], and outer-to-outer diameter[150,151]). Few studies have reported precision parameters such as inter- and intra-observer variability. Since the echogenic yolk sac wall-thickness varies with transducer-dependent point spread function and gain settings, some authors have advocated a center-to-center caliper placement[148], while others recommended outer-to-outer placement provided magnification and gain settings are optimal and enhanced gamma level is ensured[152].

Conversely, it has been reported that true yolk sac size is more accurately estimated when employing volumetric approaches because a more detailed external surface evaluation can be achieved[153]. Two examples of transvaginal volumetric measurements are the multiplanar method[154–156] and virtual organ computer-aided analysis (VOCAL)[153,157].

However, regardless of the method used, the pattern was similar. The YS size increased weekly from the fifth to the 11th week of gestational age (MA) in accordance with the CRL *(Fig. 18)*. In addition, reference intervals based on *in vitro* fertilization studies demonstrated the same pattern[158]. Between five and 10 gestational weeks, the YS growths in a linear fashion and longitudinal studies with prospective measurements reported a growth-rate of ≈0.4 mm per week[148,159].

After 10–11 weeks, the YS size remains for a short period constant, before size decreases, and the YS finally disappears[148,159] *(Table 1).*

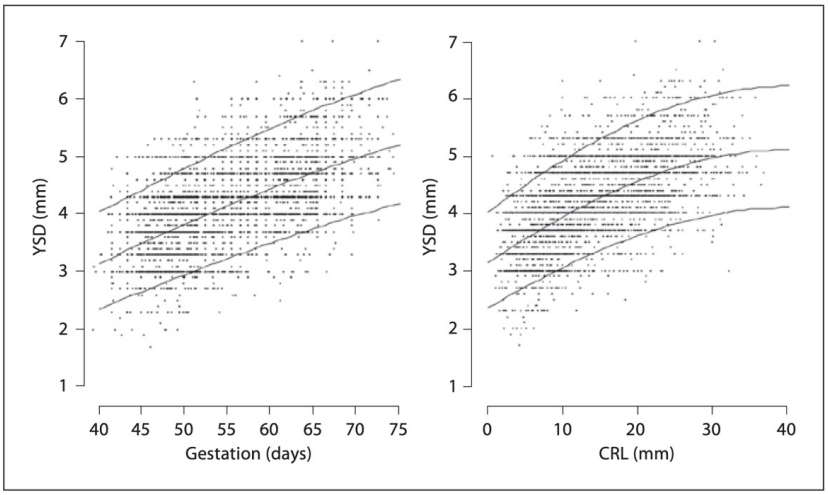


Fig. 18 Yolk sac (YS) growth-curve[149]. Relationship between YS diameter (center-to-center) and gestational age (MA) in days (left) and according to embryonic CRL (right); median, 95th, and 5th percentiles. *Figure adapted (original annotations have been reworded using the Affinity Designer 2, v.2.0.3) with permission. © Karger AG, published by S. Karger AG.*

Secondary yolk sac variations and associated factors

YS size is not only variating according to gestational age. In several studies, abnormal YS size findings have been associated with miscarriage[144,146,147,150,157,159–165]—not only YS absence, but also the presence of a small (<5th percentile) or large (>95th percentile) YS[147] *(Fig. 19).*

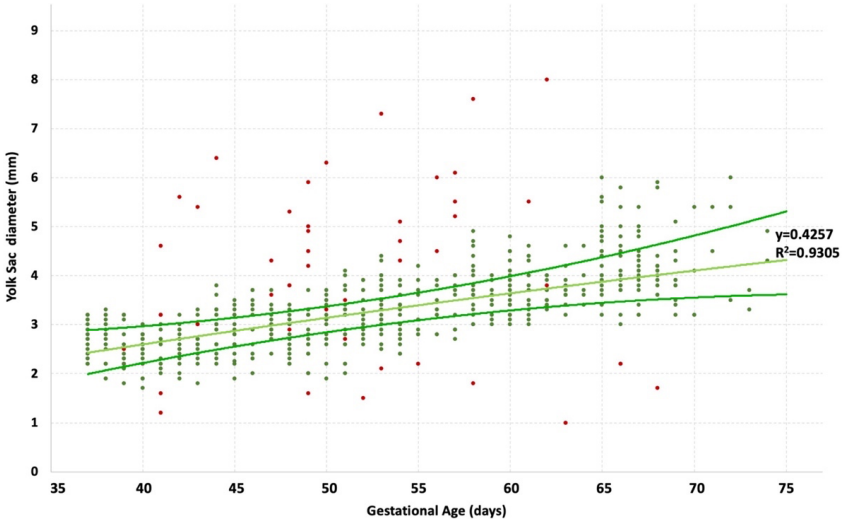


Fig. 19 Longitudinal changes in yolk sac diameter in normal and abnormal pregnancies. Each dot represents a measurement at a given gestational age, and the three green lines represent the median diameters in ongoing pregnancies with the first and third quartiles (green dots, ongoing pregnancies; red dots, pregnancies destined to be lost). *Figure reused from Detti et al. (2020)[159] with permission. © John Wiley & Sons – Books.*

Other abnormal ultrasound findings, including an irregularly shaped and calcified YS, have also been associated with miscarriage, and one study reported that an abnormal YS shape was more specific than an abnormal size[165]. However, it is noteworthy that abnormal YS findings do not always indicate a miscarriage. Although less common, pregnancies can successfully progress with an oval and enlarged yolk sac[166]. Furthermore, serious pathology and unfavorable outcomes have been associated not only with YS shape and size but also with other sex-specific sonographic differences. Even though fetal sex alone has not been associated with the size of the YS, differences such as the distance to the embryonic pool have been described for male and female embryos[167].

However, in addition to miscarriage, other fetomaternal pathologies, such as aneuploidy[161,168–170] and insulin-dependent diabetes[155,171], have been associated with abnormal YS size. Results regarding the association between YS size and diabetes are conflicting, though[172].

A more recent review[173], however, described how hyperglycemia can influence the structure and function of the YS—i.e., visceral YS capillaries and vitelline vessels become sparse, patchy, and not uniformly located in rodents[174]. There are also

reduced numbers of rough endoplasmic reticulum, ribosomes, and mitochondria in endodermal visceral YS cells[174]. Furthermore, experiments on the transport function of these cells in rats, have shown that the cellular uptake of peroxidase is diminished when conceptuses are cultured under hyperglycemic conditions[175]. Findings from human studies corroborated both structural alterations and alterations in prostaglandin E2 levels of YSs associated with maternal diabetes mellitus[155,176–178].

Nevertheless, the question remains, whether these structural and functional changes related to fetomaternal pathology may also lead to varying YS sizes.

A comparison of ultrasound and morphological findings demonstrated significant degenerative changes in the YS wall when its maximum sonographic size was reached, and it has been suggested that the YS diameter may increase between nine and 10 weeks secondary to these morphological changes[136].

This suggests that degenerative changes within the YS membrane have an impact on stability, elasticity, and permeability.

Another factor could be the intracavitary pressure of the secondary YS or external pressure within the surrounding exocoelomic cavity. One relevant biological factor for the variation in pressure, especially in a low-pressure environment, is the difference in osmolarity. In plasma, it depends mainly on salt concentrations, that is, sodium (chloride and bicarbonate), and non-electrolyte glucose and urea[179]. Notably, the concentration of pre-albumin in the coelomic fluid was positively correlated with the YS volume[180]; however, plasma osmolarity depends only to a minor degree on the protein concentration (i.e., 0.5%)[179]. Furthermore, the coelomic and YS cavity protein concentrations are similar[107] which contradicts the hypothesis of a protein-caused osmotic gradient, while other osmotic agents may still play a significant role.

With regard to the thickness of the YS wall itself, pathological conditions could also be associated with hydropic cell degeneration or edema resulting from circulatory pathology within the embryo proper[136].

Finally, vascularization, number of cells within the YS wall, or cellular content could differ, thereby contributing to differences in wall thickness.

However, when mechanisms occur in adaption to maternal and environmental factors and are related to normal embryonic development, they may be considered

physiological responses. Data from studies in rodents suggest, for example, that the histotrophic function of the yolk sac is adaptable to developmental changes influenced by diet[181]. This suggests it offers a mechanism for embryos to react to inadequate nutrition, thereby safeguarding fetal growth and "competitive fitness"[181]. This is in line with the 2018 study by Karlsen et al., who reported that YS size in low-risk pregnancies was positively correlated with maternal size[151]. These effects were traceable in later fetal size development[151].

Table 1 Overview of early yolk sac (YS) and related embryonic development according to gestational age as the number of weeks from LMP (MA), embryonic age as the number of days from conception (E), Carnegie stage (CS), and embryonic/fetal crown-rump length (CRL*) in millimeters with 5th and 95th percentiles. The median YS diameter in mm, with its 5th and 95th percentiles, was based on center-to-center measurements using transvaginal ultrasound (TVS).

Menstrual age	Embryo age	Carnegie stage	CRL	(Development Embryo/ YS)	YS size
2–3	E2-3	CS2	—	None – **global embryonic gene activation** occurs at 4–8 cell stadium[41,42].	—
	E4–5	CS3	—	None – first cavity in morula.	—
	E6	CS4	—	None – blastocyst breaks through and escapes from the zona pellucida (free blastocyst). **Implantation** occurs in the time window between day 6–10)[48]	—
	E7–8	CS5a	—	None – formation of the primary YS endoderm– pre-villous implantation (trophoblast without lacunae). Epiblast differentiates into the amnion and the pluripotent embryonic disc. Formation of the syncytiotrophoblast and its penetration into the endometrium[43].	—
3–4	E8–10	CS5b	—	Continued development of the primary YS; first formed gaps (lacunae) in the syncytiotrophoblast. Expansion of the amniotic cavity[43].	—
	E11–12	CS5c	—	**Completed** development of the **primary YS**; lacunae form a nearly completed sphere[43]; **some mesoblast** intrude the surrounding cytotrophoblast into prospective primary chorionic villi[43]. Local thickening of the visceral endoderm[43]	—
4–5	E13	CS6A	—	**Initiation of gastrulation** in the embryonic disc and primary chorionic villi formation[43].	
	E14–15	CS6B	—	Primitive streak becomes clearly visible[43]. **"Pinch off" as small secondary YS** enclosed by visceral (embryonic) and parietal (extraembryonic) endoderm. The mesodermal cells have started to form the mesothelium (lining secondary YS and trophoblast) of the coelomic cavity.	+/-

Menstrual age	Embryo age	Carnegie stage	CRL	(Development Embryo/ YS)	YS size
4–5	E14–15	CS6B	—	Primitive erythroblasts expressing embryonic globin genes, surrounded by endothelium, appear in the YS[78,182]	+/-
5–6	E16–17	CS7	**0.4	**Hematopoietic foci appear** within the mesodermal mid-layer of the secondary YS[50] and the **development of vascular plexus** begins[57] Early gastrulation	*3.01 (1.8, 4.2)
	E17–19	CS8	**1–1.5	Notochord formation and **lateral folding, narrowing the YS base** resulting later in formation of the YS stalk[108]**, YS hematopoiesis**[183]	
	E22-23	CS9	2.4 (1.1, 4.1)	**Embryonic heart activity** begins (still without competent valvular system and development of a conduction system and the umbilical arteries connected to the primitive dorsal aorta[61] **Vitellin circulation** may be visualized with ultrasound Doppler[100] **Liver development as thickened diverticulum of the foregut**[54,134]	3.2 (2.4, 4.1)
6	E24-25	CS10-13	3.4 (1.9, 5.5)	**Early heart and established fetal circulation, but not connected to maternal circulation**[57] Hematopoietic progenitors and macrophages are detectable at CS11[79]. Macrophages, monocytes, mast cells, and innate lymphocytes are also reported[79,184]. **Liver** subsequently presented with buds and ducts[54,134]	3.4 (2.6, 4.3)
7	E29			**AFP-production** at the endodermal part of the YS wall[133,185]	
	E35	CS14–15	8.5 (5.9, 11.6)	Definitive hematopoietic stem and progenitor cells (HSPCs), capable of long-term multilineage repopulation, arise in the AGM region at CS14 [186]. Vitelline duct begins progressively to narrow[187]. **Vitellin blood-flow detection is high**[100]	3.8 (3.0, 4.8)

Menstrual age	Embryo age	Carnegie stage	CRL	(Development Embryo/ YS)	YS size
8	E36–42	CS16 CS17	15.0 (11.4, 19.1)	**Liver-hematopoiesis begins.** Fetal adrenal cortex originates from the mesothelium next to the dorsal mesentery. Medulla develops from neural crest cells associated with nearby sympathetic ganglia[183]. Separation of common cardiac outflow (aortic arch and pulmonary aorta). Definitive hematopoietic stem and progenitor cells (HSPCs) cells equivalent to AGM produced at CS11 are found in the YS (at CS16) and the liver (from CS17)[186,188].	4.3 (3.4, 5.3)
9	E44–45 E48	CS18 CS19	22.4 (18.0, 27.3)	**YS protein synthesis ceases**[58] Biliary ductuli developed in periportal connective tissue produces ductal plates that receive biliary capillaries[189] Accessory olivary nucleus (neural system development)[190]	4.7 (3.7, 5.8)
10	E56	CS20–23	30.1 (24.9, 35.7)	**Fetal pancreatic and endocrine function begins.** **Hepatic production AFP**[138] Straightening of trunk, intestines herniated at umbilicus[183] **Sharp increase of placental circulation** with rise in the intra-placental oxygen tension and antioxidant gene expression[82]	5.0 (4.0, 6.2)
10-11	E63	—	35.5 (29.9, 41.6)		5.3 (4.2, 6.4)
12	E70	—			*4.3 (3.3, 5.3)
13					+/-
14					

* Before week 12 reference values (CRL and YS) from Papaioannou et al., 2010[149] were used. After week 12, reference values from Jauniaux et al., 1991[136].

** Reference value for the embryonic length is not based on ultrasound[183].

1.6 Maternal body composition, sleep, and physical activity in pregnancy

Physical activity, sleep, and nutrition constitute essential components of physiological homeostasis. These interconnected factors are directly and indirectly related to human body composition, fitness, health (including mental health), and disease.

Maternal body composition

There is consensus that body composition has a significant impact on overall health. As people age, their body composition changes, typically resulting in an increased fat mass and a decrease in lean tissue, and an increased risk of morbidity and mortality[191,192]. In particular, increased fat mass is linked to obesity-related health risks, including type-2 diabetes, cardiovascular disease, and mortality[193]. Body composition changes physiologically during pregnancy to support fetal development[194]. The fetal unit contributes to the formation of amniotic fluid, placenta, and fetus, with approximately one-third of the physiological weight gain at the time of delivery[195].The other two-thirds are derived from the maternal unit—i.e., mammary glands, uterus, fat tissue, and extracellular fluids, and due to blood volume expansion[196]. Pregnancy and the associated changes with separate contributions from fetal and maternal units are dilemmas for body composition measurements, which is reflected in the strengths and limitations of the different methods[194,196–199] *(Table 2)*.

Table 2 Overview of the different methods of body composition measurement and their advantages (pros) and disadvantages (cons) regarding their use throughout pregnancy.

Method	Description	Pros	Cons
Anthropometric measurements[194]	-Circumference and caliper skinfold measurements in different body regions combined with other factors (e.g., body weight, gender, age, and height).	-Relatively easy applicable -Inexpensive -Safe to use in pregnancy	-Only site-specific measurements. -Operator variability -No estimate of the fetal compartment.
Ultrasound	-Maternal and or fetal skinfold thickness measurements with ultrasound -Fetal weight, placenta, and amnion fluid estimation by ultrasound.	-High correlation with estimates by a caliper[194]. -Enables also measurement of visceral adipose tissues[194]. -Data from the fetal compartments accessible.	-Only site-specific measurements. -Depending on skilled ultrasound operator.
Bioelectric impedance analysis (BIA)[194]	Method that relies on the electrical properties (the conductivity) of different biological tissues	-Fast, relatively inexpensive, and user-friendly non-invasive method -Large body of evidence with reports from different studies and fields including pregnancy -Low voltage and current considered safe in pregnancy -Valid estimates of total body water in early pregnancy compared to deuterium dilution[200]	-Not discriminating maternal and fetal components[197] Segmental body analysis is possible[196] -Overestimation of fat mass changes in normal and obese women during 1st trimester compared to other methods -In later pregnancy (32 weeks), BIA may underestimate the total body water[200]
Deuterium dilution	-Exploiting the dilution and physiological body enrichment properties of the water isotope deuterium (D_2O) to estimate total body water and hence fat free mass (lean mass)[201]	-Considered safe in pregnancy[202]. Easy to applicate and to collect samples in various settings	-Laboratory needed for analysis after sample collection.

Method	Description	Pros	Cons
Quantitative magnetic resonance	-Nuclear magnetic resonance[203] is used to measure fat mass, lean mass, free water, and total body water of the maternal–fetal unit[204]	-Fat, free water and lean mass calculation[205] -Whole body measurement -Differentiation between maternal and fetal compartment	-High costs. -Non-diagnostic scanning protocols are not recommended before second trimester[194] -Limited field of view in some women
Densitometry	-Utilizes underwater weighing and air displacement plethysmography. -Based on the constant density principle of fat mass and fat-free mass. -Measures both maternal and fetal tissues[196].	-Considered safe in pregnancy—specific equations for pregnancy considering estimated changes in free fat mass, hydration, and density have been developed and validated[206–208]	-Laboratory necessary -Two-compartment model that is challenged by increased hydration and change in density of fat free mass during pregnancy[197] -equation based estimation necessary to estimate changes in free fat mass hydration
Dual-energy X-ray absorptiometry		-Results for fat mass and bone mineral content[196]	-Although small risks to mother and fetus; ionizing radiation should be avoided
Three-compartment model	-Combined body composition estimate using: 1-body weight, 2-desitometri, 3-degree of hydration of fat free mass by total body water[196]	-Safe in pregnancy	-Time consuming -Laboratory necessary
Four-compartment model	-Combined body composition is estimate using: 1- body weight, 2-densitometry, 3- total body water by means of isotope dilution, and 4- bone mineral content before and after pregnancy[196]	-Considered as gold standard today[196]	-Time consuming -Laboratory necessary -Expensive

Nevertheless, variations in maternal body composition and gestational weight gain are also linked to pregnancy complications, such as gestational diabetes[209–212], hypertensive disease in pregnancy[213,214], cesarean section[215] even when controlling for macrosomia[216], birth weight[217,218], fetal growth[219,220], and twinning[221]. In addition, data suggests an influence on the offspring's health[222] and disease in a life-long perspective[33,223,224]. Note that not only the body composition during pregnancy but also body composition before pregnancy and around conception have effects on pregnancy[195,225], on birth outcomes[226], and on the offspring[220,227–229]; some of these can even be traced within physiological body composition ranges[225,228]. However, it is crucial to consider the time perspective (duration of pregnancy/ gestational age) when assessing alterations in both body composition and overall weight gain. Associations with pregnancy outcome and prematurity can otherwise easily be biased; for example, the total amount of weight gain in pregnancy would be less in women with shorter pregnancies (i.e., those who experience miscarriage or premature birth)[230]. Conversely, studying the effect of weight gain in term pregnancies excludes these important subgroups and their body composition changes[230].

Sleep in pregnancy

Sleep is involved in the most basic physiological regulation; thus, sleep deprivation generally has severe health consequences[231,232]. This also applies to pregnancy[233,234], in which many maternal and fetal pregnancy complications have been associated with sleep disturbances. These complications include preeclampsia and hypertension[235,236], hyperglycemia and gestational diabetes[237,238], miscarriage[239], preterm birth[240,241], macrosomia[233], prolonged birth duration[242], and assisted operative delivery or cesarean section[233,241]. The suggested pathophysiological mechanisms are often based on *in vitro* experiments in assisted reproduction and animal studies. These studies have demonstrated a potential link between the circadian rhythm, embryonic development, and epigenetic programming[243–251]. Although several methods have been developed for monitoring, recording, and classifying sleep, studies on the effects of sleep on human embryonic physiology and development are scarce[234,252]. Sleep diaries and questionnaires are good tools but succumb to participant recall bias and

subjectiveness[27]. It is only through subjective measures, however, that one can capture sleep satisfaction[29]—namely the sense of feeling rested, alert, and well enough to perform daily tasks and activities. Other methods, such as polysomnography and actigraphy, are often categorized as objective because the data are generated by different sensors or probes in combination with a software package. These methods avoid recall bias and have performed better in terms of reproducibility[27] *(Fig. 20)*.

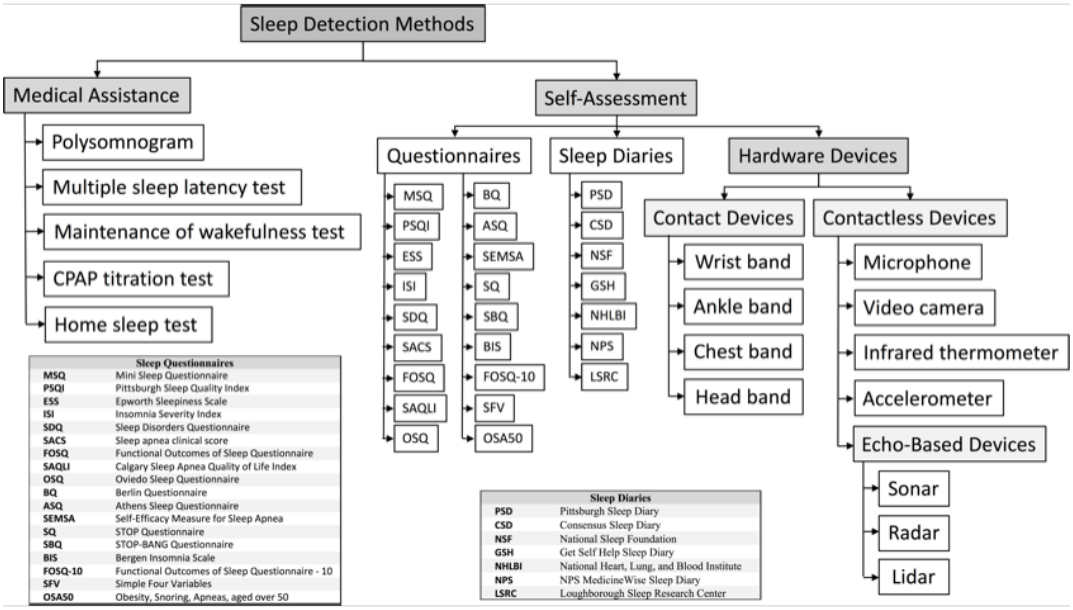


Fig. 20 Taxonomy of sleep detection methods[27]. Gray boxes represent categories. White boxes represent the methods or technologies used to assess sleep. *Reused with permission under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY 4.0) © 2018, Ibáñez et al.*

In 2014, actigraphy was implemented in the third edition of the International Classification of sleep disorders[253]. This is because sleep duration during pregnancy seems to be presented more accurately by actigraphy than by questionnaires[254]. Moreover, the technique is especially suited for studying sleep-wake rhythms by continuously recording complete days, particularly for longer periods (up to weeks and months) and in various environments, which is not possible when using polysomnography. Conversely, polysomnography allows differentiation between

sleep stages and cycles using electroencephalography (measurement of local brain activity generated potentials over time), electrooculography (eye movements), electromyography (measurement of muscle movement), and other methods[a]. It is the most advanced diagnostic tool for sleep disorders[27] and often used as "gold-standard" in validation studies[255–258].

In women, descriptive, cross-sectional studies, using questionnaires, actigraphy, or polysomnography, have reported a sleep duration of 6.8 to 7.8 h/night before pregnancy, increasing during the 1st trimester, but then decreasing during the 2nd and 3rd trimesters[259]. These findings are consistent with those of longitudinal studies[260–262]. Nevertheless, there is little data and only data from small studies describing sleep variations of healthy women from before conception[260,263,264]. However, the concept of fetal plasticity implies that early influences on a developing fetus, in particular, have the greatest potential to affect future health[265]. Given the known effects of sleep patterns and sleep duration on human physiology and their links to human pregnancy, their alterations during early pregnancy may also affect the developing fetus. The significant changes in sleep duration that occur from preconception to the end of the first trimester make this possibility particularly intriguing.

Physical activity

Widely recommended for promoting overall health and well-being[266], physical activity recommendations also extent to pregnancy, where regular maternal physical activity is beneficial for the health of the mother, fetus, and newborn[266–269]. It has been associated with healthy weight gain, improved control of maternal glucose levels[270,271], and favorable obstetric outcomes[271–275]—more specifically, maternal physical activity has been found to contribute to increased placental parenchymal volume with a larger villous surface area and vascular volume[276] and modulates placental angiogenesis[277,278]. In addition, physical activity during pregnancy reduces oxidative stress and downregulates genes involved in placental fatty acid and insulin

[a] An exhaustive list of the polysomnography channels can be found in the glossary.

transport. Genes involved in amino acid transport across the placenta are upregulated[271,279].

Similar to sleep, physical activity during pregnancy can be measured using several validated methods. These include indirect calorimetry, the doubly labelled water method, activity or activity recall logs, motion sensors (i.e., actigraphs or pedometers), heart rate monitors, and multiphasic devices, such as the one used in the current study[280,281]. Combining questionnaires with actigraphy has been suggested as advantageous for measuring moderate-to-vigorous activity during pregnancy[282]. Due to their simplicity and accessibility questionnaires and actigraphy have frequently been employed for assessing physical activity in pregnancy[281,283,284]. Triaxial accelerometers seem to represent energy expenditure better than other standard methods (indirect colorimetry and doubly labeled water)[285]. The advantages of using actigraphy over questionnaires for measuring physical activity during pregnancy[286] align with those of sleep, as described in the preceding section.

Several studies employing different measurement methods have consistently reported a reduction in daily physical activity during pregnancy[287–292]. This decline begins in the first trimester and continues throughout pregnancy, with the lowest activity levels observed in the last trimester[288,291]. Previous studies have indicated substantial individual variation and significant changes in activity levels compared with preconception measurements. These changes are already evident during the first trimester[288,291], a critical period characterized by numerous sequential developmental milestones. Therefore, it is important to start measurements before pregnancy to quantify physical activity changes, particularly during this period.

1.7 Maternal factors and epigenetic programming in early pregnancy

Forsdahl[293] and Barker[294] originally linked nutritional conditions during pregnancy to cardiovascular disease in the next generation of humans. Subsequently, several possible mechanisms were identified through animal studies, *in vitro* experiments, and prospective cohort studies[295,296]. Here, epigenetic mechanisms play a major role

in regulating the expression of DNA-encoded information and determining the specific "identity" of a cell, while the genetic code is not altered. The mechanisms involved are linked to changes in development or environmental signals. These changes regulate epigenetic chromatin markers through post-translational protein-DNA interactions, such as histone modifications, or by chemical modifications of DNA bases, for example through methylation. Additionally, non-coding RNAs, such as microRNA-mediated repression, can regulate mRNA transcription through base pairing. This can result in mRNA degradation or translational inhibition.
Currently, there is a robust body of evidence for links between epigenetic programming and maternal factors such as maternal body composition[224], diet[33], activity[297], sleep, and circadian rhythm[251]. The phase of life when epigenetic DNA imprinting is most active extends from conception until the second year of life, commonly known as "the 1,000-day period"[298]. Here, the principle of the DOHaD concept is the initiation of phenotypic alterations, typically within the bounds of normal physiology, which enable a modified reaction to subsequent challenges that also generally fall within normal physiological limits[33]. From this perspective, epigenetic changes can be viewed as swift responses to environmental challenges, in contrast to genetic adaptations, which require significant time to adjust to the environment through random alterations in the genetic code, such as mutations. Compared with epigenetic adaptations, which directly affect the phenotype of the subsequent generation in response to the maternal environment, the probability of a DNA-gene mutation conferring an evolutionary advantage and simultaneously influencing reproduction is considerably lower.
Finally, epigenetic mechanisms also include the environmental influences on gametes before conception. If, for example, one of the two inherited alleles (one maternal and one paternal) is turned off or "stamped" and does not show in the offspring, that gene is imprinted[12,299]. These mechanisms could also be traced in the human YS, as studies on mRNA expression provided evidence for genetic imprinting of sex-specific parental alleles, such as the maternal insulin and IGF-2 allele, which has been assumed to contribute to embryonic and fetal growth regulation and insulin growth-related disease later in life[300].

Epigenetics and early sexual dimorphism

Generally, the impact of sex on mammalian phenotypes and diseases is substantial[301]. However, genetic sex plays a significant role in gene expression patterns, developmental trajectories, and interactions with the maternal environment, even during the early stages of preimplantation embryonic development and before gonadal differentiation[302]. At this stage, male and female embryos primarily differ in their sex chromosome content, resulting in differential expression of sex chromosome-encoded genes through transcriptional sexual dimorphism[303,304].

One mechanism is based on a reversible and dynamic process known as X chromosome inactivation. While both X chromosomes are active at the beginning (i.e., after embryonic gene activation at day 3), the subsequent physiological process of inactivation is not fully accomplished during early development, resulting in higher expression of X-linked genes in female embryos[302–304].

As such, studies conducted in mouse[305,306], bovine[307–311], and human embryos[312] have reported increased expression of selected X-linked genes such as X-inactive specific transcript (XIST), glucose-6-phosphate dehydrogenase (G6PD), hypoxanthine phosphoribosyl transferase 1 (HPRT1), phosphoglycerate kinase (PGK), X chromosome-linked inhibitor of apoptosis protein (XIAP), and monoamine oxidase A (MAOA).[a]

Notably, X-linked genes also play a significant role in the transcriptional regulation of autosomal genes[302]. Because of their greater abundance compared to Y-linked genes, X-linked genes may have a more pronounced impact on this regulation[302]. This notion is supported by the reversal of transcriptional sexual dimorphism in autosomal genes when X chromosome inactivation is completed[302]. A notable sex difference that is linked to the environment is, for example, observed in the speed of development, particularly under suboptimal conditions such as high glucose concentration[313,314]. This is supported by the notion of sex-specific differences in glucose metabolism, with males exhibiting higher glucose metabolism[315]— that includes human embryos[316].

[a] Explanation of the function of these genes can be found in the glossary.

Another mechanism for sex-biased gene expression is gene imprinting. As mentioned above, it refers to the process of maternal or paternal alleles being turned off or "stamped" and not being expressed in the offspring. In particular, when it involves X-linked genes, it can contribute to the biased expression of genes based on sex[302]. This phenomenon is especially significant because male embryos lack the X chromosome inherited from the father[302].

Additionally, it has been observed in mice that the paternal X chromosome, which is actively transcribed during the two- to four-cell stages, undergoes progressive silencing through imprinting mechanisms in the early stages of preimplantation[317,318]. In these cases, the X chromosome inherited from the mother is inactive in certain cells, whereas the X chromosome inherited from the father is inactive in others. The preferential inactivation of the X chromosome derived from the father is restricted to cells of extraembryonic lineages[313]. Embryonic YS cells originate from the same cell lineage.

In conclusion, accumulating evidence suggests that epigenetic differences occur before gonadal differentiation and result from the presence of one or two X chromosomes. Inactivation of one X chromosome is one of the primary drivers of male and female disparities during preimplantation development, along with other mechanisms such as preferential imprinting of parental alleles. In female embryos, this inactivation introduces a delay in transcription, allowing for specific epigenetic events in female cells[319]. The variations in growth, metabolism, and genetic and epigenetic programming during the early stages before implantation suggest that males and females might respond distinctly to environmental factors. Disruptions at this stage could lead to effects that are specific to each gender, impacting not just development before implantation but also after birth[313].

1.8 Research challenges

Embryonic assessments during early human pregnancy pose significant challenges resulting in the scarcity of in vivo studies on normal pregnancies. Strict ethical regulations and concerns limit research in this area—ensuring the safety and well-

being of the mother and fetus is paramount. Additionally, the limited frequency of healthy women seeking clinical care prior to pregnancy typically leads to a lengthy participant recruitment process, which adds to the scarcity of information in these populations. This is a dilemma, since data from healthy women are needed to establish physiological ranges or new mechanisms, which, in turn, lay the foundation for understanding pathology and disease. Studies conducted on infertile populations, such as those on assisted reproduction (AR)[320], cannot be directly extrapolated to the pregnancies of healthy women who conceived naturally and under physiological conditions. Such investigations are indispensable and may extend basic knowledge. They also hold the potential to generate novel insights with implications for public health initiatives and improved clinical management practices.

2. Aims of the study

2.1 Overall aim

To study how maternal health factors (body composition, activity, and sleep) before conception and during gestation interact with human embryonic development and the sequence of events in healthy women.

2.2 Specific aims

To assess the influence of sleep, activity, and maternal body composition on embryonic and YS size in a healthy cohort with low-risk pregnancies.

Paper I

The study aimed to quantify normal daily sleep and activity duration, as well as variation, at preconception, during the first trimester, and throughout the remainder of the pregnancy (i.e., during the second and third trimesters). Furthermore, our goal was to estimate the proportion of changes from pre-pregnancy levels that can be attributed to gestation.

Paper II

The objective of this study was to test whether there is an association between maternal pregestational and early first trimester sleep duration with embryonic YS size and crown-rump length.

Paper III

This study intended to determine whether there is a connection between the duration or intensity of maternal physical activity and the size of the human YS. Additionally, the study aimed to evaluate the intra- and inter-observer variability of the current method for measuring YS.

3. Data Collection and Methods

3.1 Study population (CONIMPREG project)

Participants for Paper I were enrolled between 2014 and 2018, while for Papers II and III, enrollment spanned from 2014 to 2020. Additionally, in 2023, we generated a second dataset for Paper III that incorporated extra data to assess the intra- and inter-observer variability of the YS size measurements. These studies are embedded in the larger CONception-IMplantation interval in PREGnancy (CONIMPREG) project, which set out to prospectively gather data from over 300 healthy women who conceived naturally. Participants were recruited through targeted Facebook® advertisements and posters before pregnancy. In the CONIMPREG project, these women were followed until they conceived, then, along with their fetuses, through birth, and their offspring will be tracked until they reach five years of age. Women were eligible for the study if they were healthy, without chronic diseases, had a regular menstrual cycle, were non-smokers, were aged between 20 and 35 years, had a BMI of 18–30 kg/m^2, and had no history of complicated pregnancies or fertility problems (i.e., three or more miscarriages). Contraceptives were not used for one month before the start of the study. Women who did not conceive during the six monitored cycles allocated to them were excluded from the study. This study was approved by the Regional Committee for Medical Research Ethics, Southeast Norway (REK Southeast, ref. 2013/856a), and written informed consent was obtained from all the participants. All studies were conducted in accordance with applicable guidelines and regulations. The study is characterized by longitudinal and prospective data collection that commences before pregnancy, enabling the study of pre-conception maternal and environmental effects on the offspring. In addition, hormonal determination of conception and implantation enables precise gestational age determination (± 24h)[321–323] and allows for the study of maternal effects on the conception-to-implantation interval or, vice versa, the effect of that interval on fetal, maternal, and offspring parameters. Extensive sampling of material (stored in a biobank) and serial data collection from the mother, father, fetus, placenta, umbilical

cord, newborn, and later the child provides the opportunity for successive comprehensive studies of developmental physiology and parental or environmental effects *(Fig. 21)*.

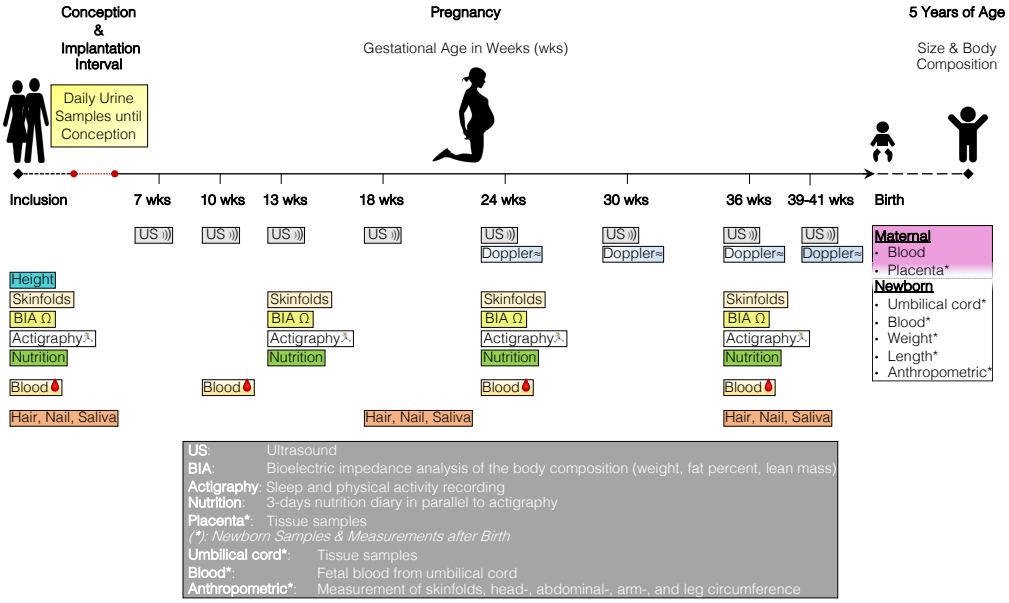


Fig. 21 Study protocol for the CONIMPREG project: Inclusion of women and their partners when planning to conceive. Determination of prepregnant body composition, average daily sleep and activity duration, nutrition records, and bio sampling (blood, saliva, hair, and nails). Daily urine samples were collected until pregnancy to determine the conception-to-implantation interval. Follow-up through gestation until birth – repeated body composition measurement, sleep, activity, and nutrition record, as well as bio samples in each trimester. Ultrasound and Doppler flow measurements of the fetus were also performed. After birth, newborn assessment and follow-up until five years of age were performed.

3.2 Study design

Paper I

Height, weight, and maternal body composition were measured at the first study visit (i.e., before conception). Physical activity and sleep recording were started on the same day using actigraphy. *(The measurement methods are described in detail below)* When pregnancy was achieved, women were scheduled for a first-trimester ultrasound based on the last menstrual period, and gestational age was adjusted

according to fetal crown-rump length. The actigraphy recording was then conducted again and repeated twice during the remainder of the pregnancy, resulting in serial actigraphy measurements of maternal sleep and physical activity before conception and during the first, second, and third trimesters *(Fig. 22)*.

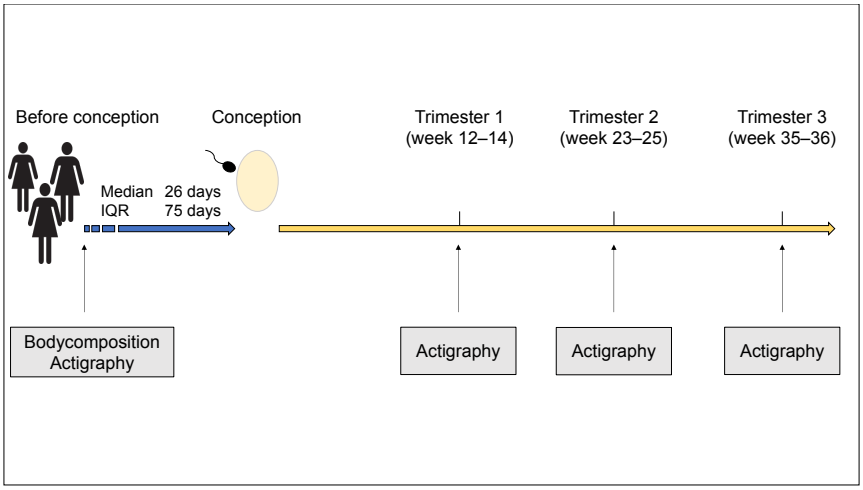


Fig. 22 Timing of actigraphy recordings in Paper I. *Material from: Vietheer A et al., Sleep and physical activity from before conception to the end of pregnancy in healthy women: A longitudinal actigraphy study, Sleep medicine, 2021, Elsevier Science*[324] *with permission. © Elsevier Science and Technology Journals.*

Papers II–III

The protocols employed for the two subsequent studies shared similarities (Papers II–III). However, their scope was limited to the timeframe encompassing the first trimester until 13 weeks and six days after LMP *(Fig. 23)*.

Four study visits were conducted in both studies. During the initial pre-pregnancy visit, eligible participants were assessed for height, weight, and body composition. Sleep and physical activity durations were recorded on the following days using actigraphy. At the second visit, the gestational age was confirmed, and measurements of the CRL and secondary YS size were obtained through transvaginal ultrasound. Ultrasound measurements were repeated at the third visit (10 ± 1 weeks). The fourth visit (13 ± 1 weeks) reassessed the activity and sleep durations, along with another sonographic measurement of the CRL.

The second paper investigated the effects of sleep duration on YS size and CRL, incorporating measurements from 7 ± 1 weeks into the analysis. In contrast, the third paper focused on physical activity and explored the impact of different intensity levels (light and moderate-to-vigorous) on YS size. However, CRL measurements were exclusively used to confirm gestational age and were not included in the analysis of the third study.

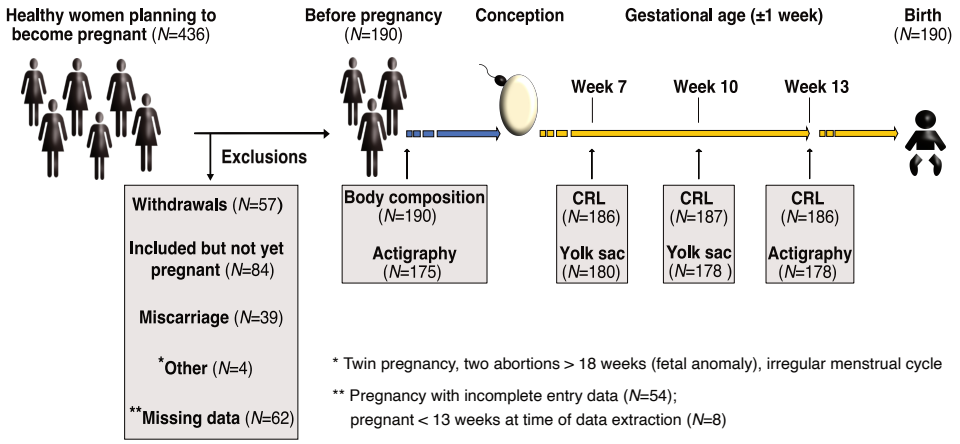


Fig. 23 Timing of measurements and recordings in Papers II–III. Note that CRL measurements were only included in the analysis in Paper II, whereas the effect of physical activity on yolk sac size was more extensively analyzed in terms of physical activity intensity levels. *Modified material from: Vietheer A et al., Effect of Maternal Sleep on Embryonic Development, Scientific Reports, 2022, Springer Nature*[325] *© The authors*

Inter- and intra-observer variability of the yolk sac measurement

Embryonic YSs were assessed at either week 7 or week 10, and video sequences (ultrasound loops) were generated and stored in the machine's local archive.

All seven ultrasound operators were instructed to select the best YS image from the sequence and measure YS size using transvaginal ultrasound. After a minimum of one day, the procedure was repeated.

3.3 Body composition, activity, and sleep measurements

3.3.1 Body composition measurement

After standardized body height measurement of the participants with a wall-mounted stadiometer[326], body weight, body fat percentage (BFP), and lean body mass (LBM) were measured digitally using a hand-to-foot multi-frequency bioelectrical impedance analyzer (BIA); Modell BC-418, Tanita, Tokyo, Japan (the present BIA-device reported the LBM as fat-free mass).

Individuals were measured in their undergarments, standing barefoot on toe and heel conductive pads, and holding hand electrodes with their arms suspended a short distance from the hip. The eight-electrode instrument is a segmental BIA, measuring body regions specifically, which presupposed information on the participant's height, age, and body type (set as standard) before weight was measured automatically, and a low amperage current was sent throughout the body. By examining electrical conductance, the BIA assesses the quantity of water in various tissues, with water-rich tissues like skeletal muscle conducting electricity better than fatty tissue or bone. The specific volume is then inferred from the electrical signal's resistance as it passes through different areas of the body[194]. Measurements were performed with standardization of the time of day as recommended by the manufacturer[327] because fluid shifts throughout the day can affect BIA measurements[328].

3.3.2 Actigraphy

Actigraphy involves recording the sensor-detected acceleration forces necessary to generate movements. Some monitors incorporate the notion of context by integrating information from multiple sensors. This integration provides increased sensitivity for detecting postural changes, subtle variations in energy expenditure associated with complex lifestyle tasks, and increased energy expenditure related to carrying loads, walking up inclines, or engaging in non-ambulatory activities. Today, these devices are also used to record sleep duration[329], especially when continuous measurements over extended periods in nonlaboratory environments are desired. Beyond sleep duration, several other sleep parameters have been defined and can be calculated

depending on the monitor and companion software. One example is sleep efficiency (SE), which represents the proportion of the total sleeping time (TST) relative to the time spent in bed. The latter is often operationalized as the time spent resting or lying down.

The current study was conducted using the SenseWear® mini Armband (model: MF-SW, BodyMedia, Inc., Pittsburgh, PA), a pattern recognition device designed for advanced activity monitoring in research and clinical settings. This monitor overcomes many limitations associated with single-axis accelerometers by integrating information from a triaxial accelerometer with other physiological sensors, such as heat flux, temperature, and galvanic skin response sensors, to provide more accurate estimates of energy expenditure. By combining these sensors with demographic parameters, the armband can also achieve accurate resting metabolic rate (RMR) estimates over long periods[330]. In this study, the monitor was configured individually for each participant and worn on the upper posterior aspect of the nondominant arm throughout the entire recording period *(Fig. 24)*.

A total recording time of 4 days or more was attempted[331], detaching the monitor at least once daily (as per the manufacturer's instructions). This usually occurs during daily body cleansing because the monitor is not waterproof. Finally, the monitor was returned to our laboratory for data extraction using the manufacturer's software package (SenseWear®pro analysis software, version 8.0 algorithm v.5.2, © 2001-2013 Body Media, Inc.). Each individual recording period, including raw data, was exported into Excel workbooks (Microsoft Office, Excel version 2016) and then merged into a single file for further

Fig. 24 Placement of the SenseWear® Actigraph

analysis using other statistical programs, such as SPSS (version 24, Armonk, NY, USA), R (Foundation for Statistical Computing, version 4.1, Vienna, Austria), and RStudio (Integrated Development for R, Boston, MA, USA). The start and endpoint of the analysis for a single day were set at midnight (i.e., 24 h, from 24:00 to 0:00 the following day), and the average sleep duration was calculated for the entire recording period. Sampling days with a data loss exceeding 6% were excluded from the calculations.

3.3.3 Activity measurements

The total daily energy expenditure for each minute of data was calculated using proprietary complex pattern-recognition algorithms. These algorithms are composed of two components: "activity classification" and "energy expenditure estimation" (SenseWear® Pro analysis software, version 8.0, algorithm v.5.2, © 2001-2013 Body Media, Inc.). An estimated basal resting metabolic rate was added to the measured energy expenditure (MEE) to determine the total energy expenditure (TEE). The measured energy expenditure estimates were then converted to METs, calculated as the ratio of the work metabolic rate ($kcal \cdot h^{-1} \cdot kg^{-1}$) to a resting metabolic rate ($kcal \cdot h^{-1} \cdot kg^{-1}$)[332]. Subsequently, physical activity-associated energy expenditure, or active energy expenditure (AEE), was computed for any physical activity above three METs. Similarly, the total physical activity duration can be categorized by intensity into light, moderate, vigorous, and very vigorous physical activities. The threshold settings for the different activity levels are predefined in the software but can be changed if required. Additionally, the percentage of time spent on various intensities of physical activity was computed. In the three current papers, the original predefined setting was applied in accordance with the Sedentary Behaviour Research Network (SBRN) consensus and American College of Sports Medicine (ACSM) guidelines[333,334]. In other words, activity was classified as light when the level of metabolic equivalents (MET's) was ≥1.5, moderate at ≥3.0, and vigorous at ≥6.0. According to the literature and international recommendations for physical activity, moderate and vigorous activities were computed as one compound variable, summarizing all activities ≥3.0 METs[335,336].

3.3.4 Sleep measurements

Although the performance of the SenseWear® actigraph as a sleep measurement device during gestation has not been compared to other methods or devices, this actigraph has been recommended for sleep measurements by the manufacturer BodyMedia©[337]. It has also been validated for measuring sleep in various non-clinical and clinical settings, such as sleep measurements in obstructive sleep apnea and measurements under different ambient temperature conditions. These validations resulted in an acceptable agreement compared with polysomnography (PSG) and other actigraphy devices[257,338]. Additionally, several studies have used other actigraphs for objective sleep measurements during pregnancy[260], and more recently, there has been an agreement study comparing the results of actigraphy with PSG[339].

3.4 Imaging

After the detection of fetal echoes at 14 gestational weeks (MA) in 1958 [340], static ultrasound scanners have become widely used in obstetrics and gynecology. Although it is possible to detect the gestational sac as early as four weeks and one day through transvaginal high-resolution ultrasound (7.5 MHz)[145], the gestational sac is typically detected today between four and a half and five weeks[341]. Ultrasound implicates almost no risk to the fetus, is reliable, and is widely available as a standard diagnostic tool in obstetrics and gynecology[342–347].

3.4.1 Embryonic and fetal measurements with ultrasound

In the present study the crown-rump-length and YS size were examined by seven obstetricians and one certified midwife using a transvaginal ultrasound transducer, 6–12 MHz, Voluson Expert E8; (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). The results were plotted using SPSS (version 24, Armonk, NY, USA) and R (Foundation for Statistical Computing, version 4.1, Vienna, Austria). The transducer output power was set to be low, with a maximum thermal index (TI) below 1.0 [345], and the viability of the embryo was ensured[348].

Yolk sac

The YS size was determined by taking the average of two perpendicular diameters, using an outer-to-outer wall caliper placement, and measured thrice (for a total of six diameters)[151]. The YS growth rate was calculated as the difference in YS size between the measurements at weeks 7 and 10 and divided by the number of days between the two measurements *(Fig. 25)*.

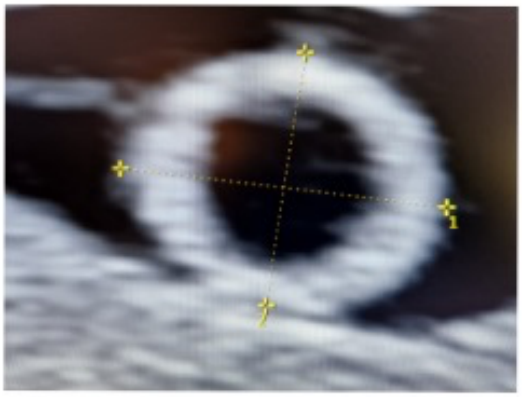


Fig. 25 Caliper placement during the yolk sac measurement

Intra- and inter-observer variability of the yolk sac measurements

In 2023, we expanded our study to calculate the intra- and inter-observer variability of YS size measurements using prospectively collected data from the CONIMPREG cohort. Ultrasound loops, including images of the embryonic YS, were generated for 19 pregnancies and stored in the local archive of the machine. These loops were created at either week 7 or week 10. Subsequently, all seven ultrasound operators were instructed to select the best YS image from this sequence. YS size was then determined according to the previously described method. Finally, the measurement was repeated at least one day later to assess intra-observer variability.

Embryo or fetus

The CRL was determined by averaging three measurements, specifically the distance in millimeters between the calipers placed at the outer side of the crown and the rump of the embryo *(Fig. 26)*.

Because it aligns with our local clinical practice and because the original formula by Robinson and Fleming is still ranked among the best four[349], we used this formula to determine or confirm the gestational age. The intra- and inter-observer variability for CRL measurements using high-resolution transvaginal ultrasound has been reported earlier, with low variability and good agreement between the two observers[343].

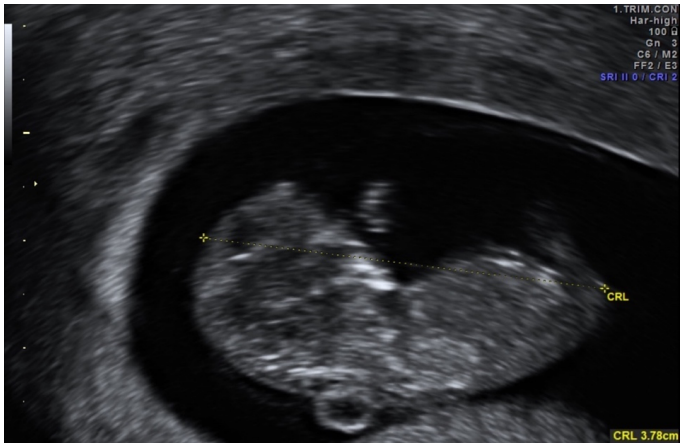


Fig. 26 Caliper placement for the Crown-Rump Length (CRL)

3.5 Statistics

General

Statistical analyses were performed using the R software (version 4.1, Vienna, Austria), RStudio software (Boston, MA, USA), and IBM SPSS (version 24, Armonk, NY, USA). Descriptive statistics were used to describe the cohort characteristics. The medians with interquartile ranges and means with standard deviations, including the 95% confidence interval, were calculated for continuous measurements. The minimum and maximum values were also reported for continuous variables, whereas frequencies and proportions were used for categorical variables. The Akaike information criterion was used to evaluate the model fit and to compare different regression models. Differences in model performance were tested using an analysis of variance. Linearity assumptions and the normal distribution of residuals were assessed, and calculations were conducted with and without outliers (>2SD).

The variance inflation factor was calculated to identify potential multicollinearity issues. A significance threshold of $p < 0.05$ was applied to all statistical analyses.

Paper I

Before the main analyses, a power analysis was conducted using a paired design with n=113 participants, accounting for similar variability as reported in previous studies[260,264,339]. With a statistical power of 0.8, we were able to detect a mean difference of 15 minutes in the total daily sleep duration between the two trimesters. Random intercept models were used to analyze serial measurements of average daily sleep duration (TST) and maternal physical activity duration (PAD). The three pregnancy trimesters were treated as time categories and included as fixed effects in the models using measurements prior to pregnancy as the reference. Regression models were adjusted for age, parity, height, lean body mass, and fat percentage. The inclusion of a random slope by pregnancy trimester was also examined to determine its effect on the model. Subanalyses were performed using paired and unpaired Student's t-tests or nonparametric tests.

Papers II–III

Ordinary least square linear regression (OLS) was used to analyze the association between YS size and maternal sleep duration (Paper II), as well as the duration of all physical activity levels (Paper III) before pregnancy and at the end of the first trimester (week 13). Regression models were fitted with and without stratification by fetal sex. In Paper II, stratification into sleep duration quartiles was conducted. Additionally, the interaction terms (fetal sex : maternal sleep) and (fetal sex : physical activity) were added to the respective OLS models. These were used to assess the effect of fetal sex on the relationship between sleep and YS size (Paper II) as well as the relationship between activity and YS size (Paper III). Results from OLS regressions were compared with those from quantile regressions, iterated reweighted least squares regressions, and heteroskedastic methods. When the analysis involved serial measurements, such as two YS measurements and two or three CRL measurements, random intercept models were used.

In the subanalysis of the main findings, the YS diameter was replaced with the YS Z-score calculated using multilevel growth models. The original OLS models for sleep (Paper II) and physical activity (Paper III) effects were also controlled for the GA. Maternal age, parity, and body composition parameters (height, weight, body mass index, lean body mass, and body fat percentage) were individually added to the primary model to assess their impact on the association.

In Paper III, stratification by the time of inclusion was performed, and the regression model was adjusted accordingly. Furthermore, regression analyses were conducted both with and without participants who experienced complications, such as hypertensive complications, gestational diabetes, preterm birth, and low five-minute Apgar scores. Intra- and inter-observer variability assessments, along with the calculation of the associated standard error of measurement (SEM-intra-observer and SEM-inter-observer), as well as the minimum detectable difference, were performed using a two-way analysis of variance (ANOVA) method, as outlined by Popović and Thomas[350]. The necessary variances were either derived directly or indirectly from the variances expressed as multiple squares for various factors in the model, including the observer, subject, interaction between the observer and subject, and residual variation.

4. Main results

Paper I

During the assigned period, 123 of 249 eligible participants conceived with a successful pregnancy and were included in the present study *(Paper I; p. 92, Fig.2).* At the time of inclusion, participants were aged between 20 and 35 years (mean 28.9 years SD 3.3). Their height ranged from 149 to 185 cm (mean 167.9 cm SD 6.5), and their weight varied between 47.1 and 89.8 kg (mean 64.7 kg SD 8.4). Their lean body mass was between 36.0 and 54.9 kg (mean 45.5 kg SD 3.7), and their body fat percentage ranged from 15.9% to 41.9% (mean 29.1% SD 5.5). Most of these women expected either their first (48.8%) or second (41.5%) child. Based on a non-validated questionnaire, the majority (43.9%) exercised twice a week at the start of the study. Meanwhile, 26.8% reported exercising three or more times per week, and an equal proportion considered walking effortlessly as their sole exercise. Only a small percentage (2.3%) of participants reported no prior training habits.

Sleep and physical activity duration

During actigraphy, 94.1% of days were successfully recorded. The mean duration per recording was 3.6 days (95%CI [3.56–3.69]). Variations in daily sleep and physical activity durations were substantial *(Fig. 27)*.

Before conception the ± 2 SD sleep-range was 307–523 min and ± 2 SD physical activity ranged between 119.7 and 607.7 min. While sleep variation was even higher from the end of the first trimester, compared to pregestational levels, physical activity variation declined and remained lower throughout pregnancy.

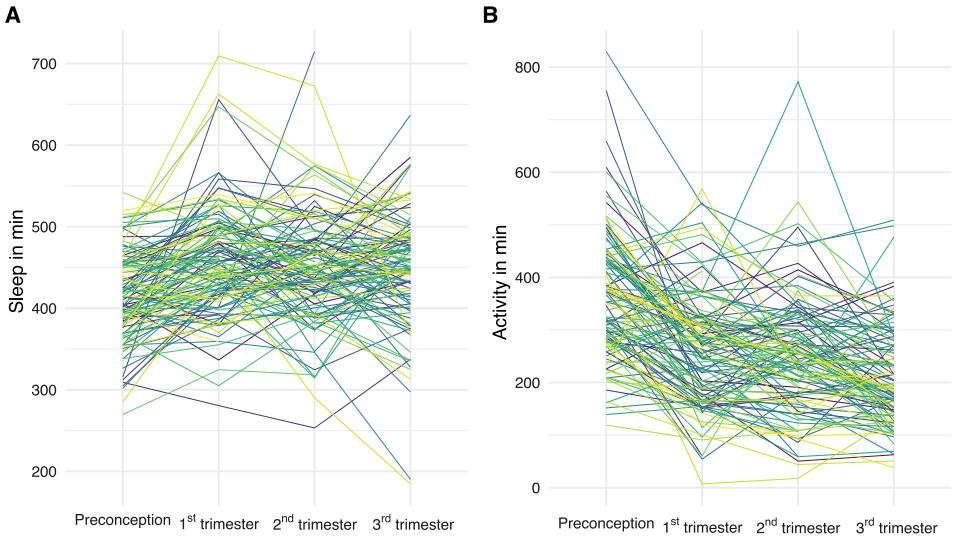


Fig. 27 (A) Variation in daily sleep duration and (B) physical activity duration in serial measurements of 123 women from before conception to the end of pregnancy. *Material from: Vietheer A et al., Sleep and physical activity from before conception to the end of pregnancy in healthy women: A longitudinal actigraphy study, Sleep Medicine 2021, Elsevier Science*[324] *with permission. © Elsevier Science and Technology Journals.*

Sleep

The unadjusted daily sleep duration increased by 10.3% (42.7 min) from before conception to the end of the 1st trimester, reaching a peak. Throughout the remainder of the pregnancy (2nd and 3rd trimesters), the duration remained consistently high. After adjusting for maternal age, parity, and body composition factors, the same pattern was observed *(Fig. 28)*.

Sleep efficiency tended to decrease slightly. The unadjusted and unadjusted means for all four measurements (preconception, 1st, 2nd, and 3rd trimester) of daily sleep duration as well as sleep efficiency, including their SD and 95% CI, are reported in *Table* 3. In the multivariate regression model, total daily sleep duration was significantly associated with age, parity, lean body mass, and pregestational body fat percentage *(Paper I; Appendix B)*.

Table 3 Unadjusted mean ± standard deviation (SD) of total daily sleep time, sleep efficiency, total daily physical activity, light physical activity, and vigorous-moderate physical activity duration in minutes (i.e., before conception and in each trimester). Adjusted mean (adj. mean) and adjusted 95% confidence interval (adj. 95%CI) were calculated for the main outcomes (total sleep time and total daily physical activity duration). Adjustments in the regression model were made based on cohort medians for age, parity, and body composition parameters (i.e., for a 29-year-old nulliparous woman with a height of 167 cm, lean body mass of 45 kg, and body fat 30%).

Time point	*n*	Mean ± SD	Range	adj. mean	adj. 95%CI
Daily total sleep time, min					
Before conception	117	415.3 ± 54.0	(269.8–542.0)	429.1	(414.5–443.7)
1st trimester	117	458.0 ± 68.8	(280.7–709.3)	471.6	(457.0–486.2)
2nd trimester	115	450.9 ± 68.2	(253.2–714.7)	463.4	(448.7–478.1)
3rd trimester	113	446.1 ± 70.4	(184.7–636.8)	459.8	(444.9–474.6)
Daily sleep efficiency, %					
Before conception	117	82.71 ± 6.34	(64.87–94.30)	88.69	(61.22–116.15)
1st trimester	117	81.17 ± 6.51	(61.49–95.23)	87.29	(59.83–114.75)
2nd trimester	115	82.44 ± 7.07	(52.62–96.41)	88.44	(61.0–115.89)
3rd trimester	113	79.73 ± 8.31	(50.23–95.61)	85.55	(58.09–113.01)
Daily total physical activity duration, min					
Before conception	117	363.7 ± 122.0	(119.0–829.5)	331.7	(307.6–355.8)
1st trimester	117	262.1 ± 108.7	(7.3–568.5)	231.6	(207.4–255.7)
2nd trimester	115	250.6 ± 114.2	(17.8–772.0)	218.9	(194.6–243.2)
3rd trimester	113	214.8 ± 95.4	(38.2–509.0)	179.1	(154.7–203.6)
Daily light activity duration, min					
Before conception	117	267.2 ± 90.6	(94.3–530.8)		
1st trimester	117	207.7 ± 87.0	(7.2–436.0)		
2nd trimester	115	197.5 ± 86.6	(18.2–448.0)		
3rd trimester	113	172.7 ± 81.0	(33.2–439.0)		
Daily vigorous or moderate activity duration, min					
Before conception	117	98.6 ± 58.4	(17.2–356.2)		
1st trimester	117	56.4 ± 37.5	(0.0–218.8)		
2nd trimester	115	54.3 ± 47.9	(0.0–324.0)		
3rd trimester	113	43.5 ± 29.2	(0.0–123.8)		

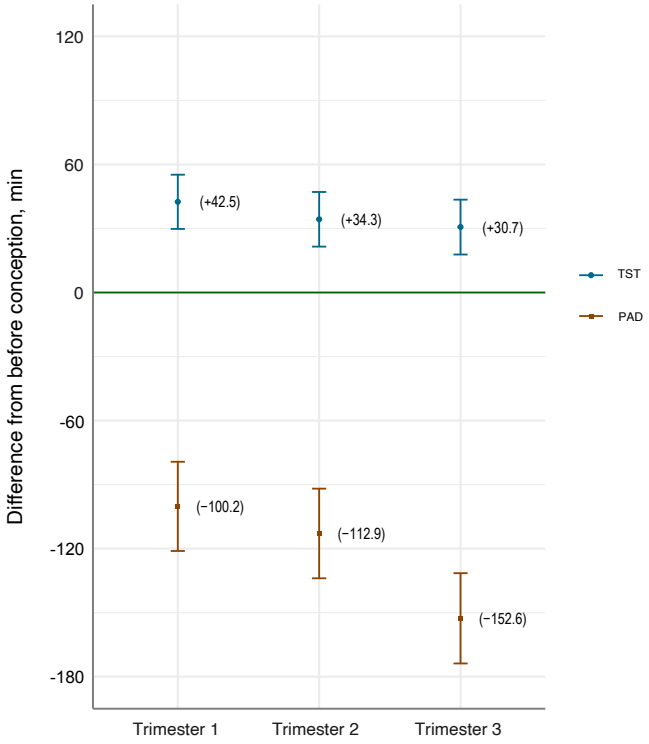


Fig. 28 Changes in mean total sleep time (TST) and total physical activity duration (PAD, min/24 h) in the first, second, and third trimesters of pregnancy over pregestational levels (set to zero in this graph). Data are mean and standard deviation values for the increases in sleep duration (TST) and decreases in total physical activity duration (PAD) relative to pregestational levels, adjusted for maternal age before conception, parity, height, lean body mass, and body fat percentage. *Material from: Vietheer A et al., Sleep and physical activity from before conception to the end of pregnancy in healthy women: A longitudinal actigraphy study, Sleep Medicine 2021, Elsevier Science*[324] *with permission. © Elsevier Science and Technology Journals.*

Physical activity

The unadjusted mean PAD before conception was 363.7 min, decreasing sharply to 262.1 min in the first trimester and gradually thereafter *(Table 3)*.

Adjustment for maternal age, parity, and body composition parameters did not alter this pattern, resulting in a similar reduction in daily physical activity duration compared to pre-conception measurements in the 1st (30.2%), 2nd (34.0%), and 3rd trimester (46.0%) *(Fig. 28)*.

Vigorous and moderate activities decreased more than light activity *(Table 3)*.

Similar to daily sleep duration, physical activity was associated with age, parity, and pregestational body fat percentage. However, the relationship with lean body mass was not significant *(Paper I; Appendix C).*

Subanalyses examining the effect of seasonal or weekday variations on sleep and physical activity measurements revealed that despite seasonal variations in physical activity before pregnancy, there was no confounding *(Paper I; Appendices E and F)*.

Paper II

Body composition and actigraphy sleep measurements were performed in 436 women. Of these, 190 women with a regular menstrual cycle (median, 28 days; range, 24–35 days; IQR, 1 day) had successful pregnancies, resulting in live births *(Fig. 23).* Descriptive statistics of the maternal characteristics are provided in *Table* **4**.

Table 4 Descriptive statistics of the participants are presented as mean, standard deviation (SD), range (Min, Max), and interquartile range (IQR).

***n*=190 (no missing)**	**Frequency**	**Mean**	**SD**	**Min**	**Max**	**IQR**
Age (years)		29.0	3.1	20.0	35.0	(27–31)
Height (cm)		167.7	6.2	149.0	185.0	(164–172)
Weight (kg)		64.7	8.3	47.1	89.8	(58.9–71.2)
BMI		23.0	2.6	17.8	29.9	(21–24.8)
Lean body mass (kg)		45.7	3.8	36.0	55.6	(43.0–48)
Body fat (%)		28.8	5.5	15.9	41.9	(25–32.9)
Cycle length (days)		28.5	1.7	24	35	(28–29)
Parity						
- 0	89 (46.8%)					
- 1	79 (41.6%)					
- ≥2	22 (11.6%)					
Training efforts*						
- None	3 (1.6%)					
- Effortless walking	46 (24.2%)					
- <3 times·week^{-1}	90 (47.4%)					
- ≥3 times·week^{-1}	51 (26.8%)					

The majority conceived within the two first menstrual cycles, and the median time from the first sleep recording before pregnancy to the estimated day of conception

(i.e., 14 days after LMP) was 36 days (IQR, 10–75). The median pregnancy length was 281 days (IQR, 12 days) by LMP or 278.5 days (IQR, 11.8 days) based on embryonic CRL[351]. Maternal or fetal complications during pregnancy, such as gestational hypertension (3.2%), gestational diabetes (3.7%), preterm birth (3.2 %), and five-minutes Apgar score less than seven (1.1%), were rare *(Paper II; p.4, Table 2).*

Using actigraphy, over 92% of all recorded days met our reliability standards, resulting in no participant being excluded from the analysis. With significant individual variation, sleep duration was 38 min shorter before pregnancy than at the end of the first trimester (95%CI [28.6–47.2], $P < 0.01$) *(Paper II; p. 5, Table 3).* However, the total length of actigraphy and the frequency of recorded weekend days did not differ between measurements.

From ultrasound assessments (at weeks 7 and 10), 358 YS measurements were obtained. The 7, 10, and 13-week assessments provided 559 CRL measurements. Both sets of measurements exhibited typical growth patterns *(Fig. 29).* An overview, with average values specific for fetal sex and gestational age, SD, and 95% CI for each measurement point, is shown in *Table 5*.

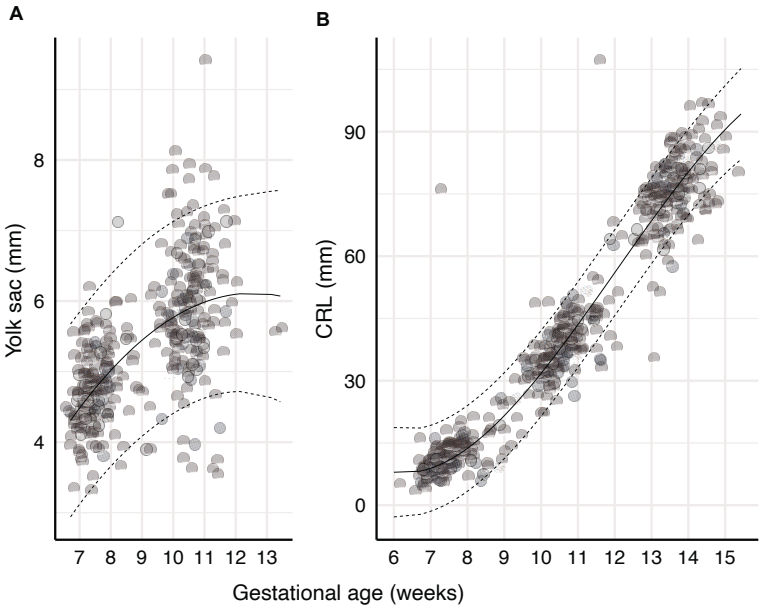


Fig. 29 (A) First (*n* = 180) and second (*n* = 175) yolk sac measurements, and (B) the three serial Crown-Rump Length (CRL) measurements (*n* = 171, 173, and 164), presented with the predicted mean (mixed model with random intercept) and 95% prediction band. The gestational age was determined based on the last menstrual period. *Material adapted from: Vietheer A et al., Effect of maternal sleep on embryonic development, Scientific Reports 2022, Nature*[325] *with permission. © Elsevier Science and Technology Journals.*

Table 5 Summary statistics of the ultrasound data of 190 low-risk pregnancies presented with subgroups (male and female), number of measurements (*n*), mean, standard deviation (SD), 95% confidence interval of the mean (95% CI), and *p*-value for the between-group tests. Gestational age was based on last menstrual period.

Term	Sex	*n*	Mean	SD	95% CI	**p*-value
1st measurement, gestational age (weeks)	All	180	7.6	0.7	(7.5–7.7)	
	♂	89	7.5	0.5	(7.4–7.6)	0.08
	♀	91	7.7	0.9	(7.5–7.8)	
Yolk sac diameter (mm)	All	180	4.7	0.6	(4.7–4.8)	
	♂	89	4.7	0.6	(4.6–4.8)	0.39
	♀	91	4.8	0.6	(4.7–4.9)	
Crown-rump-length (mm)	All	186	11.4	3.8	(10.8–11.9)	
	♂	93	10.9	3.6	(10.2–11.6)	0.09
	♀	93	11.9	4.0	(11–12.7)	
2nd measurement, gestational age (weeks)	All	178	10.6	0.8	(10.5–10.7)	
	♂	87	10.6	0.7	(10.5–10.8)	0.65
	♀	91	10.7	0.9	(10.5-10.9)	
Yolk sac diameter (mm)	All	178	5.9	0.9	(5.8–6.0)	
	♂	87	5.9	0.8	(5.7–6.1)	0.99
	♀	91	5.9	1.0	(5.7–6.1)	
Crown-rump-length (mm)	All	187	37.8	6.9	(36.8–38.8)	
	♂	93	37.6	6.3	(36.3–38.9)	0.71
	♀	94	38.0	7.5	(36.5–39.5)	
1st – to – 2nd measurement, **yolk sac growth rate** ($mm \cdot week^{-1} \cdot h^{-1}$)	All	170	0.38	0.33	(0.33–0.43)	
	♂	81	0.37	0.29	(0.30–0.43)	0.67
	♀	89	0.39	0.36	(0.32–0.46)	
3rd measurement, gestational age (weeks)	All	185	13.6	0.8	(13.5–13.7)	
	♂	93	13.6	0.9	(13.4–13.7)	0.41
	♀	92	13.7	0.8	(13.5–13.8)	
Crown-rump-length (mm)	All	186	76.4	8.1	(75.2–77.6)	
	♂	92	77.2	8.2	(75.5–78.9)	0.18
	♀	94	75.6	8.1	(74.0–77.3)	

* Unpaired *t*-test was conducted for the yolk sac and crown-rump-length (CRL) data, and Mann-Whitney U test (Wilcoxon rank-sum test) for the gestational age data.

Maternal sleep and yolk sac size

At the initial measurement (week 7), the YS size decreased by 0.12 mm for every additional hour of preconception sleep (95% CI [0.22–0.03], p=0.01). With the average sleep duration, this reduction equated to 11.3%. After sex stratification, this association was not observed in female embryos. However, in male embryos, this reduction nearly doubled to 20.7% (0.22; 95% CI [0.35–0.09], p<0.01).

No association was found between the preconception sleep duration and the second YS measurement at 10 weeks *(Fig. 30 A)*.

Notably, the pattern above—i.e., sleep-YS association dependent on gestational age and embryonic sex—was still traceable in the association of YS size with sleep duration at 13 weeks *(Fig. 30 B)* and remained consistent across the YS percentiles. *(Linear regression results are provided in Paper II; p.6, Table 5, and results from quantile regression in Paper II; Supplementary Fig. S3)*

Influence of other maternal characteristics and variations of gestational age

Overall, preconception maternal body size (weight and height) and body composition parameters (specifically, body fat percentage and lean body mass) were not associated with YS size at 7 and 10 weeks of gestation, as shown in *Table 6*. In subanalyses that accounted for these factors in regression models assessing the association between maternal sleep and YS size, the results remained substantially unchanged *(Paper II; Supplementary Tables S4 and S5)*.

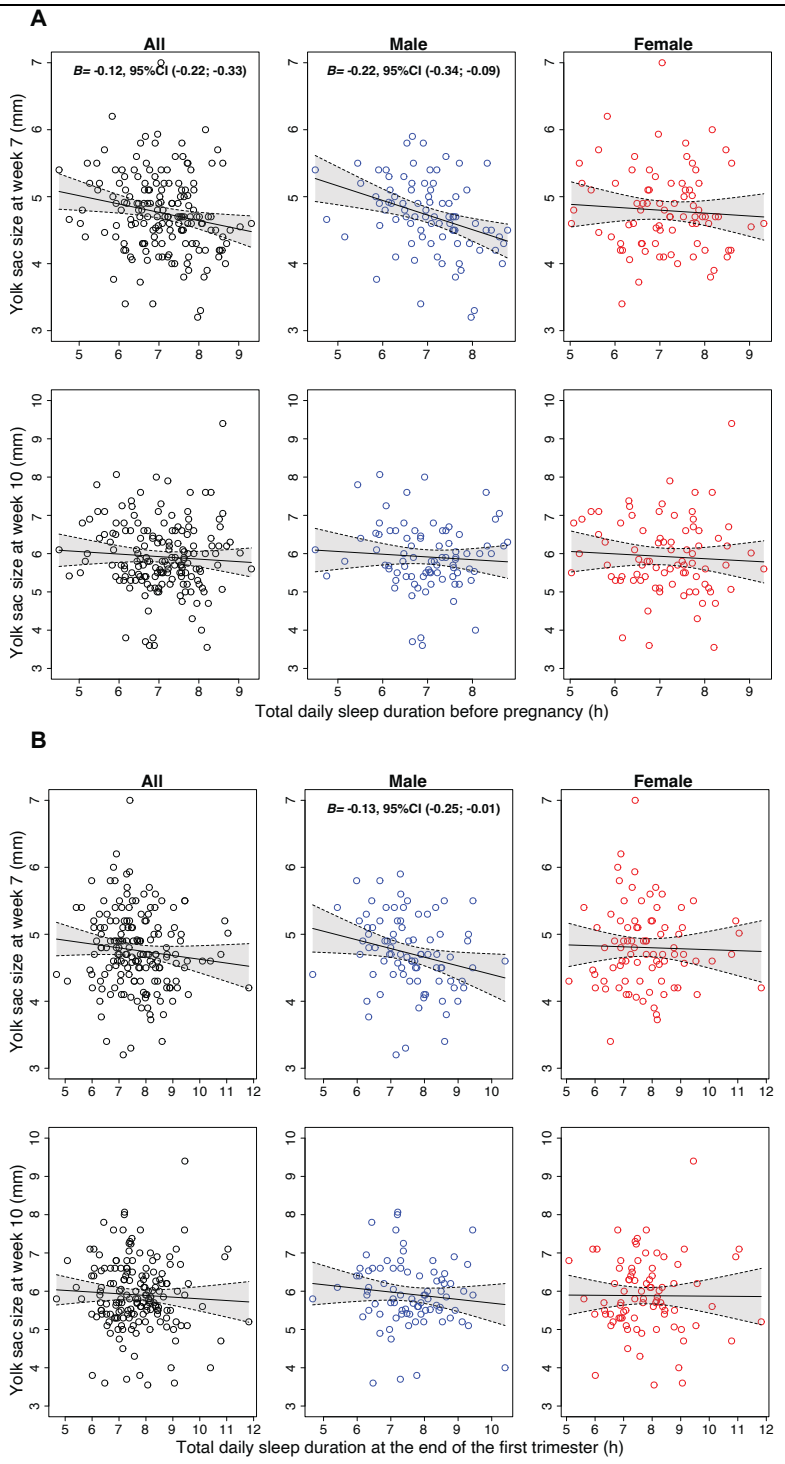


Fig. 30 Effect of daily total sleeping time before pregnancy (A), and the effect of the daily total sleeping time at 13 weeks (B) on the first (upper row) and second yolk sac measurement (lower row); presented with regression line and its 95% confidence interval. The first column represents the total dataset, and the second and third columns represent the analyses according to embryonic sex. *Material adapted from: Vietheer A et al., Effect of maternal sleep on embryonic development, Scientific Reports (2022)*[325], *Springer Nature. © The authors.*

Table 6 Estimated yolk sac size in mm by prepregnant metrics of maternal size and body composition: Ungrouped (all) or stratified by fetal sex (male, female), modeled using ordinary least square regression—degrees of freedom (DF); unstandardized regression coefficient (Effect); adjusted R squared (adj.R2); 95% confidence interval (95% CI); AIC (Akaike information criterion), *p*-value.

Term	Sex	DF	Effect	SD	95% CI	adj.*R*2	AIC	*p*-value
Height on yolk sac size (mm·1cm^{-1})								
1st measurement	All	178	0.007	0.007	(-0.007–0.021)	0.000	319.53	0.32
	♂	87	0.017	0.009	(-0.001–0.034)	0.027	152.13	0.07
	♀	89	-0.004	0.011	(-0.026–0.017)	-0.009	169.73	0.69
2nd measurement		176	0.000	0.011	(-0.022–0.023)	-0.006	479.11	0.98
	♂	85	-0.005	0.014	(-0.034–0.023)	-0.010	222.63	0.72
	♀	89	0.007	0.018	(-0.029–0.042)	-0.010	260.17	0.71
Weight on yolk sac size (mm·1kg^{-1})								
1st measurement	All	178	0.001	0.005	(-0.009–0.011)	-0.005	320.49	0.84
	♂	87	0.009	0.007	(-0.006–0.023)	0.006	154.06	0.23
	♀	89	-0.006	0.007	(-0.020–0.009)	-0.004	169.24	0.43
2nd measurement	All	176	-0.003	0.008	(-0.020–0.013)	-0.005	478.94	0.69
	♂	85	0.012	0.012	(-0.011–0.035)	0.001	221.68	0.31
	♀	89	-0.016	0.012	(-0.040–0.008)	0.008	258.54	0.19
Body fat percent (BFP) on yolk sac size (mm·1%$^{-1}$)								
1st measurement	All	178	-0.007	0.008	(-0.022–0.009)	-0.001	319.74	0.38
	♂	87	-0.003	0.011	(-0.025–0.019)	-0.011	155.50	0.79
	♀	89	-0.011	0.011	(-0.033–0.011)	-0.001	168.94	0.34
2nd measurement	All	176	-0.010	0.013	(-0.035–0.015)	-0.002	478.54	0.45
	♂	85	0.022	0.017	(-0.012–0.055)	0.008	221.06	0.20
	♀	89	-0.038	0.018	(-0.075–0.002)	0.035	256.04	0.04
Lean body mass on yolk sac size (mm·1kg^{-1})								
1st measurement	All	178	0.013	0.011	(-0.009–0.036)	0.002	319.16	0.25
	♂	87	0.031	0.015	(0.001–0.062)	0.035	151.39	0.04
	♀	89	-0.005	0.017	(-0.039–0.029)	-0.010	169.82	0.79
2nd measurement	All	176	0.002	0.019	(-0.035–0.039)	-0.006	479.10	0.92
	♂	85	0.009	0.025	(-0.041–0.059)	-0.010	222.63	0.72
	♀	89	-0.005	0.028	(-0.060–0.051)	-0.011	260.29	0.87

Influence on yolk sac growth-rate

We did not find a significant influence of daily maternal sleep duration on the average YS growth rate (mm/week·h^{-1}) between the two YS measurements at weeks 7 and 10 *(Paper II; Supplementary Table S3).*

Maternal sleep and embryonic size

In week 7, embryonic CRL decreased with increased prepregnant sleep duration, particularly in males. The effect size was 33% larger (-0.92 mm·h-1·d-1; 95% CI [-1.77 to -0.08]) after sex stratification, a pattern that was similar to the sleep effects on the YS. By week 13, however, sleep duration lacked significant ties to the CRL, even after sex-stratification. Adjustments for maternal age, parity, and body composition did not significantly alter these findings *(Paper II; p.6, Table 6).*

Paper III

The study population, YS measurements, and actigraphy recording times were consistent with those reported in Paper II.

The repeatability and reproducibility of YS size measurements obtained through transvaginal ultrasound were determined using an additional set of 266 measurements. These were conducted by the seven ultrasound operators from the CONIMPREG study, with each operator contributing to 38 measurements (19x2) *(Table 7).*

Table 7 Repeatability and reproducibility of yolk sac measurements (transvaginal ultrasound).

Observer variability	Value (%)	Standard error of measurements (mm)	Minimum detectable difference (mm)
Intra-observer variability	0.08	0.029	0.08
Inter-observer variability	0.09	0.030	

Physical activity

Daily physical activity duration was 5h and 55 min before conception (95% CI [5h 37 min–6h 13 min]) and 1h 36min shorter at the end of the first trimester (95% CI [1h 19 min–1h 55 min]) *(Paper III; Table 2 and Supplementary Fig. S1)*. This pattern was similar for different activity intensities (light and moderate-vigorous activity) *(Paper III, Table 2)*.

Effect of daily physical activity duration on yolk sac size

When considering both YS measurements (*n*=355) without adjusting for gestational age (GA) and fetal sex, both prepregnant maternal physical activity and 13 weeks' gestation maternal physical activity (at the end of the first trimester) did not significantly influence the YS size—preconception maternal activity: 95% CI [-0.006–0.079 $mm \cdot h^{-1}$]; 13 weeks' maternal physical activity: 95% CI [-0.049–0.05 $mm \cdot h^{-1}$]. In the following analysis, stratifying YS size by gestational age (i.e., by the measurements at 7 and 10 weeks' gestation), the influence on YS size did not reach the level of statistical significance either *(Table 8; term "all")*.

However, the stratified analysis, accounting for both gestational age and sex, revealed that in males, the average amount of daily preconception activity was associated with a 10% larger YS at 7 weeks (0.08 mm·h-1; 95% CI [0.02–0.13]), and a smaller YS at 10 weeks. Conversely, in female embryos, the YS increased at 10 weeks (0.16$mm \cdot h^{-1}$; 95% CI [0.06–0.26]), and was 24% larger at average maternal physical activity duration compared to males *(Table 8, term "Male.PAD" and Paper III; Supplementary Fig.4)*

The activity duration recorded at 13 weeks was similarly linked to the YS size measured at weeks 7 and 10 *(Fig. 31)*.

In the subanalysis, we adjusted the analysis for maternal age, parity, and body composition, as well as gestational age and gestational age-adjusted YS Z-scores. Additionally, we stratified by time of inclusion due to the long study period and repeated the analysis after excluding the few women with pregnancy complications (for each complication < 4%). None of these measures altered the conclusions *(Paper III; Supplementary Tables S2–S8)*.

Table 8 Estimated yolk sac diameter at gestational week 7 (upper half) and week 10 (lower half) by daily prepregnant maternal physical activity duration and physical activity duration at the end of the first trimester (week 13): Ungrouped (all), grouped according to fetal sex (male, female), or by the interaction term male embryo and prepregnant maternal physical activity duration (Male:PAD). Modeled using ordinary least square regression—degrees of freedom (DF); unstandardized regression coefficient (Effect); adjusted R-squared (adj.R2); 95% confidence interval (95% CI); AIC (Akaike information criterion, *p*-value).

Term	DF	Effect	95% CI	adj.*r*2	AIC	*p*-value
Yolk sac at week 7 by *prepregnant* daily physical activity duration						
All	165	0.04 mm·h^{-1}	(-0.00–0.08)	0.014	295.2	0.07
Male	82	0.08 mm·h^{-1}	(0.02–0.13)	0.077	137.2	**<0.01**
Female	81	0.00 mm·h^{-1}	(-0.06–0.07)	-0.012	158.7	0.93
Male:PAD	163	0.07 mm·h^{-1}	(-0.01–0.16)	0.024	295.4	0.09
Yolk sac at week 7 by the *end of 1st trimesters'* daily physical activity duration						
All	167	0.02 mm·h^{-1}	(-0.03–0.07)	-0.002	301.9	0.47
Male	83	0.04 mm·h^{-1}	(-0.04–0.11)	-0.000	146.0	0.34
Female	82	0.01 mm·h^{-1}	(-0.07–0.08)	-0.011	159.8	0.88
Yolk sac at week 10 by *prepregnant* daily physical activity duration						
All	164	0.03 mm·h^{-1}	(-0.04–0.09)	-0.002	446.1	0.42
Male	81	-0.09 mm·h^{-1}	(-0.18 to -0.00)	0.038	209.8	**0.04**
Female	81	0.16 mm·h^{-1}	(0.06–0.26)	0.094	227.9	**<0.01**
Male:PAD	162	-0.24 mm·h^{-1}	(-0.38 to -0.12)	0.064	436.7	**<0.01**
Yolk sac at week 10 by the *end of 1st trimesters'* daily physical activity duration						
All	166	-0.01 mm·h^{-1}	(-0.10–0.07)	-0.005	451.1	0.72
Male	82	-0.09 mm·h^{-1}	(-0.21–0.02)	0.020	214.8	0.10
Female	82	0.05 mm·h^{-1}	(-0.07–0.17)	-0.003	238.1	0.41

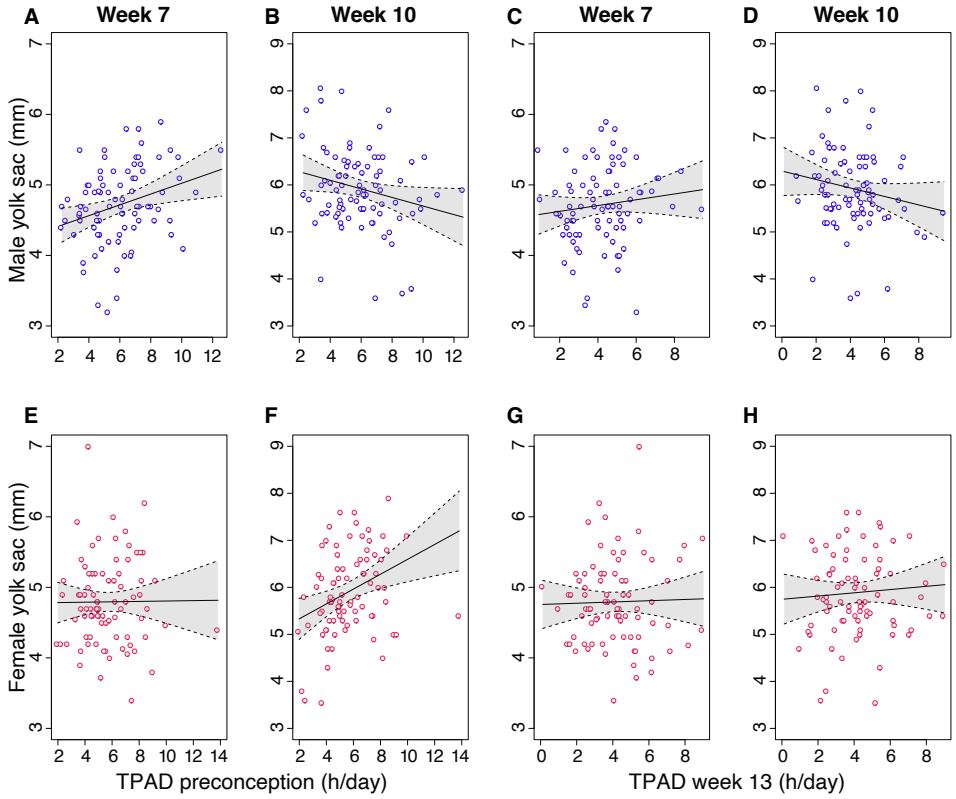


Fig. 31 Effect of total daily activity duration (TPAD) on yolk sac size at 7 and 10 weeks in male **(A–D)** and female embryos (**E–H**). Activity recordings before pregnancy are shown in the left half **(A)**, **(B)**, **(E)**, and **(F),** and at week 13 in the right half **(C)**, **(D)**, **(G)**, and **(H)**. Presented with regression lines and its 95% confidence intervals and grouped according to embryonic sex: male (blue) and female (red). *Material adapted from Vietheer et al., Scientific Reports (2023), Springer Nature. © The authors.*

Effect of maternal physical activity on yolk sac growth (mm·week^{-1}·h^{-1})

The average growth rate of YSs between the two measurements (at 7 and 10 weeks) demonstrated large individual variations *(Fig. 32)*.

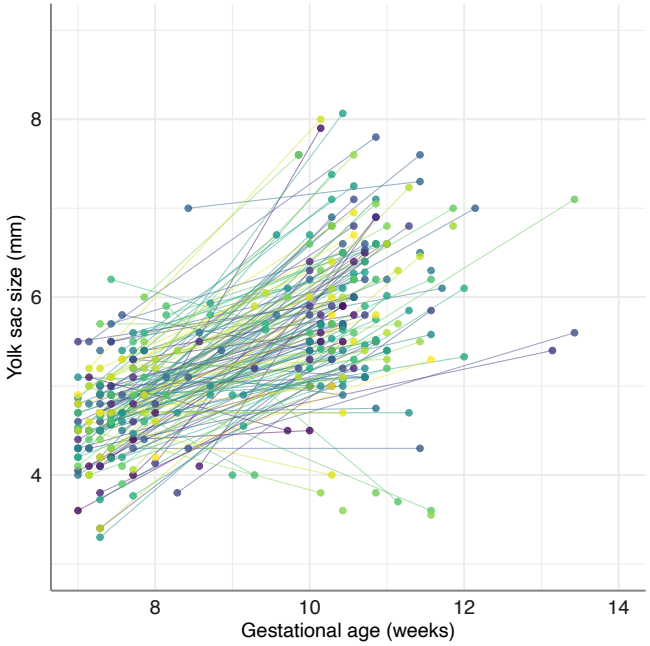


Fig. 32 Line plot demonstrating variation in yolk sac growth between the two measurements. Gestational age (GA) is based on menstrual age. (No line but sole points when only one measurement was available; Participant-id 370 is not part of that plot as menstrual age of the second measurement was an outlier beyond 14 gestational weeks.) *Material adapted from Vietheer et al., Scientific Reports (2023), Springer Nature. © The authors.*

In both male and female embryos, the recorded maternal physical activity before pregnancy was associated with variation in YS growth (mm·week^{-1}·h^{-1}) but differed between the sexes. In contrast to male embryos, where YS growth was slower at longer activity durations, females showed faster growth between 7 and 10 weeks (p<0.001) *(Table 9 and* Fig. *33)*.

Table 9 Estimated *Yolk sac growth rate (mm·week^{-1}·h^{-1})* by total daily physical activity duration (TPAD) before pregnancy and at the end of the first trimester (week 13): Ungrouped (all), grouped according to fetal sex (male, female), or by the interaction term male sex and TPAD (Male:TPAD). Modeled using ordinary least square regression—degrees of freedom (DF); unstandardized regression coefficient (Effect); adjusted *R* squared (adj.*R*2); 95% confidence interval (95% CI); Akaike information criterion (AIC), *p*-value.

Model	DF	Effect	95% CI	adj.*R*2	AIC	*p*-value
Yolk sac growth rate by physical activity duration before pregnancy						
All	156	-0.00 mm·week^{-1}·h^{-1}	(-0.03–0.02)	-0.006	103.3	0.77
Male	75	-0.05 mm·week^{-1}·h^{-1}	(-0.08 to -0.02)	0.126	26.4	**<0.01**
Female	79	0.05 mm·week^{-1}·h^{-1}	(0.01–0.09)	0.064	62.4	**0.01**
Male:TPAD	154	-0.1 mm·week^{-1}·h^{-1}	(-0.15 to -0.05)	0.084	90.5	**<0.01**
Yolk sac growth rate by physical activity duration at the end of first trimester						
All	158	-0.01 mm·week^{-1}·h^{-1}	(-0.04–0.02)	-0.004	96.2	0.53
Male	76	-0.03 mm·week^{-1}·h^{-1}	(-0.07–0.01)	0.021	34.6	0.11
Female	80	0.01 mm·week^{-1}·h^{-1}	(-0.03–0.05)	-0.010	63.3	0.66
Male:TPAD	156	-0.04 mm·week^{-1}·h^{-1}	(-0.10–0.02)	-0.003	98.1	0.15

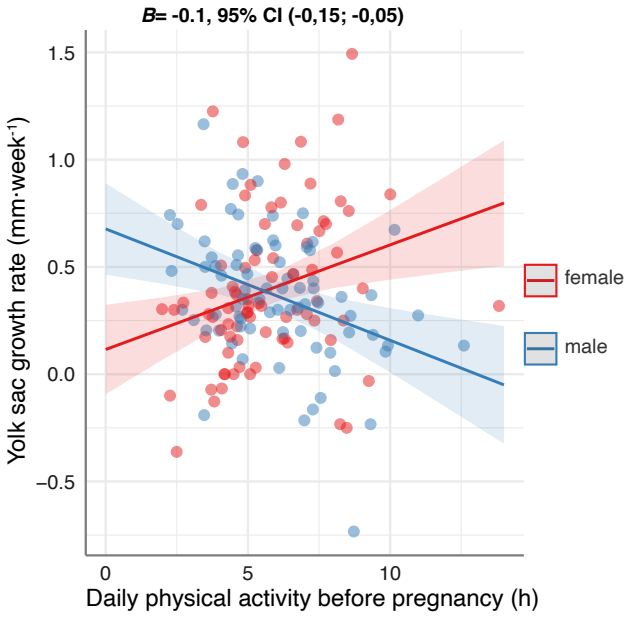


Fig. 33 Effect of preconception maternal physical activity on the growth rate of the yolk sac between weeks 7 and 10: regression lines with 95% confident bands according to embryonic sex.

Effect of maternal physical activity intensity on yolk sac size

Analysis of the association between subcategories of physical activity (light vs. moderate-vigorous) and YS size showed effects similar to those observed for total physical activity duration *(Fig. 34)*.

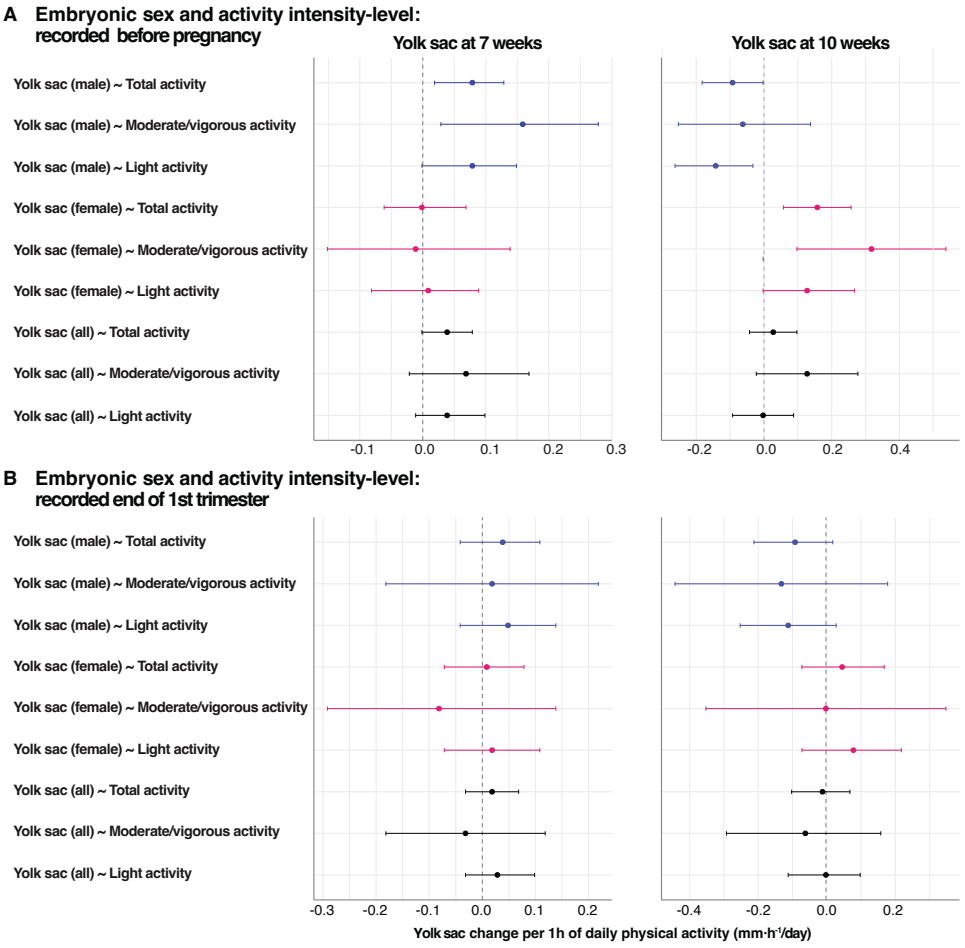


Fig. 34 Forest plot showing the effect of physical activity intensity on yolk sac size according to embryonic sex and the time of actigraphy recording: **(A)** before pregnancy and **(B)** at the end of the 1st trimester. The coefficients are presented with 95% confidence intervals. *Material adapted from Vietheer et al., Scientific Reports (2023), Springer Nature. © The authors.*

5. Discussion

This study revealed significant variations in maternal sleep and physical activity durations from preconception to the end of the first trimester (Paper I) and examined their associations with embryonic development in a cohort of healthy women (Papers II–III). Specifically, the data suggest that both sleep duration and physical activity, particularly prior to conception, interact in a factor-specific manner and are dependent on the sex of the embryo. These interactions have measurable effects on developmental sequences at weeks 7 and 10 of menstrual age. Maternal body composition did not appear to have a significant effect at these time points. Because the data were derived from a healthy cohort, the observed age- and sex-dependent changes were considered to be physiological. Possible biological mechanisms will be discussed subsequently, along with the specific findings in Papers I–III.

5.1 Discussion of the main results papers (I–III)

Paper I

The initial paper served as the foundational basis for the two subsequent studies, as it was essential to quantify both the duration and quality or intensity of sleep and physical activity to assess their effects on pregnancy. The assessment started before pregnancy, which is particularly important when studying early developmental structures such as the human YS and embryo.

Consequently, the large variation in sleep and activity duration among participants *(Fig. 27)*, along with the significant differences observed in our longitudinal measurements from preconception to the end of the first trimester, appeared promising with regard to the measurable effects on early development.

Sleep patterns [236,262,264,352–354] and activity duration patterns [287,292,335,355]were consistent with previously reported actigraphy measurements during pregnancy, thereby corroborating the validity of our measurements.

However, the large number of participants and unique design of the present study, which started as early as preconception, enabled us to report machine-recorded longitudinal changes in both sleep and physical activity from before pregnancy and through each trimester *(Fig. 28)*. Comparing the present results with data from sleep studies using other methods, such as questionnaires[259] or polysomnography[260,356], generally showed similar patterns, although the absolute numbers could differ. Here, it is important to be aware of study population, cultural, and working life differences, as well as methodological differences, such as the mode of sleep recording and sleep determination—sleep duration in absolute numbers measured by actigraphy is for example not the exactly the same as sleep duration determined by polysomnography or questionnaires, though correlation is high[255–257,329,339,357,358]. However, sleep quality, different sleep stages, or the more subjective but not less important measure of sleep satisfaction[29] are not detected by actigraphy. It is important to be aware of this when discussing the association between sleep and embryonic development and looking for plausible explanations.

Another reason for discrepancies regarding other measurement methods is the daily recording time. Polysomnography studies are often not performed in a home environment and are based on nocturnal recordings, thereby lacking daytime sleep registration such as napping. Although napping plays an important role through pregnancy[261,352,359,360] and is associated with pregnancy outcome[361–365], it does not necessarily affect sleep duration at night in pregnancy[354]. As such, it contributes to a higher measurement of the daily sleep duration. Data from questionnaires or sleep diaries that are not limited to night or laboratory recordings and correlate well in terms of nap registration with actigraphy[358] might still be affected by potential recall bias[27], which contributes to discrepancies in results compared to other methods[329,357,358]—also in pregnancy[254].

However, why is the total sleep duration particularly increased during the first trimester? Sleep efficiency was reduced in the 1st and 3rd trimester, a possible sign of lower sleep quality, supporting the results of an earlier questionnaire study[259]. Sleep quality deteriorates, particularly in the 3rd trimester due to the increased prevalence of sleep disturbances[366]. These disturbances arise from pregnancy-related maternal

changes such as a growing uterus, musculoskeletal shifts leading to back pain, heightened voiding frequency, and respiratory alterations. Nonetheless, significant physiological changes—hormonal, metabolic, and cardiovascular—also occur during the first trimester of normal pregnancy. We speculate that extended Total Sleep Time (TST) and daytime napping are part of physiological adaptation to these factors.
Regarding maternal physical activity, we recorded in the present study not only the total daily physical activity duration, but also determined the different intensity levels. The previously reported shift from structured exercise to lighter activities during pregnancy[290,291] is confirmed by the present results *(Table 3)*.
Similar to the decreased sleep quality during pregnancy, it has been suggested that increasing weight, cardiac load, abdominal expansion, edema, joint pain, and altered sleep patterns impair the quality of life during pregnancy[367] and contribute to the altered physical activity described herein.
As the employed statistical model accounted for probable confounders *(Fig. 35)*, it provided insights into the cumulative effects of pregnancy-related changes in each trimester on daily sleep and physical activity duration. These effects were found to vary significantly with maternal factors, such as parity, age, and pregestational body composition *(Paper I; Supplementary Appendices B and D)*. Therefore, these factors were also considered potential confounders during the analysis of the relationship between sleep, physical activity, and early embryonic development *(Papers II–III)*.

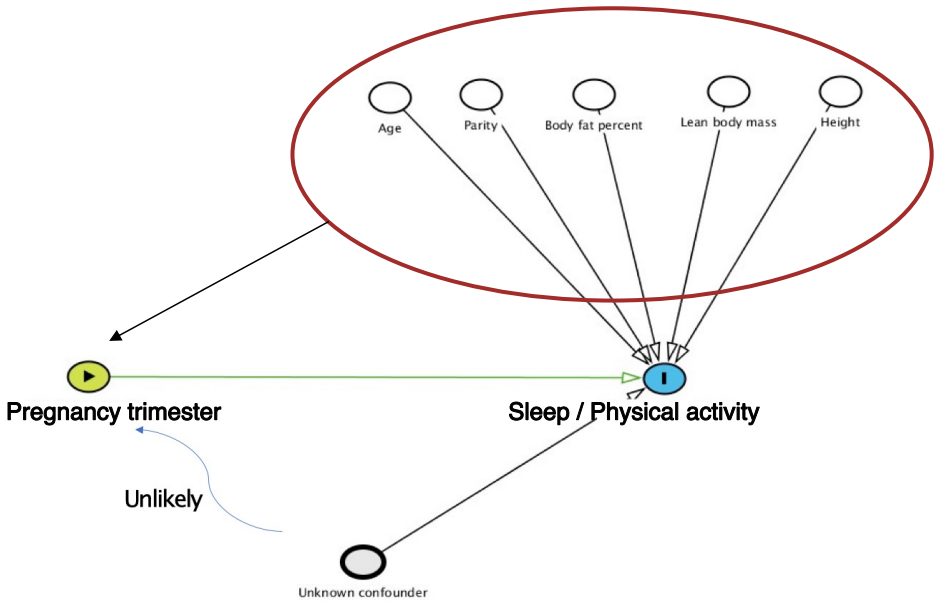


Fig. 35 Directed acyclic graph (DAG) illustrating the basis for adjustments in our regression model, including the key variables: pregnancy trimester (predictor), sleep or physical activity duration (outcome), as well as age, parity, body fat percentage, lean body mass, and height (controlled covariates).

Paper II

The present study demonstrated a sex- and time-dependent relationship between pre-conception sleep duration and human YS size as well as embryonic crown-rump length *(Fig. 30 and Paper II; p. 6, Tables 5 and 6).*

Specifically, the shorter maternal sleep duration before pregnancy was associated with a larger YS and CRL in male embryos at seven weeks of gestation. The effect on the YS of 7-weeks (MA) embryos could be traced later in pregnancy—i.e., although the sleep duration assessed at 13 weeks was 38 min longer, it still had a distributional pattern that was significantly linked to YS size at 7 weeks.

Regarding the effect of environmental factors on YS size, there is some evidence from animal studies supporting that environmental factors can affect the size of the YS (e.g., temperature[368], nutrition[368–370], and noise[371]). However, YS development and implantation mechanisms differ among species[16,43,59,372], making it difficult to

extrapolate these findings to humans. Is it yet biologically plausible that sleep can influence YS size?

The following section describes two biological models that could potentially explain these findings: "YS compensation" and "ovulation-implantation bias".

Yolk sac compensation

The first model aligns with the hypothesis put forward by Karlsen et al. (2018)[151]. In response to an exocoelomic cavity with diminished nutrient content, the YS volume increases, which results in a compensating larger endodermal and mesothelial surface that can provide sufficient access to nutrients, contributing to a favorable outcome and early fetal growth, as evident in the pregnancy outcomes of this low-risk cohort *(Paper II; p. 4, Table 2 and p. 6, Table 6)*.

The secondary YS is directly connected to the embryonic gut and has a rich vascular plexus, supporting the theory that the YS plays a nutritional role during early pregnancy[15]. Its absorptive role has been demonstrated, confirming that the exocoelomic cavity and secondary YS form a transfer interface between the extra-embryonic and embryonic compartments of the human gestational sac[96]. Indeed, it has been proposed that alternative hemochorial nourishment of the embryo through the human placenta cannot be considered effective prior to the end of the first trimester because the intervillous space is filled with clear fluid only[87]. In addition, spiral arteries are still blocked by trophoblast cells[89,90] and remain unconnected[86,88]. This is consistent with the results of Hustin et al. (1987 and 1988)[81,84], who were unable to detect significant maternal blood flow within the placenta when they used ultrasound Doppler before 12-weeks' gestation which has been confirmed in other studies[373,374]. Measurements of oxygen tension within the human placenta and extraembryonic cavities[82,83,375] in vivo support this claim. It is not before 10 to 12 weeks of gestation (MA), when the oxygen tension in the placenta rises from 20 mmHg to 50 mmHg, which is, in turn, associated with changes in oxygenation at the cellular level (i.e., increased activity of antioxidant enzymes in placental tissues)[15]. Although it was initially considered that the intervillous fluid originated from plasma filtering through the trophoblastic plugs, subsequent evidence showed that secretions from the uterine glands fill the intervillous space up to at least the 10th week of

gestation. This implies that these glands might play a role in producing the fluid[15]. As such, uterine glands could represent a primary source of nutritional support for the embryo throughout the period of organ formation, which aligns with the pathway of the majority of eutherian mammalian species[15]. Nutrition from histotroph that relies on anaerobic glycolysis can satisfy the metabolic needs throughout this developmental stage[376] and reduce the potential for DNA damage or interference with signaling pathways caused by reactive oxygen species produced in aerobic metabolism[377]. This is supported by studies in sheep, which demonstrated that the growth of uterine glands plays a critical role in the likelihood of embryo survival and implantation, as well as in initiating and sustaining pregnancy[91]. Thus, the histotrophic pathway may provide an environment that benefits the correct cell differentiation during early development[378–380].

The hypothesis of YS compensation is corroborated by studies in rodents, which suggest that YS's histotrophic function, mediated by diet, provides a mechanism for embryos to respond to poor nutrition, thereby protecting fetal growth and competitive fitness[181].

However, to serve as a plausible model for the current findings, it is also necessary to consider how variations in sleep could affect the intrauterine environment and potentially diminish nutrient availability in the exocoelomic cavity; for instance, reduced maternal sleep duration may disturb nutrient secretion by the uterine glands. Additionally, biological mechanisms that may lead to changes in the YS volume are required.

Influence of sleep pattern on the intrauterine-environment

Evidence suggests that sleep patterns can influence female neuroendocrinology, intrauterine environment, and reproductive outcomes[381]. For example, observational studies have reported a higher frequency of miscarriages among women working night shifts[382,383]. Recently, a large Danish register study that included 22 744 pregnant women[239] found a 32% increase in the frequency of miscarriages among women who worked two or more night shifts per week. This link to shift work suggests an association with circadian rhythms and their regulation of melatonin production[243–251]. The roles of melatonin [N-acetyl-5-methoxytryptamin] in

reproduction have gained increasing attention[123,245,384]. This small neurohormone, secreted by the pineal gland, is amphiphilic and permeates every cell, compartment, and body fluid[385]. It plays a pivotal role in coordinating circadian and seasonal rhythms, particularly the sleep-wake cycle[386], and is considered a molecule that dates back to the origin of life[387]. In humans and many mammals, the suprachiasmatic nucleus (SCN) within the hypothalamus is the primary mediator of the circadian clock[388,389]. It regulates circadian physiology and behavioral rhythms, including sleep patterns, body temperature, appetite, and neuroendocrine and autonomic functions, and orchestrates the secretion of melatonin into the bloodstream by the pineal gland[390]. However, melatonin is also non-cyclically produced in the mitochondria of all cell types[391]. Recent findings have suggested that melatonin plays a role in blastocyst development and implantation[120]. Histological analysis of the endometrium in melatonin-treated mice showed an enlarged endometrial area and increased density of uterine glands[384]. Melatonin also appears to facilitate the interaction between adhesion proteins on endometrial cells and blastocysts through melatonin receptors (MT1 and MT2) present in both cell types[121]. It directly regulates several molecules involved in uterine receptivity during implantation, including growth factors, cytokines, lipid mediators, and transcription factors, which are also affected by steroid hormones[392]. At the molecular level, melatonin is postulated to regulate the function of p53, a downstream element of MT1 and MT2 activation[121], which is crucial as a transcription factor and tumor suppressor, and is associated with cellular apoptosis, cell cycle arrest, senescence, and DNA repair[393–396]. Notably, p53 also plays a significant role in reproduction, exerting sex-specific effects[397] and regulating leukemia inhibiting factor (LIF) expression, a cytokine essential for implantation[398,399].

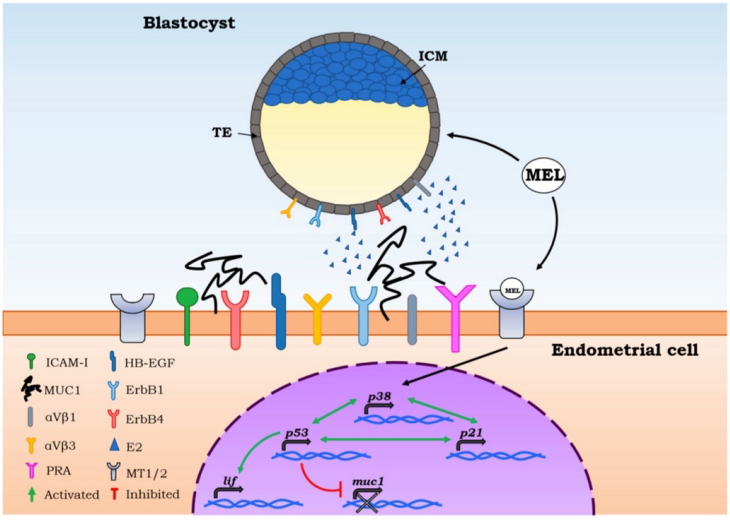


Fig. 36 Physiological secretion of melatonin facilitates implantation. Melatonin membrane receptors (MT1 and MT2) are present in endometrial cells and blastocysts. Melatonin signaling creates a positive feedback loop between *p53*, *p38,* and *p21*, activating leukemia inhibiting factor (*lif)* transcription and inhibiting mucin 1 (*muc1*) secretion, resulting in a better interaction between adhesion proteins at the membrane level in endometrial cells and blastocysts. In addition, HB-EGF expression is upregulated. *Reused with permission under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International (CC BY-NC-ND 4.0). © Carlomagno et al.*[121]*, MDPI 2018, Basel, Switzerland.*

Furthermore, sleep and circadian rhythms are involved in the regulation of sex steroids[381], and in vivo studies in mice have shown that melatonin treatment can increase estradiol levels, favoring implantation while simultaneously shortening the uterine receptivity period[400].

Melatonin treatment has also improved the uterine microenvironment by promoting the expression of antioxidant enzymes such as superoxide dismutase, glutathione, and catalase[401]. This is significant, given that ROS can cause loss of membrane integrity and alterations in functional structures[402]. Accordingly, multiple clinical studies have indicated that adequate melatonin concentrations in women can enhance reproductive processes and decrease negative events[123]. In a mouse model, Asgari et al.[403] showed that melatonin treatment at the 2-cell stage resulted in a higher percentage of morula formation, improved blastocyst development rates, and increased hatching frequency. The treatment significantly elevated the total cell count, encompassing both the trophectoderm and the inner cell mass. Moreover, evidence suggests that melatonin is involved in growth metabolism, enhancing both the quality and quantity of

developing embryos, with treated embryos showing higher implantation rates than control embryos[403].

However, other mechanisms involving the links between insomnia, HPA-axis activation, plasma cortisol levels[404], and the association of deep sleep with human growth hormone secretion[405,406] also have the potential to influence fertility and the intrauterine environment[406], and may play a role in the availability of nutrients and implantation.

Biological mechanisms that may account to changes in yolk sac volume

Regarding the potential mechanisms accounting for size changes of the YS, several processes may be involved. These include early epigenetic regulation of cell metaplasia, cell differentiation, hypertrophy, or hydropic degeneration within the YS membrane. An imbalance, characterized by low antioxidant levels, may lead to overproduction of ROS, a primary factor in oxidative stress responsible for embryonic damage[407] through the induction of apoptosis[408].

Alternatively, these changes may also stem from variations in mesenchymal volume or in the number, volume, and flow of the vitelline vessels. Epigenetic programming during the earliest stages of gestation has been associated with sleep[243–251,409] and embryonic cellular development, including the production of immune progenitor cells within the YS[409]. Such links might represent early mechanisms that could condition offspring health.

Additionally, mechanical changes due to smooth muscle cell contractions in the YS membrane and extraembryonic motor activity may also play a role[410]. For example, circadian rhythms have been shown to influence uterine contractility in humans and other primates[411,412]. Melatonin, intricately connected to the circadian rhythm, has been observed to have inhibitory effects on uterine contractility by modulating the expression of smooth muscle myometrial melatonin receptors MT1 and MT2[412]. These receptors, also identified in human trophoblast cells[244], may be present in the human YS. Furthermore, the dynamics of osmotic pressure between the chorion and the YS cavities could be a contributing factor.

Despite these possibilities, the exact processes leading to increased YS size remain speculative, as the data presented in this study could not identify the precise mechanisms.

Time specific influence on yolk sac size

The present findings also demonstrate a temporal and early influence on YS size, observable specifically at the 7-weeks' gestation, but not at 10-weeks' gestation. This is in line with the histotrophic nutrition of the embryo from the YS in early pregnancy[97] and a gradual shift to hemotrophic nutrition provided by the placenta at the end of the first trimester[43,97] when the nutritional significance of the YS is reduced. In growth models, it has also been shown that there is a breakpoint at a CRL of 20 mm, referring to day 46, where the growth velocity increases. It has been suggested that this might be caused by the change from histotrophic to hemotrophic nutrition and the initial connection to maternal circulation[413]. In addition to acting as a source of nutrients, histotroph also contains a number of cytokines, such as TNF-α[414], uteroglobin[415], epidermal growth factor, colony stimulating factor, and vascular endothelial growth factor, which are powerful regulators of trophoblast proliferation and migration in vitro[416,417]—as such, these factors may accelerate or increase the remodeling of uteroplacental arteries[15] and contribute to the diminished role of the YS at an earlier stage. In contrast, in a nutrient-rich environment, the YS could have accomplished its functional means at an earlier stage and thus proceed faster through development, resulting in earlier involution with decreasing size. However, the lack of placental circulation before weeks 10 to 12 could also mean that YS variation would rather be seen at the second measurement and not as early as six–eight weeks of gestation because the larger embryo needs more nourishment.

The observed association between compensatory enlargement of the YS and embryonic CRL at week 7 *(Paper II; p. 6, Table 6)* might also seem to challenge the compensatory model. If the YS compensation were entirely effective, the expectation would be that the CRL would not be affected. However, this inconsistency may be attributed to a delayed compensatory response from the YS.

Ovulation-implantation bias

Another model that may explain our findings involves systematic errors attributable to variations in ovulation or implantation timing.

Errors due to ovulation timing can occur if the sleep duration affects the length of the follicular phase. Among women with regular menstrual cycles, the follicular phase can vary by several days (Mode = 13 ± 4 days in 75% of the cases)[39]. If sleep patterns influence this phase, embryonic age, which corresponds to conception (within 24h of ovulation)[37,38], would also be affected. This can lead to a systematic deviation from MA, which assumes a standardized conception on the 14th day of the cycle. Since both the CRL and secondary YS increase in size with embryonic age, sonographic measurements during gestation could be skewed relative to MA. This is supported by the inverse correlation observed between the interval from ovulation to implantation and the first-trimester measurements of fetal CRL[418]. Consequently, the embryonic CRL and YS diameters may appear systematically larger if ovulation occurs earlier than day 14, or conversely, smaller if ovulation occurs after the standard estimated day 14 *(Fig. 37)*.

Numerous studies have elucidated the mechanisms by which alterations in sleep can influence the menstrual cycle[123,419–421] and may induce early or delayed ovulation. Influences on the hypothalamic-pituitary-adrenal (HPA) axis[404,419,421], alterations in the secretion of luteinizing hormone (LH)[422,423], estradiol[421], growth hormone (GH)[405], and other hormones[381,420–422] are implicated, as well as variations in the levels of natural molecules such as myo-inositol and antioxidants[424]. For instance, melatonin has been shown to suppress human ovulation when combined with progesterone[425]. Additionally, melatonin levels are positively correlated with follicular growth, indicating a significant role of melatonin in the ovulation process[426].

In the current study, a systematic skew in embryonic age can indeed account for the observed correlation between sleep patterns and both YS size and CRL, but it does not explain the lack of an association between sleep duration and the second YS measurement. However, at later gestational ages, increased size variability due to individual embryonic and YS growth may obscure this relationship. Additionally,

variations in YS size may be accentuated by degenerative processes, which eventually lead to dissolution by the end of the first trimester[43,148].

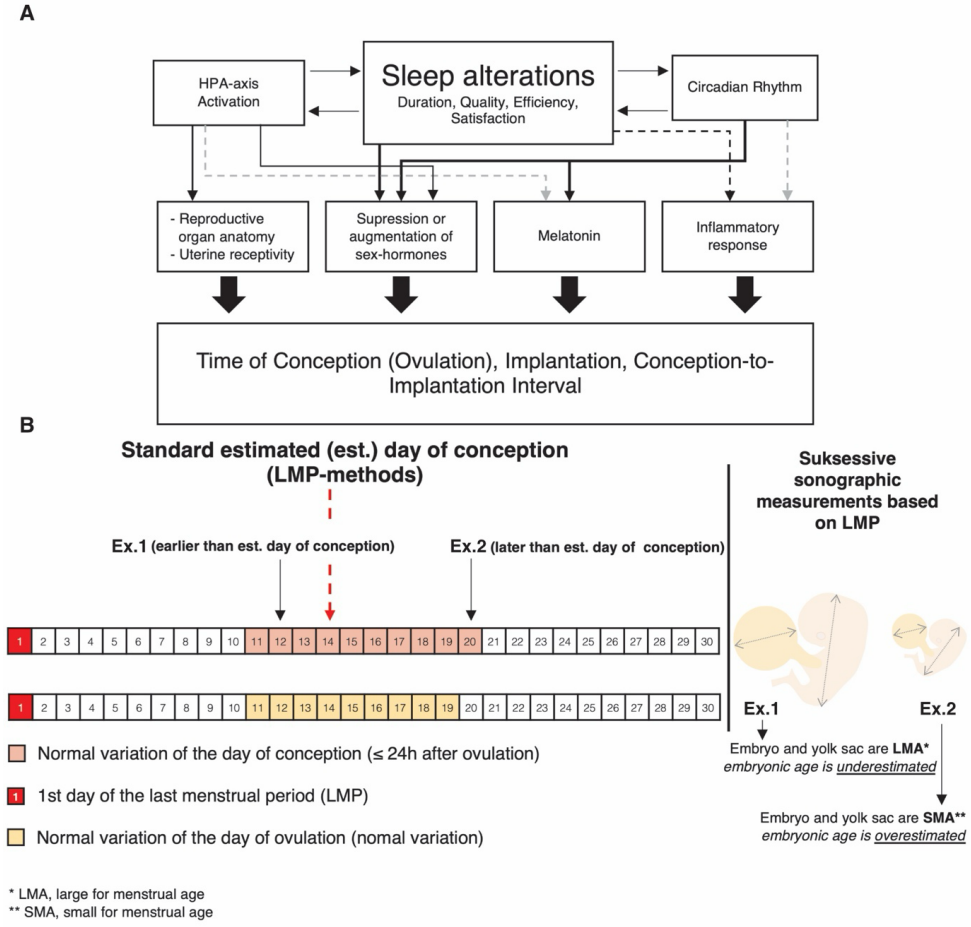


Fig. 37 Ovulation- and implantation-bias. (A) Possible pathways from sleep alterations to female reproduction and timing of ovulation and implantation during the menstrual cycle. **(B)** Illustration of ovulation-implantation-bias using two examples (Ex.1 and Ex.2)—Left side, Ex.1: Sleep alterations lead to an earlier ovulation (conception) than assumed by the LMP method (red arrow) and left side, Ex.2: Sleep alterations lead to a delayed ovulation (conception) compared to that assumed by the LMP method. Right side, corresponding to Ex.1: Conception was earlier than that estimated using the LMP method. Consequently, the embryo is older, and the CRL and yolk sac size are larger than expected by the LMP method at successive sonographic measurements. Right side, corresponding to Ex.2: Conception is later than estimated by the LMP method, and the embryo is younger with a shorter CRL and smaller yolk sac size than expected by the LMP method at successive sonographic measurements.

Similarly, systematic skew can be attributed to the mechanisms that involve the time of implantation. As discussed above, sleep patterns can also affect the intrauterine

environment *(refer to "Influence of sleep pattern on the intrauterine environment")* and may thereby modify the timing of implantation.

The two suggested mechanisms, YS compensation and ovulation-implantation bias, are based on nutrient availability and differences in ovulation and implantation timings. However, other mechanisms could explain variations in YS size, such as vascular changes or YS membrane degeneration due to metabolites. This is discussed in conjunction with the findings presented in Paper III.

Paper III

While Paper II primarily assessed the effects of maternal sleep duration on embryonic development, this study demonstrated that maternal physical activity prior to pregnancy is associated with YS size *(Table 8 and Fig. 31)*. The YS exhibited a graded response under varying durations of maternal physical activity, evident across all activity levels, including light and moderate-vigorous activities *(Fig. 34)*. Consistent with the previous study (Paper II), the effect was dependent on the timing of the measurement and the sex of the embryo. However, the direction and timing of the influence, specifically whether it was observed in the first or second YS measurement, differed *(Fig. 31)*. In male embryos, increased physical activity was associated with a larger YS at the first measurement (7 weeks), while in female embryos, a comparable response was not observed until the second measurement (10 weeks). At this stage, male and female embryos exhibited the opposite response *(Table 8, Term Male:PAD)*, leading to a sex-specific and inverse effect on the growth rate of the YS between 7 and 10 weeks of gestation *(Table 9 and Fig. 33)*.

Physical activity as a natural stress factor and yolk sac size

Physical activity in expectant mothers improves gestational metabolism[270,271], placental development[271,276–279], and obstetric outcomes[271–275]. These responses manifest relatively late during pregnancy compared with the developmental phase of the YS. Moreover, the present yolk sac responses differed from those associated with maternal sleep patterns reported in (Paper II). Despite these differences in timing and response compared to preconception maternal sleep duration, the underlying

mechanism affecting YS size may be analogous. Physical activity could act as a natural stressor, prompting a physiological adaptive response in the YS—an increase in size and, consequently, surface area to facilitate gas exchange and nutrient uptake before the placenta has fully developed[151].

In non-pregnant individuals, exercise is known to reduce visceral blood flow to meet the metabolic demands of working muscles[427]. Physical activity has also been suggested to induce shear stress and intermittent fluctuations in substrate and oxygen delivery, resulting in hypoxic strain that generates a repetitive stimulus that triggers a fetomaternal response with increased placental vascularization[279,428]. These activity-related substrates and oxygen fluctuations may also influence histotrophic nutrition in early pregnancy stages through the vasculature and uterine glands.

However, physical activity can lead to more than just circulatory and metabolic changes. It has also been associated with variable steroid levels in women[429]. These are recognized to affect the menstrual cycle and the timing of ovulation, as well as the composition of the endometrium and its glands, and could play an essential role both in the model of ovulation-implantation bias and the model of YS compensation.

Degenerative changes of the yolk sac due to intrauterine-environment

YS size has also been associated with significant degenerative changes in its wall. As the maximum sonographic size coincides with these degenerative morphological changes, it has been suggested that the notable increase in YS diameter observed between nine and ten weeks of gestation is secondary to these changes[136].

Furthermore, pathological conditions such as miscarriage[147,160,430] and chromosomal anomalies[168], have been linked to abnormal YS size. Specifically, a YS diameter above or below two standard deviations according to menstrual age is a specific predictor of miscarriage[147,160,430]. In a small study[164], changes in the diameter of the secondary YS preceding miscarriage by a few days were related to abnormal heart activity, suggesting that hypoperfusion could result in progressive loss of structure and necrosis or edema, ultimately leading to miscarriage and cessation of heart activity. This is consistent with a study reporting a larger YS diameter relative to crown-rump length (CRL)[146] after miscarriage.

In addition, animal experiments have shown that various metabolites can damage YS structures in utero[174,431]. Hyperglycemic conditions during organogenesis, for instance, have been associated with YS damage and subsequent embryonic malformations[174], which are more prevalent in infants born to diabetic mothers[432]. Based on studies in rats with experimental hyperglycemia, mechanisms of macroscopic and microscopic YS membrane injury and patterns of aberrant cellular communication have been suggested as explanations for the observed anomalies[433]. Such membrane injuries, along with various defective signaling mechanisms[434,435], have also been proposed as the origin of findings in a study involving pregnant women with insulin-dependent type I diabetes[155]. In this study, the secondary YS, measured by 2D- and 3D-ultrasound between five and 10 weeks of gestation, reached its maximum diameter between one and two weeks earlier than that in the control group and was associated with an approximately two-week earlier involution.
Diabetes has also been linked to specific changes in YS vessels within the YS wall, termed diabetic YS vasculopathy[173]. These alterations are thought to contribute to transport malfunctions and are suggested to be another mechanism underlying diabetes-associated malformations[173]. Oxidative stress is considered a significant factor in these mechanisms.
Increased oxidative stress has been implicated in diabetes-induced teratogenicity of post-implantation embryos, because hyperglycemia includes deficiencies in myoinositol and arachidonic acid, leading to disturbed prostaglandin metabolism[432,436]. This hypothesis is corroborated by a study where increased arachidonic acid (but not prostaglandin E2) levels were found in the YS structures of pregnant women with diabetes, as opposed to normal levels in the control group[176]. There is also evidence that insufficient prostaglandin levels adversely affect membrane development and functionality[437]. As prostaglandin insufficiency allows for the excessive entry of glucose into cells, the formation of free oxygen radicals is triggered and can harm the embryo[437].
However, studies have also shown a reduction in the activity of antioxidant enzymes such as SOD and CAT in rat embryos and their YS structures under diabetic conditions, along with an increased rate of birth defects, growth inhibition, and

diminished protein levels[438]. As such, the surplus of free radicals could be due to both their overproduction and deficiency in their enzymatic neutralization[115].
When interpreting the present results, it is critical to recognize that they were derived from normal pregnancies under physiological conditions. Therefore, caution is warranted when comparing them with the results of pathological pregnancies. Nevertheless, variations in sleep and physical activity could still contribute to changes in ROS levels, albeit probably to a lesser extent. These changes may influence translational or post-translational processes such as the expression of receptors, enzymes, or transport complexes. Consequently, this could not only affect the availability of nutrients in the coelomic cavity but also alter the composition and structure of the YS membrane, eventually causing cell death[439] and leading to changes in the size and structure of the YS.

Papers II–III

Maternal influences on yolk sac size are both factor- and GA-specific

Before examining the association between sleep and activity parameters and measures of embryonic development, we sought to corroborate the findings of an earlier study conducted by our group. In a similar cohort, Karlsen et al. (2018) reported an association between maternal body composition parameters and YS size at approximately eight weeks of gestation, particularly in female embryos[151]. They also observed that the YS-to-CRL ratio affected ultrasound measurements of fetal growth parameters, with effects persisting up to 24 weeks of gestation, especially in the Z-scores of abdominal circumferences and estimated fetal weight. Notably, these effects were not observed in the current study *(Table 6)*.
Nevertheless, considering these results together with the effects of sleep described in Paper II and of physical activity in Paper III, a distinct pattern emerges: pregestational maternal cues appear to influence the size of the human YS and the embryonic CRL in a manner specific to each factor, and this influence also seems to depend on the gestational timing. Indeed, these observations should not be unexpected, given that the 1st trimester is a period of rapid embryonic development during which there are many developmental steps. Additionally, the intrauterine

environment undergoes significant changes and the source of embryonic nutrition shifts from early histotroph nutrition, which relies on the endometrium and uterine glands, to placental hemotroph nutrition at the end of the 1st trimester[59,97]. Finally, although the physiological effects of maternal factors, such as body composition, sleep, and physical activity, are interconnected within a broader physiological context, they may still exert distinct influences on development with varying effects.

Sex-specific effects of maternal cues

The current study not only demonstrated a factor-specific and gestational age (GA)-dependent impact on yolk sac size but also revealed sex-specific associations between sleep and yolk sac size, as well as physical activity and yolk sac size. These associations were only marginally significant when fetal sex and GA were not considered *(Table 8 and Paper II; p. 6, Tables 5 and 6)*. Moreover, the study by Karlsen et al.[151] indicated that the effect of maternal body composition parameters on yolk sac size was confined to female fetuses. Figure 38 represents both the time- and sex-specific effects on YS for each specific maternal factor (i.e., sleep, physical activity, and body composition) and gives an insight in the dynamic regulation of development *(Fig. 38)*.

In 1995, Weinberg et al.[440] reported that women with a short follicular phase before conception were more likely to give birth to males, which could explain why the observed effects in the present study were influenced by both GA and fetal sex. This also corroborates the theory of ovulation-implantation bias introduced, along with the findings of Paper II.

However, the influence of sex on mammalian phenotype and disease susceptibility is not a novel concept[301]. There has been a growing focus on how genetic sex influences gene expression patterns and embryonic interactions with the maternal environment[302]. Through transcriptional sexual dimorphism[303,304] or gene imprinting[302], maternal and environmental factors can affect preimplantation embryos, even before gonadal differentiation. This could explain the observed sex-specific maternal and environmental effects based on sex-specific plasticity, which depends on embryonic age[441]. Essentially, developmental time windows for influence

may vary between sexes, similar to the divergent developmental trajectories observed in postnatal life between girls and boys.

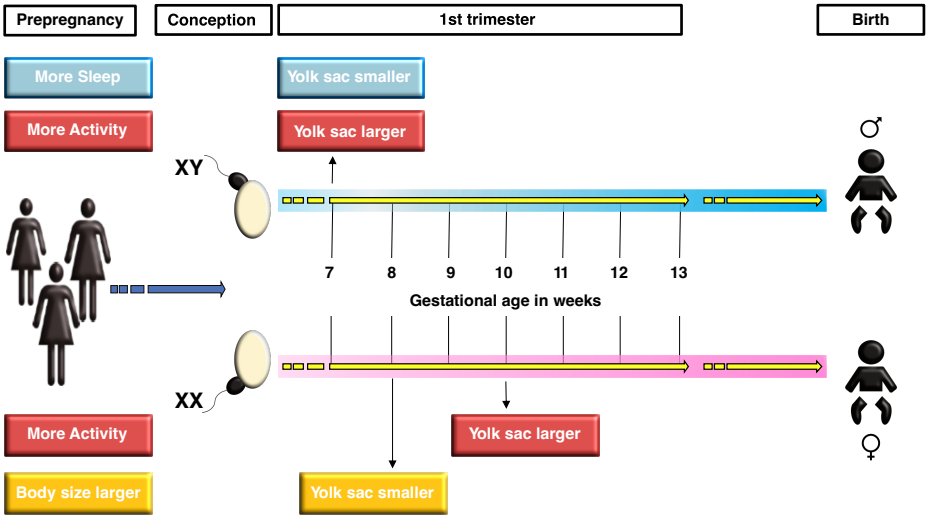


Fig. 38 Effect of preconception maternal factors on yolk sac size: i.e., the effect of maternal sleep duration (Paper II), total maternal physical activity duration (Paper III), and maternal body size (weight and height)[151]. The figure illustrates the sex- and time-dependent effects during the 1st trimester. The time windows in which these effects could be observed were short. *Material adapted from Vietheer et al., Scientific Reports (2023), Springer Nature. © The authors.*

Strengths and weaknesses

Strengths of the study

Design

This study is unique because of its prospective longitudinal design, which started prior to conception and continued throughout pregnancy until birth. This granted access to maternal data from a large number of healthy women who conceived naturally as well as data on embryos, fetuses, and newborns. However, collecting such data from healthy women poses significant challenges and is time-consuming, because this cohort usually does not seek clinical services when planning to conceive. Conversely, data from assisted reproduction involve fertility problems and the confounding influence of hormonal treatments. These factors can lead to

consequences such as increased growth beyond a CRL of 20 mm in IVF pregnancies[413].

Methods

Actigraphy, involving machine measurements, is superior in the measurement of sleep duration and circadian rhythms, providing continuous data over extended periods in an ecologically valid environment (i.e., at home or work)[442]. It also uniquely records both sleep duration and daily physical activity levels, including intensity, using a single device.

A summary of both advantages and disadvantages of the different methods for measuring maternal body composition is shown in *Table 2.* In the current study, the focus was exclusively on the effects of pre-conception body composition, as it offers benefits such as reducing the risk of inaccurately distinguishing between maternal and fetal compartments.

To ascertain the accuracy of YS measurements through transvaginal ultrasound, the study calculated both intra- and inter-observer variability, confirming that the measurements were precise and unbiased by the observer. Additionally, the likelihood of confounding observer effects is minimal, as maternal physical activity is not inherently connected to the ultrasound procedure, and ultrasound operators were blinded to embryonic sex at the time of observation.

Statistics

The combined use of actigraphy, BIA, and a stadiometer not only allowed for the assessment of their associations with measures of embryonic development but also enabled controlling for preconception body composition parameters in the statistical analysis. These parameters are known to affect pregnancy[209] and its outcomes[215,216,221]. Considering a previous study where prepregnant body composition was correlated with measures of embryonic and fetal development, particularly the yolk sac[151], subanalyses controlling for these parameters (papers II–III) was deemed essential. Focusing on preconception measurements is advantageous, as they are less prone to systematic errors compared to those taken during pregnancy,

which can fluctuate due to various factors such as hormonal changes, activity limitations, edema, sleep disturbances, and nausea.

To assess repeated maternal sleep and activity measurements before and during pregnancy (Paper I), growth curve calculation of the CRL and YS, and determination of the YS Z-score (Papers II–III), mixed-model regression analysis was employed. These models are beneficial because they account for autocorrelation in serial measurements through the inclusion of random effects for participant-specific variations, while the fixed effects assess factors that are consistently influential across the study population[443], thereby enhancing the generalizability of the findings. Furthermore, mixed-models are advantageous in the case of missing data as they use all available information, resulting in more precise estimates and enhancing the study's robustness[443].

Results

The current findings suggest that the actigraphy monitor effectively captures specific characteristics of sleep and physical activity by integrating data from a triaxial accelerometer with other physiological sensors. Notably, the absence of an inverse relationship between the effects of physical activity and sleep duration on yolk sac size at week 10 suggests that actigraphy data provides a more comprehensive measure than merely counting movements *(Fig. 38)*. Furthermore, the variations in yolk sac size corresponded to different classes (intensities) of physical activity, similar to the pattern observed for the total daily activity duration *(Fig. 34)*. This alignment reinforces the notion that similar diametric effects on yolk sac size, as for example at week 7, are not merely byproducts of inverse sleep and activity movement recording over a 24-hour period.

The validity of the actigraphy measurements in the current study was further strengthened by their consistency with previous findings[236,262,264,287,292,335,352–355]. Additionally, the measurement results in Paper I are in line with those in Papers II–III despite the increased sample size, demonstrating the study's internal validity. Another factor supporting the validity of our data is the YS size and CRL measurements that correspond with established growth patterns[144,148,149,159,351] *(Fig. 29)*. Reported sex-dependent maternal effects on yolk sac

size have also been corroborated[151]. In Paper III, the influence of embryonic sex was substantiated by demonstrating the opposite effect of maternal physical activity on the yolk sac size of male and female embryos at 10 weeks of gestation *(interaction analysis, Table 8)* as well as the sex-specific growth rates of the YS *(Fig. 33)*.

Finally, this study utilized several alternative statistical models for the analysis in Papers II–III, such as quantile regression models, which consistently produced the same results. Adjusting these models for observer effects (Paper III), effects of maternal age, parity, weight, height, BMI, lean body mass, body fat percentage, and GA (Paper II–III), as well as time of inclusion or inclusion of a few participants with pregnancy complications (Paper III) did not change our conclusions. This suggests that the results were neither biased by these factors, nor affected by skewed data, systematic distribution differences, or extreme values.

Weaknesses and limitations

Studypopulation

The study's extensive data collection required significant commitment from the participants to adhere to the protocol and complete the study. Furthermore, the inclusion criteria were strict. This could lead to selection bias, resulting in a cohort of exceptionally healthy women particularly interested in health possessing the capacity and determination to complete the study. On the other hand, some participants might have joined the study because of fertility issues that were not covered by the exclusion criteria. Nonetheless, the collected data, including variations in maternal characteristics, actigraphy, and yolk sac measurements, appeared normal and aligned with previous findings.

Study design

For Papers II–III, maternal sleep and physical activity were recorded only twice, first before pregnancy and then not again before 13±1 weeks of gestation. This recording schedule implied that sleep or activity patterns observed before pregnancy may not have persisted into early pregnancy. Additionally, the YS was measured only twice

during the first trimester (at 7 and 10 weeks). Consequently, it is uncertain whether the variations in YS observations originated from pre- or peri-conceptional sleep and activity patterns. These may also be attributable to concurrent processes influenced by prevailing sleep and physical activity patterns at 7 and 10 weeks. Utilizing sleep and activity diaries or a continuous actigraphy measurement, along with more frequent YS measurements, could have provided more definitive conclusions and deeper insights. Nonetheless, the average actigraphy recordings were close to the time of conception *(Paper III; Table 2)*, and the impact of various levels of physical activity on the YS was still observable at 13 weeks' gestation (Paper III). The latter observation suggested that the distribution of physical activity patterns was consistent across the population throughout the first trimester.

Finally, sex-steroid levels, mental stress, and maternal nutrition are potential confounders. Stress causes a hormonal response that is comparable to that of exercise, and nutrition is closely related to energy metabolism. However, our study population composed of healthy women with no history of chronic diseases or risk factors, and the likelihood of experiencing chronic psychological stress in this group was expected to be minimal. Additionally, the potential for confounding factors related to maternal nutrition seemed low, as maternal body composition, which is closely associated with energy metabolism and nutrition, did not significantly affect our findings *(Paper II; Supplementary Information 1, Table S4 and Paper III; Supplementary Tables S4–S7).*

Sleep measurements

It is important to be aware of the limitations of actigraphy for sleep measurements. Although additional parameters, such as sleep onset latency (SOL), wakefulness after sleep onset (WASO), and sleep efficiency (i.e., the proportion of time spent asleep after going to bed)[329,357], can provide insight into the quality of sleep, these measurements do not capture all dimensions. For example, they do not represent sleep satisfaction (feeling well rested upon waking up). Furthermore, our analysis did not include circadian rhythm, and the sleep architecture (i.e., the cycles of different sleep stages throughout the night) was not determined by the actigraphy monitor

used—both factors are significantly correlated with variations in the HPA axis[381,404,405].

Another aspect to consider is that weekday variations can significantly impact sleep and activity duration, particularly for shorter recording periods (less than seven days)[357]. However, in subanalysis of our actigraphy recordings, those with a higher proportion of weekends did not differ from those with a lower proportion *(Paper I; Appendix F)*. Furthermore, neither the total number of days recorded nor the frequency of weekend days recorded differed between the pre-pregnancy and 1st trimester measurements (Papers II–III).

Activity measurements

Although triaxial accelerometers produce valid energy expenditure measurements compared to standard methods (indirect calorimetry and doubly labeled water measurements), they may not always yield accurate measurements of energy expenditure for individual physical activities, especially at low and high intensities[285]. Enhancing these measurements could involve combining methods, for example, with activity diaries, as has been suggested for measuring moderate-to-vigorous physical activity during pregnancy[282], which could strengthen the results.

Yolk sac measurements

It is critical to be aware of that the measurement of the outer-to-outer YS size in the present study is a compound outcome since we have summarized two different YS compartments as one. The intracavitary diameter might not necessarily correspond to the wall thickness of the membrane, and *vice versa*. In other words, we were not able to distinguish these sizes in our study because the measurement method and technical ultrasound protocol did not account for these measurements.

Interpretation

Based on the current data, we cannot definitively claim that a larger YS diameter indicates a benefit to the embryo, nor can we ascertain that the sleep and activity durations measured using actigraphy in this study confer such advantages. However, both extended sleep duration and regular physical activity within the observed range

are associated with health benefits. Given that the study participants were low-risk pregnant women with primarily normal pregnancies, it is likely that the observed YS variations reflect a physiological mechanism. Thus, it is plausible to infer that these YS changes related to activity and sleep are due to a regulatory mechanism that is advantageous to the embryo's early development, similar to the "YS compensation" model. Conversely, ovulation-implantation bias, which seems to exert a neutral impact on the potential health benefits for the embryo, is not unlikely compared to other mechanisms that include cell and membrane damage. These latter mechanisms seem less probable, as they would imply more extreme effects induced by the observed sleep and activity patterns, indicative of an unphysiological intrauterine environment.

6. Conclusion

The potential lifelong consequences of early developmental processes are being increasingly acknowledged, with evidence supporting the pivotal role of the yolk sac in delivering essential organismal functions[43,59]. This study uncovered significant changes in sleep and activity from preconception levels, along with their individual variation, independent of maternal factors, such as age, parity, and body composition (Paper I). Furthermore, a direct and measurable impact of maternal sleep and physical activity on the size and growth dynamics of the human YS has been demonstrated (Papers II–III). Occurring during a critical embryonic development phase between six and 11 weeks of gestation, these observations suggest an interaction between gestational age and embryonic sex. The effects of both sleep and physical activity may stem from initial variations in the intrauterine environment, leading to an adaptive physiological response of the YS.

Considering the period of significant organ development and shifts in organ function allocation, along with epigenetic programming mechanisms, these findings could have profound implications for the developing child. This study highlights sex- and time-specific windows of embryonic developmental sensitivity and reinforces the importance of maternal health factors, including the often-underrated aspect of maternal sleep, from preconception onward.

7. Future perspective

The current study highlights the importance of considering fetal sex and gestational age in research on maternal and environmental influences. The integration of fetal sex determination and gestational age stratification into research protocols and statistical analyses is recommended.

Furthermore, this study poses questions for future research. It has yet to be conclusively determined whether the observed variations in yolk sac size are a result of maternal sleep and physical activity effects on the length of the luteal phase, which influences the timing of ovulation or implantation. Additionally, whether these early embryonic changes persist into later fetal development and impact epigenetic regulation remains to be elucidated. The CONIMPREG project collected extensive data to enable these assessments. The participants provided daily urine samples to facilitate precise determination of ovulation and implantation. Moreover, a broad array of biological samples was collected from women before and during pregnancy and from their partners. The fetus was monitored using serial ultrasound and Doppler measurements during gestation. After birth, a range of biological samples from newborns, including blood, saliva, hair, and nails as well as umbilical cord and placental tissues, were archived in a biobank. This repository supports comprehensive investigations into the enduring effects, including maternal or paternal epigenetic footprints, that propagate into later stages of fetal development and into the neonatal phase.

8. References

1. Ivanovs, A. *et al.* Human haematopoietic stem cell development: from the embryo to the dish. *Development* **144**, 2323-2337 (2017). DOI: 10.1242/dev.134866
2. Nature Education, Definition allele. *Scitable* (2014). https://www.nature.com/scitable/definition/allele-48/
3. Buffe, D., Rimbaut, C. & Gaillard, J. A. in *The human yolk sac and yolk sac tumors* (ed FF, N.) 109-125 (Springer-Verlag, Berlin-Heidelberg, 1993).
4. Hafez, S. Comparative Placental Anatomy: Divergent Structures Serving a Common Purpose. *Prog Mol Biol Transl Sci* **145**, 1-28 (2017). DOI: 10.1016/bs.pmbts.2016.12.001
5. Favaron, P. O., Carvalho, R. C., Borghesi, J., Anunciação, A. R. & Miglino, M. A. The Amniotic Membrane: Development and Potential Applications - A Review. *Reprod Domest Anim* **50**, 881-892 (2015). DOI: 10.1111/rda.12633
6. Silverman, A. J. Embryonic cephalocaudal and lateral flexion/folding. *INTRODUCTION TO EMBRYOLOGY II* 1-8, *Columbia.edu* (2020). http://www.columbia.edu/itc/hs/medical/humandev/2006/HD2/Flexion.pdf
7. Schulz, K. N. & Harrison, M. M. Mechanisms regulating zygotic genome activation. *Nature Reviews Genetics* **20**, 221-234 (2019). DOI: 10.1038/s41576-018-0087-x
8. Hsu, Y. C. Theory and Practice of Lineage Tracing. *Stem Cells* **33**, 3197-3204 (2015). DOI: 10.1002/stem.2123
9. Freeman, S. C., Malik, A. & Basit, H. Physiology, Exocrine Gland. *StatPearls* (2022). https://www.ncbi.nlm.nih.gov/books/NBK542322/
10. Wang, Y. & Zhao, S. in *Vascular Biology of the Placenta* (Morgan & Claypool Life Sciences, San Rafael (CA), 2010). https://www.ncbi.nlm.nih.gov/books/NBK53245/
11. Jauniaux, E. & Gulbis, B. Fluid compartments of the embryonic environment. *Human reproduction update* **6**, 268-278 (2000). DOI: 10.1093/humupd/6.3.268
12. Bajrami, E. & Spiroski, M. Genomic Imprinting. *Open Access Maced J Med Sci* **4**, 181-184 (2016). DOI: 10.3889/oamjms.2016.028
13. Richardson, S. R. & O'Malley, G. F., Glucose-6-Phosphate Dehydrogenase Deficiency, in *StatPearls* (StatPearls Publishing, Treasure Island (FL), 2023). https://pubmed.ncbi.nlm.nih.gov/29262208
14. Moore, K. L., Persaud, T. V. N. & Torchia, M. G. in *The developing human* 65-84 (Elsevier, Edinburgh, 2020). ISBN: 978-0-323-61154-1
15. Burton, G. J., Watson, A. L., Hempstock, J., Skepper, J. N. & Jauniaux, E. Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. *J Clin Endocrinol Metab* **87**, 2954-2959 (2002). DOI: 10.1210/jcem.87.6.8563
16. Burton, G. J., Cindrova-Davies, T. & Turco, M. Y. Review: Histotrophic nutrition and the placental-endometrial dialogue during human early pregnancy. *Placenta* **102**, 21-26 (2020). DOI: 10.1016/j.placenta.2020.02.008
17. Ueda, S., Hirata, T. & Sakasegawa, S. I. Hypoxanthine-guanine phosphoribosyltransferase is activated via positive cooperativity between guanine and IMP. *FEBS Lett* **596**, 1072-1080 (2022). DOI: 10.1002/1873-3468.14306
18. Aumailley, M. *et al.* A simplified laminin nomenclature. *Matrix Biol* **24**, 326-332 (2005). DOI: 10.1016/j.matbio.2005.05.006
19. Miner, J. H. & Yurchenco, P. D. Laminin functions in tissue morphogenesis. *Annu Rev Cell Dev Biol* **20**, 255-284 (2004). DOI: 10.1146/annurev.cellbio.20.010403.094555

20. Mentis, A. A., Dardiotis, E., Katsouni, E. & Chrousos, G. P. From warrior genes to translational solutions: novel insights into monoamine oxidases (MAOs) and aggression. *Transl Psychiatry* **11**, 130 (2021). DOI: 10.1038/s41398-021-01257-2
21. Yao, N. J. *et al.* Interaction Between Prematurity and the MAOA Gene on Mental Development in Children: A Longitudinal View. *Front Pediatr* **8**, 92 (2020). DOI: 10.3389/fped.2020.00092
22. Lundwall, R. A. & Rasmussen, C. G. MAOA Influences the Trajectory of Attentional Development. *Front Hum Neurosci* **10**, 424 (2016). DOI: 10.3389/fnhum.2016.00424
23. Himabindu, A. & Srilatha, B. R. Potency of Various Types of Stem Cells and their Transplantation. *Journal of Stem Cell Research & Therapy* **1**, 0-0 (2011). DOI: 10.4172/2157-7633.1000115
24. Cho, R. H., Sieburg, H. B. & Muller-Sieburg, C. E. A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. *Blood* **111**, 5553-5561 (2008). DOI: 10.1182/blood-2007-11-123547
25. Rojas-Pirela, M. *et al.* Phosphoglycerate kinase: structural aspects and functions, with special emphasis on the enzyme from Kinetoplastea. *Open Biol* **10**, 200302 (2020). DOI: 10.1098/rsob.200302
26. Matoso, A. Testis and paratestis; germ cell tumors; polyembryoma. *PathologyOutlines.com* (2021). https://www.pathologyoutlines.com/topic/testispolyembryoma.html
27. Ibáñez, V., Silva, J. & Cauli, O. A survey on sleep assessment methods. *PeerJ* **6**, e4849 (2018). DOI: 10.7717/peerj.4849
28. Schoenwolf, G. C., Bleyl, S. B., Brauer, P. R. & rancis-West, P. H. *Larsen's human embryology* (Elsevier, Philadelphia, 2021). ISBN: 978-0-323696043 https://pageburstls.elsevier.com/reader/books/9780323811392/epubcfi/6/4%5B%3Bvnd.vst.idref%3Dtitle%5D!/4/2
29. Buysse, D. J. Sleep health: can we define it? Does it matter. *Sleep* **37**, 9-17 (2014). DOI: 10.5665/sleep.3298
30. Harley, V. R. & Goodfellow, P. N. The biochemical role of SRY in sex determination. *Mol Reprod Dev* **39**, 184-193 (1994). DOI: 10.1002/mrd.1080390211
31. Chaudhary, A. K. *et al.* A potential role of X-linked inhibitor of apoptosis protein in mitochondrial membrane permeabilization and its implication in cancer therapy. *Drug Discov Today* **21**, 38-47 (2016). DOI: 10.1016/j.drudis.2015.07.014
32. Loda, A. & Heard, E. Xist RNA in action: Past, present, and future. *PLoS Genet* **15**, e1008333 (2019). DOI: 10.1371/journal.pgen.1008333
33. Hanson, M. A. & Gluckman, P. D. Early developmental conditioning of later health and disease: physiology or pathophysiology. *Physiol Rev* **94**, 1027-1076 (2014). DOI: 10.1152/physrev.00029.2013
34. Gakidou, E. *et al.* Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet* **390**, 1345-1422 (2017). DOI: 10.1016/S0140-6736(17)32366-8
35. Clark, H. *et al.* A future for the world's children? A WHO-UNICEF-Lancet Commission. *Lancet* **395**, 605-658 (2020). DOI: 10.1016/S0140-6736(19)32540-1
36. Inoue, Y., Qin, B., Poti, J., Sokol, R. & Gordon-Larsen, P. Epidemiology of Obesity in Adults: Latest Trends. *Curr Obes Rep* **7**, 276-288 (2018). DOI: 10.1007/s13679-018-0317-8

37. Wilcox, A. J., Weinberg, C. R. & Baird, D. D. Timing of sexual intercourse in relation to ovulation. Effects on the probability of conception, survival of the pregnancy, and sex of the baby. *N Engl J Med* **333**, 1517-1521 (1995). DOI: 10.1056/NEJM199512073332301
38. Wilcox, A. J., Weinberg, C. R. & Baird, D. D. Post-ovulatory ageing of the human oocyte and embryo failure. *Hum Reprod* **13**, 394-397 (1998). DOI: 10.1093/humrep/13.2.394
39. Baird, D. D. *et al.* Application of a method for estimating day of ovulation using urinary estrogen and progesterone metabolites. *Epidemiology* **6**, 547-550 (1995). DOI: 10.1097/00001648-199509000-00015
40. Moore, K. L., Persaud, T. V. N. & Torchia, M. G. in *The developing human* 11-36 (Elsevier, Edinburgh, 2020). ISBN: 978-0-323-61154-1
41. Braude, P., Bolton, V. & Moore, S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature* **332**, 459-461 (1988). DOI: 10.1038/332459a0
42. Tesařék, J., Kopečný, V., Plachot, M. & Mandelbaum, J. Early morphological signs of embryonic genome expression in human preimplantation development as revealed by quantitative electron microscopy. *Developmental Biology* **128**, 15-20 (1988). DOI: 10.1016/0012-1606(88)90261-8
43. Ross, C. & Boroviak, T. E. Origin and function of the yolk sac in primate embryogenesis. *Nature Communications* **11**, 3760 (2020). DOI: 10.1038/s41467-020-17575-w
44. Enders, A. C. & King, B. F. in *The human yolk sac and yolk sac tumors* (ed FF, N.) 33-47 (Springer, Berlin, Heidelberg, 1993).
45. Moore, K. L., Persaud, T. V. N. & Torchia, M. G. in *The developing human* 37-46 (Elsevier, Edinburgh, 2020). ISBN: 978-0-323-61154-1
46. Brawand, D., Wahli, W. & Kaessmann, H. Loss of egg yolk genes in mammals and the origin of lactation and placentation. *PLoS biology* **6**, e63 (2008). DOI: 10.1371/journal.pbio.0060063
47. O'Rahilly, R. & Müller, F. *Developmental stages in human embryos* (Carnegie Institution, Washington, 1987).
48. Su, R. W. & Fazleabas, A. T. Implantation and Establishment of Pregnancy in Human and Nonhuman Primates. *Adv Anat Embryol Cell Biol* **216**, 189-213 (2015). DOI: 10.1007/978-3-319-15856-3_10
49. Hermitte, S. & Chazaud, C. Primitive endoderm differentiation: from specification to epithelium formation. *Philos Trans R Soc Lond B Biol Sci* **369**, 20130537 (2014). DOI: 10.1098/rstb.2013.0537
50. Luckett, W. P. Origin and differentiation of the yolk sac and extraembryonic mesoderm in presomite human and rhesus monkey embryos. *Am J Anat* **152**, 59-97 (1978). DOI: 10.1002/aja.1001520106
51. Brown, K. *et al.* A comparative analysis of extra-embryonic endoderm cell lines. *PLoS One* **5**, e12016 (2010). DOI: 10.1371/journal.pone.0012016
52. Nakamura, T. *et al.* A developmental coordinate of pluripotency among mice, monkeys and humans. *Nature* **537**, 57-62 (2016). DOI: 10.1038/nature19096
53. Enders, A. C. & King, B. F. Formation and differentiation of extraembryonic mesoderm in the rhesus monkey. *Am J Anat* **181**, 327-340 (1988). DOI: 10.1002/aja.1001810402
54. Sadler, T. W. *Langman's medical embryology* (Williams and Wilkins, Baltimore, 1998). ISBN: 978-1496383907
55. O'Rahilly, R. & Müller, F. Developmental stages in human embryos: revised and new measurements. *Cells Tissues Organs* **192**, 73-84 (2010). DOI: 10.1159/000289817

56. Shi, W. K., Hopkins, B., Thompson, S., Heath, J. K. & Luke…, B. M. Synthesis of apolipoproteins, alphafoetoprotein, albumin, and transferrin by the human foetal yolk sack and other foetal organs. *journals.biologists.com* **85**, 191-206 (1985). DOI: 10.1242/dev.85.1.191
57. Enders, A. C. & King, B. F. in *The human yolk sac and yolk sac tumors* (ed Nogales, F. F.) 84-108 (Springer, Berlin, Heidelberg, 1993). DOI: 10.1007/978-3-642-77852-0_2
58. Jones, C. J. & Jauniaux, E. Ultrastructure of the materno-embryonic interface in the first trimester of pregnancy. *Micron* **26**, 145-173 (1995). DOI: 10.1016/0968-4328(95)00002-l
59. Goh, I. *et al.* Yolk sac cell atlas reveals multiorgan functions during human early development. *Science* **381**, eadd7564 (2023). DOI: 10.1126/science.add7564
60. Exalto, N. in *The human yolk sac and yolk sac tumors* (ed FF, N.) 126-134 (Springer, Berlin, Heidelberg, 1993). DOI: 10.1007/978-3-642-77852-0_2
61. Burton, G. J. & Jauniaux, E. Development of the Human Placenta and Fetal Heart: Synergic or Independent. *Front Physiol* **9**, 373 (2018). DOI: 10.3389/fphys.2018.00373
62. Kowalski, P. J. & Ziadie, M. S. Embryonic remnants. (Pathology Outlines.com, 2023). https://www.pathologyoutlines.com/topic/placentaembryonicremn.html
63. Julien, E., El Omar, R. & Tavian, M. Origin of the hematopoietic system in the human embryo. *FEBS Lett* **590**, 3987-4001 (2016). DOI: 10.1002/1873-3468.12389
64. Migliaccio, G. & Migliaccio, A. R. in *The human yolk sac and yolk sac tumors* (ed FF, N.) 70-83 (Springer-Verlag, Berlin-Heidelberg, 1993). DOI: 10.1007/978-3-642-77852-0_2
65. Stremmel, C. *et al.* Yolk sac macrophage progenitors traffic to the embryo during defined stages of development. *Nat Commun* **9**, 75 (2018). DOI: 10.1038/s41467-017-02492-2
66. Migliaccio, G. *et al.* Human embryonic hemopoiesis. Kinetics of progenitors and precursors underlying the yolk sac----liver transition. *J Clin Invest* **78**, 51-60 (1986). DOI: 10.1172/JCI112572
67. Böiers, C. *et al.* Lymphomyeloid contribution of an immune-restricted progenitor emerging prior to definitive hematopoietic stem cells. *Cell Stem Cell* **13**, 535-548 (2013). DOI: 10.1016/j.stem.2013.08.012
68. McGrath, K. E. *et al.* Distinct Sources of Hematopoietic Progenitors Emerge before HSCs and Provide Functional Blood Cells in the Mammalian Embryo. *Cell Rep* **11**, 1892-1904 (2015). DOI: 10.1016/j.celrep.2015.05.036
69. Atkins, M. H. *et al.* Modeling human yolk sac hematopoiesis with pluripotent stem cells. *J Exp Med* **219**, e20211924 (2022). DOI: 10.1084/jem.20211924
70. Tavian, M., Robin, C., Coulombel, L. & Péault, B. The human embryo, but not its yolk sac, generates lympho-myeloid stem cells: mapping multipotent hematopoietic cell fate in intraembryonic mesoderm. *Immunity* **15**, 487-495 (2001). DOI: 10.1016/s1074-7613(01)00193-5
71. Gomez Perdiguero, E. *et al.* Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* **518**, 547-551 (2015). DOI: 10.1038/nature13989
72. Liu, K. *et al.* In vivo analysis of dendritic cell development and homeostasis. *Science* **324**, 392-397 (2009). DOI: 10.1126/science.1170540
73. Mass, E. *et al.* Specification of tissue-resident macrophages during organogenesis. *Science* **353**, aaf4238 (2016). DOI: 10.1126/science.aaf4238
74. Schulz, C. *et al.* A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* **336**, 86-90 (2012). DOI: 10.1126/science.1219179
75. Gentek, R. *et al.* Hemogenic Endothelial Fate Mapping Reveals Dual Developmental Origin of Mast Cells. *Immunity* **48**, 1160-1171.e5 (2018). DOI: 10.1016/j.immuni.2018.04.025
76. Gentek, R. *et al.* Epidermal γδ T cells originate from yolk sac hematopoiesis and clonally self-renew in the adult. *J Exp Med* **215**, 2994-3005 (2018). DOI: 10.1084/jem.20181206

77. Bloom, W. & Bartelmez, G. W. Hematopoiesis in young human embryos. *American Journal of Anatomy* **67**, 21-53 (1940). DOI: 10.1002/aja.1000670103
78. Peschle, C. *et al.* Haemoglobin switching in human embryos: asynchrony of ζ → α and ε → γ-globin switches in primitive and definitive erythropoietic lineage. *Nature* **313**, 235-238 (1985). DOI: 10.1038/313235a0
79. Bian, Z. *et al.* Deciphering human macrophage development at single-cell resolution. *Nature* **582**, 571-576 (2020). DOI: 10.1038/s41586-020-2316-7
80. Hempstock, J., Cindrova-Davies, T., Jauniaux, E. & Burton, G. J. Endometrial glands as a source of nutrients, growth factors and cytokines during the first trimester of human pregnancy: a morphological and immunohistochemical study. *Reprod Biol Endocrinol* **2**, 58 (2004). DOI: 10.1186/1477-7827-2-58
81. Hustin, J. & Schaaps, J. P. Echographic [corrected] and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy. *Am J Obstet Gynecol* **157**, 162-168 (1987). DOI: 10.1016/s0002-9378(87)80371-x
82. Jauniaux, E. *et al.* Onset of maternal arterial blood flow and placental oxidative stress: a possible factor in human early pregnancy failure. *The American journal of pathology* **157**, 2111-2122 (2000). DOI: 10.1016/S0002-9440(10)64849-3
83. Rodesch, F., Simon, P., Donner, C. & Jauniaux, E. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. *Obstet Gynecol* **80**, 283-285 (1992). PMID: 1635745
84. Schaaps, J. P. & Hustin, J. In vivo aspect of the maternal—trophoblastic border during the first trimester of gestation. *Placental Vascularization and Blood Flow: Basic Research and Clinical Applications* 39-48 (1988). DOI: 10.1007/978-1-4615-8109-3_3
85. Burton, G. J., Charnock-Jones, D. S. & Jauniaux, E. Regulation of vascular growth and function in the human placenta. *Reproduction* **138**, 895-902 (2009). DOI: 10.1530/REP-09-0092
86. HAMILTON, W. J. & BOYD, J. D. Development of the human placenta in the first three months of gestation. *J Anat* **94**, 297-328 (1960). PMID: 14399291
87. Foidart, J. M., Hustin, J., Dubois, M. & Schaaps, J. P. The human placenta becomes haemochorial at the 13th week of pregnancy. *Int. J. Dev. Biol.* **36**, 451-453 (1992). DOI: 10.1387/ijdb.1445791
88. Harris, J. W. S. & Ramsey, E. M. The morphology of human uteroplacental vasculature. *Contr. Embryol.* **38**, 43-58 (1966).
89. Burton, G. J., Jauniaux, E. & Watson, A. L. Maternal arterial connections to the placental intervillous space during the first trimester of human pregnancy: the Boyd collection revisited. *Am J Obstet Gynecol* **181**, 718-724 (1999). DOI: 10.1016/s0002-9378(99)70518-1
90. Roberts, V. H. J. *et al.* Early first trimester uteroplacental flow and the progressive disintegration of spiral artery plugs: new insights from contrast-enhanced ultrasound and tissue histopathology. *Hum Reprod* **32**, 2382-2393 (2017). DOI: 10.1093/humrep/dex301
91. Gray, C. A. *et al.* Endometrial glands are required for preimplantation conceptus elongation and survival. *Biol Reprod* **64**, 1608-1613 (2001). DOI: 10.1095/biolreprod64.6.1608
92. Armstrong, E. M., More, I. A., McSeveny, D. & Chatfield, W. R. Reappraisal of the ultrastructure of the human endometrial glandular cell. *J Obstet Gynaecol Br Commonw* **80**, 446-460 (1973). DOI: 10.1111/j.1471-0528.1973.tb15961.x
93. Burton, G. J. Review article. Placental uptake of maternal erythrocytes: a comparative study. *Placenta* **3**, 407-434 (1982). DOI: 10.1016/s0143-4004(82)80033-7

94. Burton, G. J., Jauniaux, E. & Murray, A. J. Oxygen and placental development; parallels and differences with tumour biology. *Placenta* **56**, 14-18 (2017). DOI: 10.1016/j.placenta.2017.01.130
95. King, B. F. Absorption of peroxidase-conjugated immunoglobulin G by human placenta: an in vitro study. *Placenta* **3**, 395-406 (1982). DOI: 10.1016/s0143-4004(82)80032-5
96. Jones, C. J. P., The life and death of the embryonic yolk sac; in *Embryonic medicine and therapy* (eds Jauniaux, E., Barnea, E. R. & Edwards, R. G.) 180-196 (Oxford University Press, 1997). ISBN: 9780192627292
97. Burton, G. J., Hempstock, J. & Jauniaux, E. Nutrition of the human fetus during the first trimester—a review. *Placenta* **22 Suppl A**, S70-7 (2001). DOI: 10.1053/plac.2001.0639
98. Gonzalez-Crussi, F. & Roth, L. M. The human yolk sac and yolk sac carcinoma. An ultrastructural study. *Hum Pathol* **7**, 675-691 (1976). DOI: 10.1016/s0046-8177(76)80079-2
99. Zohn, I. E. & Sarkar, A. A. The visceral yolk sac endoderm provides for absorption of nutrients to the embryo during neurulation. *Birth Defects Res A Clin Mol Teratol* **88**, 593-600 (2010). DOI: 10.1002/bdra.20705
100. Mäkikallio, K., Tekay, A. & Jouppila, P. Yolk sac and umbilicoplacental hemodynamics during early human embryonic development. *Ultrasound Obstet Gynecol* **14**, 175-179 (1999). DOI: 10.1046/j.1469-0705.1999.14030175.x
101. Takashina, T. in *The human yolk sac and yolk sac tumors* (ed Nogales, F.F.) 50-69 1993). DOI: 10.1007/978-3-642-77852-0_2
102. Hesseldahl, H. & Larsen, J. F. Ultrastructure of human yolk sac: endoderm, mesenchyme, tubules and mesothelium. *Am J Anat* **126**, 315-335 (1969). DOI: 10.1002/aja.1001260306
103. Enders, A. C., Schlafke, S. & Hendrickx, A. G. Differentiation of the embryonic disc, amnion, and yolk sac in the rhesus monkey. *Am J Anat* **177**, 161-185 (1986). DOI: 10.1002/aja.1001770205
104. Vito, T. *et al.* Cysteine cathepsins: From structure, function and regulation to new frontiers. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1824**, 68-88 (2012). DOI: 10.1016/j.bbapap.2011.10.002
105. Cindrova-Davies, T. *et al.* RNA-seq reveals conservation of function among the yolk sacs of human, mouse, and chicken. *Proc Natl Acad Sci U S A* **114**, E4753-E4761 (2017). DOI: 10.1073/pnas.1702560114
106. Beckman, D. A., Brent, R. L. & Lloyd, J. B. Sources of amino acids for protein synthesis during early organogenesis in the rat. 4. Mechanisms before envelopment of the embryo by the yolk sac. *Placenta* **17**, 635-641 (1996). DOI: 10.1016/s0143-4004(96)80082-8
107. Gulbis, B., Jauniaux, E., Cotton, F. & Stordeur, P. Protein and enzyme patterns in the fluid cavities of the first trimester gestational sac: relevance to the absorptive role of secondary yolk sac. *Mol Hum Reprod* **4**, 857-862 (1998). DOI: 10.1093/molehr/4.9.857
108. Docherty, S. M., Iles, R. K., Wathen, N. & Chard, T. The temporary anatomical structures prominent in the first trimester may be fulfilling exchange functions assigned to the placenta in the second and third trimester. *Hum Reprod* **11**, 1157-1161 (1996). DOI: 10.1093/oxfordjournals.humrep.a019346
109. Baardman, M. E. *et al.* The role of maternal-fetal cholesterol transport in early fetal life: current insights. *Biol Reprod* **88**, 24 (2013). DOI: 10.1095/biolreprod.112.102442
110. Woollett, L. A. & Heubi, J. E. Fetal and Neonatal Cholesterol Metabolism. *Endotext* (2020). https://www.ncbi.nlm.nih.gov/books/NBK395580/
111. Christenson, L. K. & Devoto, L. Cholesterol transport and steroidogenesis by the corpus luteum. *Reprod Biol Endocrinol* **1**, 90 (2003). DOI: 10.1186/1477-7827-1-90

112. Lanford, R. E., Bronson, D. L., Estlack, L. E. & Wians, F. H. Plasma protein and apolipoprotein synthesis by human yolk sac carcinoma cells in vitro. *In Vitro Cell Dev Biol* **27A**, 205-210 (1991). DOI: 10.1007/BF02630917
113. Martemucci, G. *et al.* Free radical properties, source and targets, antioxidant consumption and health. *Oxygen* **2**, 48-78 (2022). DOI: 10.3390/oxygen2020006
114. Jauniaux, E. *et al.* Distribution and transfer pathways of antioxidant molecules inside the first trimester human gestational sac. *J Clin Endocrinol Metab* **89**, 1452-1458 (2004). DOI: 10.1210/jc.2003-031332
115. Burton, G. J., Hempstock, J. & Jauniaux, E. Oxygen, early embryonic metabolism and free radical-mediated embryopathies. *Reprod Biomed Online* **6**, 84-96 (2003). DOI: 10.1016/s1472-6483(10)62060-3
116. Ornoy, A., Zaken, V. & Kohen, R. Role of reactive oxygen species (ROS) in the diabetes-induced anomalies in rat embryos in vitro: reduction in antioxidant enzymes and low-molecular-weight antioxidants (LMWA) may be the causative factor for increased anomalies. *Teratology* **60**, 376-386 (1999). DOI: 10.1002/(SICI)1096-9926(199912)60:6<376::AID-TERA10>3.0.CO;2-Q
117. Kawasaki, M., Arata, N. & Ogawa, Y. Obesity and abnormal glucose tolerance in the offspring of mothers with diabetes. *Curr Opin Obstet Gynecol* **30**, 361-368 (2018). DOI: 10.1097/GCO.0000000000000479
118. Kong, L., Chen, X., Gissler, M. & Lavebratt, C. Relationship of prenatal maternal obesity and diabetes to offspring neurodevelopmental and psychiatric disorders: a narrative review. *Int J Obes (Lond)* **44**, 1981-2000 (2020). DOI: 10.1038/s41366-020-0609-4
119. Wu, Y. *et al.* Association of Maternal Prepregnancy Diabetes and Gestational Diabetes Mellitus With Congenital Anomalies of the Newborn. *Diabetes Care* **43**, 2983-2990 (2020). DOI: 10.2337/dc20-0261
120. Russo, M., Forte, G., Montanino Oliva, M., Laganà, A. S. & Unfer, V. Melatonin and Myo-Inositol: Supporting Reproduction from the Oocyte to Birth. *Int J Mol Sci* **22**, 8433 (2021). DOI: 10.3390/ijms22168433
121. Carlomagno, G., Minini, M., Tilotta, M. & Unfer, V. From Implantation to Birth: Insight into Molecular Melatonin Functions. *Int J Mol Sci* **19**, 2802 (2018). DOI: 10.3390/ijms19092802
122. Mahal, H. S., Sharma, H. S. & Mukherjee, T. Antioxidant properties of melatonin: a pulse radiolysis study. *Free Radic Biol Med* **26**, 557-565 (1999). DOI: 10.1016/s0891-5849(98)00226-3
123. Carlomagno, G., Minini, M., Tilotta, M. & Unfer, V. From implantation to birth: Insight into molecular melatonin functions. *International Journal of Molecular Sciences* **19**, 2802 (2018). DOI: 10.3390/ijms19092802
124. Cipolla-Neto, J. & Amaral, F. G. D. Melatonin as a Hormone: New Physiological and Clinical Insights. *Endocrine Reviews* **39**, 990-1028 (2018). DOI: 10.1210/er.2018-00084
125. Ferrari, C. K., Souto, P. C., França, E. L. & Honorio-França, A. C. Oxidative and nitrosative stress on phagocytes' function: from effective defense to immunity evasion mechanisms. *Arch Immunol Ther Exp (Warsz)* **59**, 441-448 (2011). DOI: 10.1007/s00005-011-0144-z
126. Stone, J. R. & Yang, S. Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal* **8**, 243-270 (2006). DOI: 10.1089/ars.2006.8.243
127. Ward, S. J., Chambon, P., Ong, D. E. & Båvik, C. A retinol-binding protein receptor-mediated mechanism for uptake of vitamin A to postimplantation rat embryos. *Biol Reprod* **57**, 751-755 (1997). DOI: 10.1095/biolreprod57.4.751
128. Lillemoen, P. K. S. & Bjørke-Monsen, A. L. Nutritional status of vitamins and trace elements. *Tidsskr Nor Laegeforen* **140**, (2020). DOI: 10.4045/tidsskr.19.0587

129. Wintergerst, E. S., Maggini, S. & Hornig, D. H. Contribution of selected vitamins and trace elements to immune function. *Ann Nutr Metab* **51**, 301-323 (2007). DOI: 10.1159/000107673
130. Moestrup, S. K. *et al.* Megalin-mediated endocytosis of transcobalamin-vitamin-B12 complexes suggests a role of the receptor in vitamin-B12 homeostasis. *Proc Natl Acad Sci U S A* **93**, 8612-8617 (1996). DOI: 10.1073/pnas.93.16.8612
131. Stover, P. J. Physiology of folate and vitamin B12 in health and disease. *Nutr Rev* **62**, S3-12; discussion S13 (2004). DOI: 10.1111/j.1753-4887.2004.tb00070.x
132. Saitoh, K., Harada, H., Harada, K. & Inoue, K. An immunofluorescent study of α-fetoprotein. *European Surgical Research* **9**, 113-121 (1977). ISBN: 0014-312X
133. Takashina, T. *et al.* Yolk sac tumors of the ovary and the human yolk sac. *Am J Obstet Gynecol* **156**, 223-229 (1987). DOI: 10.1016/0002-9378(87)90242-0
134. Gitlin, D. & Perricelli, A. Synthesis of serum albumin, prealbumin, alpha-foetoprotein, alpha-1-antitrypsin and transferrin by the human yolk sac. *Nature* **228**, 995-997 (1970). DOI: 10.1038/228995a0
135. Jacobsen, G. K., Jacobsen, M. & Henriksen, O. B. An immunohistochemical study of a series of plasma proteins in the early human conceptus. *Oncodev Biol Med* **2**, 399-410 (1981). PMID: 7050933
136. Jauniaux, E., Jurkovic, D., Henriet, Y., Rodesch, F. & Hustin, J. Development of the secondary human yolk sac: correlation of sonographic and anatomical features. *Hum Reprod* **6**, 1160-1166 (1991). DOI: 10.1093/oxfordjournals.humrep.a137503
137. Mizejewski, G. J. Protein binding and interactions with alpha-fetoprotein (AFP): a review of multiple AFP cell surface receptors, intracytoplasmic binding, and inter-molecular complexing proteins. *J Mol Cell Biol Forecast. 2019; 2 (1)* **1016**, (2019). https://scienceforecastoa.com/Articles/JMCBF-V2-E1-1016.pdf
138. Taketa, K. *et al.* Datura stramonium agglutinin-reactive alpha-fetoprotein isoforms in hepatocellular carcinoma and other tumors. *Tumour Biol* **11**, 220-228 (1990). DOI: 10.1159/000217658
139. Xue, J. *et al.* Incomplete embryonic lethality and fatal neonatal hemorrhage caused by prothrombin deficiency in mice. *Proc Natl Acad Sci U S A* **95**, 7603-7607 (1998). DOI: 10.1073/pnas.95.13.7603
140. Fuss, A. Ueber extraregionare Geschlechtszellen bei einem menschlichen Embryo von 4 Wochen. *Anat Am.* **39**, 407-409 (1911).
141. Kobayashi, T. *et al.* Principles of early human development and germ cell program from conserved model systems. *Nature* **546**, 416-420 (2017). DOI: 10.1038/nature22812
142. Irie, N. *et al.* SOX17 is a critical specifier of human primordial germ cell fate. *Cell* **160**, 253-268 (2015). DOI: 10.1016/j.cell.2014.12.013
143. Mantoni, M. & Pedersen, J. F. Ultrasound visualization of the human yolk sac. *Journal of Clinical Ultrasound* **7**, 459-460 (1979). DOI: 10.1002/jcu.1870070608
144. Crooij, M. J., Westhuis, M., Schoemaker, J. & Exalto, N. Ultrasonographic measurement of the yolk sac. *Br J Obstet Gynaecol* **89**, 931-934 (1982). DOI: 10.1111/j.1471-0528.1982.tb05060.x
145. Timor-Tritsch, I. E., Farine, D. & Rosen, M. G. A close look at early embryonic development with the high-frequency transvaginal transducer. *Am J Obstet Gynecol* **159**, 676-681 (1988). DOI: 10.1016/s0002-9378(88)80033-4
146. Ferrazzi, E. *et al.* The yolk sac in early pregnancy failure. *Am J Obstet Gynecol* **158**, 137-142 (1988). DOI: 10.1016/0002-9378(88)90796-x
147. Detti, L. *et al.* Early pregnancy ultrasound measurements and prediction of first trimester pregnancy loss: A logistic model. *Sci Rep* **10**, 1545 (2020). DOI: 10.1038/s41598-020-58114-3 10.1023/A:1009497809176

148. Blaas, H. G., Eik-Nes, S. H. & Bremnes, J. B. The growth of the human embryo. A longitudinal biometric assessment from 7 to 12 weeks of gestation. *Ultrasound Obstet Gynecol* **12**, 346-354 (1998). DOI: 10.1046/j.1469-0705.1998.12050346.x
149. Papaioannou, G. I., Syngelaki, A., Poon, L. C., Ross, J. A. & Nicolaides, K. H. Normal ranges of embryonic length, embryonic heart rate, gestational sac diameter and yolk sac diameter at 6-10 weeks. *Fetal Diagn Ther* **28**, 207-219 (2010). DOI: 10.1159/000319589
150. Cepni, I. *et al.* Significance of yolk sac measurements with vaginal sonography in the first trimester in the prediction of pregnancy outcome. *Acta Obstet Gynecol Scand* **76**, 969-972 (1997). DOI: 10.3109/00016349709034911
151. Odland Karlsen, H. *et al.* The human yolk sac size reflects involvement in embryonic and fetal growth regulation. *Acta Obstet Gynecol Scand* **98**, 176-182 (2018). DOI: 10.1111/aogs.13466
152. Schmidt, P. *et al.* Pitfalls of ultrasonographic yolk sac measurement. *Ultraschall Med* **32 Suppl 2**, E147-50 (2011). DOI: 10.1055/s-0031-1281648
153. Rôlo, L. C., Nardozza, L. M., Araujo Júnior, E., Nowak, P. M. & Moron, A. F. Yolk sac volume assessed by three-dimensional ultrasonography using the VOCAL method. *Acta Obstet Gynecol Scand* **87**, 499-502 (2008). DOI: 10.1080/00016340802011595
154. Kupesic, S., Kurjak, A. & Ivancić-Kosuta, M. Volume and vascularity of the yolk sac studied by three-dimensional ultrasound and color Doppler. *J Perinat Med* **27**, 91-96 (1999). DOI: 10.1515/JPM.1999.010
155. Cosmi, E. *et al.* Structural-tridimensional study of yolk sac in pregnancies complicated by diabetes. *J Perinat Med* **33**, 132-136 (2005). DOI: 10.1515/JPM.2005.025
156. Bagratee, J. S., Regan, L., Khullar, V., Connolly, C. & Moodley, J. Reference intervals of gestational sac, yolk sac and embryo volumes using three-dimensional ultrasound. *Ultrasound in Obstetrics and Gynecology* **34**, 503-509 (2009). DOI: 10.1002/uog.7348
157. Figueras, F. *et al.* Three-dimensional yolk and gestational sac volume. A prospective study of prognostic value. *J Reprod Med* **48**, 252-256 (2003). PMID: 12746989
158. Ouyang, Y., Qin, J., Lin, G., Xiang, S. & Li, X. Reference intervals of gestational sac, yolk sac, embryonic length, embryonic heart rate at 6-10 weeks after in vitro fertilization-embryo transfer. *BMC Pregnancy Childbirth* **20**, 533 (2020). DOI: 10.1186/s12884-020-03186-2 10.1016/j.rbmo.2017.07.015
159. Detti, L. *et al.* Pilot study establishing a nomogram of yolk sac growth during the first trimester of pregnancy. *J Obstet Gynaecol Res* **46**, 223-228 (2020). DOI: 10.1111/jog.14173
160. Lindsay, D. J. *et al.* Yolk sac diameter and shape at endovaginal US: predictors of pregnancy outcome in the first trimester. *Radiology* **183**, 115-118 (1992). DOI: 10.1148/radiology.183.1.1549656
161. Angiolucci, M., Murru, R., Melis, G., Carcassi, C. & Mais, V. Association between different morphological types and abnormal karyotypes in early pregnancy loss. *Ultrasound Obstet Gynecol* **37**, 219-225 (2011). DOI: 10.1002/uog.7681
162. Cho, F. N., Chen, S. N., Tai, M. H. & Yang, T. L. The quality and size of yolk sac in early pregnancy loss. *Aust N Z J Obstet Gynaecol* **46**, 413-418 (2006). DOI: 10.1111/j.1479-828X.2006.00627.x
163. Moradan, S. & Forouzeshfar, M. Are abnormal yolk sac characteristics important factors in abortion rates. *Int J Fertil Steril* **6**, 127-130 (2012). PMID: 25493170
164. Datta, M. R. & Raut, A. Efficacy of first-trimester ultrasound parameters for prediction of early spontaneous abortion. *Int J Gynaecol Obstet* **138**, 325-330 (2017). DOI: 10.1002/ijgo.12231
165. Suguna, B. & Sukanya, K. Yolk sac size & shape as predictors of first trimester pregnancy outcome: A prospective observational study. *J Gynecol Obstet Hum Reprod* **48**, 159-164 (2019). DOI: 10.1016/j.jogoh.2018.10.016

166. Moradan, S. & Forouzeshfar, M. Are abnormal yolk sac characteristics important factors in abortion rates. *Int J Fertil Steril* **6**, 127-130 (2012). PMID: 25493170
167. Kurban, Y., Uyar, I., Alan, M. & Hacifazlioglu, C. Fetal sex prediction measuring yolk sac size and yolk sac-fetal pole distance in the first trimester via ultrasound screening. *J Ultrasound* **24**, 489-492 (2021). DOI: 10.1007/s40477-020-00516-0
168. Brambati, B. & Lanzani, A. A clinical look at early post-implantation pregnancy failure. *Hum Reprod* **2**, 401-405 (1987). DOI: 10.1093/oxfordjournals.humrep.a136558
169. Papaioannou, G. K., Syngelaki, A., Maiz, N., Ross, J. A. & Nicolaides, K. H. Sonographic markers of aneuploidies at 6-10 weeks of gestation. *Early Hum Dev* **87**, 453-456 (2011). DOI: 10.1016/j.earlhumdev.2011.01.045
170. Huang, J. *et al.* Do specific ultrasonography features identified at the time of early pregnancy loss predict fetal chromosomal abnormality? - A systematic review and meta-analysis. *Genes Dis* **6**, 129-137 (2019). DOI: 10.1016/j.gendis.2018.10.001
171. Ivanisevic, M., Djelmis, J., Jalsovec, D. & Bljajic, D. Ultrasonic morphological characteristics of yolk sac in pregnancy complicated with type-1 diabetes mellitus. *Gynecol Obstet Invest* **61**, 80-86 (2006). DOI: 10.1159/000088933
172. Papaioannou, G. I., Syngelaki, A., Maiz, N., Ross, J. A. & Nicolaides, K. H. Yolk sac diameter in early pregnancy in maternal diabetes mellitus. *Gynecol Obstet Invest* **73**, 16-20 (2012). DOI: 10.1159/000328690
173. Dong, D. *et al.* New development of the yolk sac theory in diabetic embryopathy: molecular mechanism and link to structural birth defects. *Am J Obstet Gynecol* **214**, 192-202 (2016). DOI: 10.1016/j.ajog.2015.09.082
174. Pinter, E. *et al.* Yolk sac failure in embryopathy due to hyperglycemia: ultrastructural analysis of yolk sac differentiation associated with embryopathy in rat conceptuses under hyperglycemic conditions. *Teratology* **33**, 73-84 (1986). DOI: 10.1002/tera.1420330110
175. Reece, E. A. *et al.* Yolk sac failure in embryopathy due to hyperglycemia: horseradish peroxidase uptake in the assessment of yolk sac function. *Obstet Gynecol* **74**, 755-762 (1989). PMID: 2812653
176. Schoenfeld, A. *et al.* Yolk sac concentration of prostaglandin E2 in diabetic pregnancy: further clues to the etiology of diabetic embryopathy. *Prostaglandins* **50**, 121-126 (1995). DOI: 10.1016/0090-6980(95)00084-4
177. Schoenfeld, A., Warchaizer, S., Erman, A. & Hod, M. Prostaglandin metabolism in the yolk sacs of normal and diabetic pregnancies. *Early Pregnancy* **2**, 129-132 (1996). PMID: 9363210
178. Reece, E. A., Pinter, E., Homko, C., Wu, Y. K. & Naftolin, F. The yolk sac theory: closing the circle on why diabetes-associated malformations occur. *J Soc Gynecol Investig* **1**, 3-13 (1994). PMID: 9419739
179. Rasouli, M. Basic concepts and practical equations on osmolality: Biochemical approach. *Clin Biochem* **49**, 936-941 (2016). DOI: 10.1016/j.clinbiochem.2016.06.001
180. Jauniaux, E. *et al.* Relationship between protein concentrations in embryological fluids and maternal serum and yolk sac size during human early pregnancy. *Hum Reprod* **9**, 161-166 (1994). DOI: 10.1093/oxfordjournals.humrep.a138308
181. Watkins, A. J. *et al.* Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. *Biol Reprod* **78**, 299-306 (2008). DOI: 10.1095/biolreprod.107.064220
182. Tavian, M., Hallais, M. F. & Péault, B. Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo. *Development* **126**, 793-803 (1999). DOI: 10.1242/dev.126.4.793

183. Hill, M. A. UNSW Embryology. (2020). https://embryology.med.unsw.edu.au/embryology/index.php/Yolk_Sac_Development
184. Popescu, D. M. *et al.* Decoding human fetal liver haematopoiesis. *Nature* **574**, 365-371 (2019). DOI: 10.1038/s41586-019-1652-y
185. Gitlin, D. Normal biology of alpha-fetoprotein. *Ann N Y Acad Sci* **259**, 7-16 (1975). DOI: 10.1111/j.1749-6632.1975.tb25397.x
186. Ivanovs, A. *et al.* Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. *J Exp Med* **208**, 2417-2427 (2011). DOI: 10.1084/jem.20111688
187. Moore, K. L., Persaud, T. V. N. & Torchia, M. G. in *The developing human* 99-130 (Elsevier, Edinburgh, 2020). ISBN: 978-0-323-61154-1
188. Calvanese, V. *et al.* Mapping human haematopoietic stem cells from haemogenic endothelium to birth. *Nature* **604**, 534-540 (2022). DOI: 10.1038/s41586-022-04571-x
189. Godlewski, G., Gaubert-Cristol, R., Rouy, S. & Prudhomme, M. Liver development in the rat and in man during the embryonic period (Carnegie stages 11–23). *Microscopy research and technique* **39**, 314-327 (1997). DOI: 10.1002/(SICI)1097-0029(19971115)39:4<314::AID-JEMT2>3.0.CO;2-H
190. Müller, F. & O'Rahilly, R. The human brain at stages 18-20, including the choroid plexuses and the amygdaloid and septal nuclei. *Anat Embryol (Berl)* **182**, 285-306 (1990). DOI: 10.1007/BF00185521
191. Francis, P. *et al.* Measurement of muscle health in aging. *Biogerontology* **18**, 901-911 (2017). DOI: 10.1007/s10522-017-9697-5
192. Peterson, S. J. & Braunschweig, C. A. Prevalence of Sarcopenia and Associated Outcomes in the Clinical Setting. *Nutr Clin Pract* **31**, 40-48 (2016). DOI: 10.1177/0884533615622537
193. Mraz, M. & Haluzik, M. The role of adipose tissue immune cells in obesity and low-grade inflammation. *J Endocrinol* **222**, R113-27 (2014). DOI: 10.1530/JOE-14-0283
194. Most, J., Marlatt, K. L., Altazan, A. D. & Redman, L. M. Advances in assessing body composition during pregnancy. *Eur J Clin Nutr* **72**, 645-656 (2018). DOI: 10.1038/s41430-018-0152-8
195. Rasmussen, K. M. & Yaktine, A. L. (eds) *Weight Gain During Pregnancy: Reexamining the Guidelines*. (National Academies Press (US), Washington (DC), 2009). DOI: 10.17226/12584
196. Bosaeus, M. *et al.* Body Composition During Pregnancy: Longitudinal Changes and Method Comparisons. *Reprod Sci* **27**, 1477-1489 (2020). DOI: 10.1007/s43032-020-00141-6 10.1016
197. Widen, E. M. & Gallagher, D. Body composition changes in pregnancy: measurement, predictors and outcomes. *Eur J Clin Nutr* **68**, 643-652 (2014). DOI: 10.1038/ejcn.2014.40
198. Lemos, T. & Gallagher, D. Current body composition measurement techniques. *Curr Opin Endocrinol Diabetes Obes* **24**, 310-314 (2017). DOI: 10.1097/MED.0000000000000360
199. Marshall, N. E. *et al.* Comparison of multiple methods to measure maternal fat mass in late gestation. *Am J Clin Nutr* **103**, 1055-1063 (2016). DOI: 10.3945/ajcn.115.113464
200. Lof, M. & Forsum, E. Evaluation of bioimpedance spectroscopy for measurements of body water distribution in healthy women before, during, and after pregnancy. *J Appl Physiol (1985)* **96**, 967-973 (2004). DOI: 10.1152/japplphysiol.00900.2003
201. International Atomic Energy Agency. *Introduction to body composition assessment using the deuterium dilution technique with analysis of saliva samples by Fourier transform infrared spectrometry.* (IAEA, Vienna, 2010). https://www-pub.iaea.org/MTCD/Publications/PDF/Pub1450_web.pdf

202. International Atomic Energy Agency. *Assessment of Body Composition and Total Energy Expenditure in Humans Using Stable Isotope Techniques* (IAEA, Vienna, 2009). https://www-pub.iaea.org/MTCD/Publications/PDF/Pub1370_web.pdf
203. Taicher, G. Z., Tinsley, F. C., Reiderman, A. & Heiman, M. L. Quantitative magnetic resonance (QMR) method for bone and whole-body-composition analysis. *Anal Bioanal Chem* **377**, 990-1002 (2003). DOI: 10.1007/s00216-003-2224-3
204. Bosy-Westphal, A. & Müller, M. J. Assessment of fat and lean mass by quantitative magnetic resonance: a future technology of body composition research. *Curr Opin Clin Nutr Metab Care* **18**, 446-451 (2015). DOI: 10.1097/MCO.0000000000000201
205. Gallagher, D. *et al.* Quantitative magnetic resonance fat measurements in humans correlate with established methods but are biased. *Obesity (Silver Spring)* **18**, 2047-2054 (2010). DOI: 10.1038/oby.2010.97
206. van Raaij, J. M., Peek, M. E., Vermaat-Miedema, S. H., Schonk, C. M. & Hautvast, J. G. New equations for estimating body fat mass in pregnancy from body density or total body water. *The American Journal of Clinical Nutrition* **48**, 24-29 (1988). DOI: 10.1093/ajcn/48.1.24
207. Hopkinson, J. M. *et al.* Body fat estimation in late pregnancy and early postpartum: comparison of two-, three-, and four-component models. *Am J Clin Nutr* **65**, 432-438 (1997). DOI: 10.1093/ajcn/65.2.432
208. Kopp-Hoolihan, L. E., van Loan, M. D., Wong, W. W. & King, J. C. Fat mass deposition during pregnancy using a four-component model. *J Appl Physiol (1985)* **87**, 196-202 (1999). DOI: 10.1152/jappl.1999.87.1.196
209. Heude, B. *et al.* Pre-pregnancy body mass index and weight gain during pregnancy: relations with gestational diabetes and hypertension, and birth outcomes. *Matern Child Health J* **16**, 355-363 (2012). DOI: 10.1007/s10995-011-0741-9
210. Liu, Y. *et al.* The Body Composition in Early Pregnancy is Associated with the Risk of Development of Gestational Diabetes Mellitus Late During the Second Trimester. *Diabetes Metab Syndr Obes* **13**, 2367-2374 (2020). DOI: 10.2147/DMSO.S245155
211. Zhang, R. Y. *et al.* Measuring maternal body composition by biomedical impedance can predict risk for gestational diabetes mellitus: a retrospective study among 22,223 women. *J Matern Fetal Neonatal Med* 1-8 (2020). DOI: 10.1080/14767058.2020.1797666
212. Henriksson, P. *et al.* Associations of body composition and physical fitness with gestational diabetes and cardiovascular health in pregnancy: Results from the HealthyMoms trial. *Nutr Diabetes* **11**, 16 (2021). DOI: 10.1038/s41387-021-00158-z 10.1161
213. Sween, L. K., Althouse, A. D. & Roberts, J. M. Early-pregnancy percent body fat in relation to preeclampsia risk in obese women. *Am J Obstet Gynecol* **212**, 84.e1-7 (2015). DOI: 10.1016/j.ajog.2014.07.055
214. Trindade, C. R., Torloni, M. R., Mattar, R. & Sun, S. Y. Good performance of bioimpedance in early pregnancy to predict preeclampsia. *Pregnancy Hypertens* **26**, 24-30 (2021). DOI: 10.1016/j.preghy.2021.08.115
215. Goldstein, R. F. *et al.* Association of Gestational Weight Gain With Maternal and Infant Outcomes: A Systematic Review and Meta-analysis. *JAMA* **317**, 2207-2225 (2017). DOI: 10.1001/jama.2017.3635
216. Stotland, N. E., Hopkins, L. M. & Caughey, A. B. Gestational weight gain, macrosomia, and risk of cesarean birth in nondiabetic nulliparas. *Obstet Gynecol* **104**, 671-677 (2004). DOI: 10.1097/01.AOG.0000139515.97799.f6
217. Kent, E. *et al.* Correlation between birth weight and maternal body composition. *Obstet Gynecol* **121**, 46-50 (2013). DOI: 10.1097/aog.0b013e31827a0052

218. Wanjohi, M. N., Ogada, I., Wekesah, F. M., Khayeka-Wandabwa, C. & Kimani-Murage, E. W. Relationship between maternal body composition during pregnancy and infant's birth weight in Nairobi informal settlements, Kenya. *BMJ Nutr Prev Health* **3**, 151-161 (2020). DOI: 10.1136/bmjnph-2019-000060 10.1136
219. Kiserud, T. *et al.* The World Health Organization Fetal Growth Charts: A Multinational Longitudinal Study of Ultrasound Biometric Measurements and Estimated Fetal Weight. *PLoS Med* **14**, e1002220 (2017). DOI: 10.1371/journal.pmed.1002220
220. O'Brien, C. M., Louise, J., Deussen, A., Grivell, R. & Dodd, J. M. The effect of maternal obesity on fetal biometry, body composition, and growth velocity. *J Matern Fetal Neonatal Med* **33**, 2216-2226 (2020). DOI: 10.1080/14767058.2018.1543658
221. Nilsen, T. S., Magnus, P. & Ørstavik, R. Maternal body composition in relation to twinning. *Norsk Epidemiologi* (2016). DOI: 10.5324/nje.v26i1-2.2019
222. Gale, C. R. *et al.* Maternal size in pregnancy and body composition in children. *J Clin Endocrinol Metab* **92**, 3904-3911 (2007). DOI: 10.1210/jc.2007-0088
223. Fleming, T. P. *et al.* Origins of lifetime health around the time of conception: causes and consequences. *Lancet* **391**, 1842-1852 (2018). DOI: 10.1016/S0140-6736(18)30312-X
224. Isganaitis, E. Developmental Programming of Body Composition: Update on Evidence and Mechanisms. *Curr Diab Rep* **19**, 60 (2019). DOI: 10.1007/s11892-019-1170-1
225. Omaña-Guzmán, L. I. *et al.* Association of pre-pregnancy body mass index and rate of weight gain during pregnancy with maternal indicators of cardiometabolic risk. *Nutrition & Diabetes* **11**, 36 (2021). DOI: 10.1038/s41387-021-00178-9
226. Syböck, K., Hartmann, B. & Kirchengast, S. Maternal Prepregnancy Obesity Affects Foetal Growth, Birth Outcome, Mode of Delivery, and Miscarriage Rate in Austrian Women. *Int J Environ Res Public Health* **20**, 4139 (2023). DOI: 10.3390/ijerph20054139
227. Forsum, E., Löf, M., Olausson, H. & Olhager, E. Maternal body composition in relation to infant birth weight and subcutaneous adipose tissue. *Br J Nutr* **96**, 408-414 (2006). DOI: 10.1079/bjn20061828
228. Harvey, N. C. *et al.* Parental determinants of neonatal body composition. *J Clin Endocrinol Metab* **92**, 523-526 (2007). DOI: 10.1210/jc.2006-0456
229. Spann, M. N. *et al.* Association of Maternal Prepregnancy Body Mass Index With Fetal Growth and Neonatal Thalamic Brain Connectivity Among Adolescent and Young Women. *JAMA Netw Open* **3**, e2024661 (2020). DOI: 10.1001/jamanetworkopen.2020.24661
230. Champion, M. L. & Harper, L. M. Gestational Weight Gain: Update on Outcomes and Interventions. *Curr Diab Rep* **20**, 11 (2020). DOI: 10.1007/s11892-020-1296-1
231. Zielinski, M. R., McKenna, J. T. & McCarley, R. W. Functions and Mechanisms of Sleep. *AIMS Neurosci* **3**, 67-104 (2016). DOI: 10.3934/Neuroscience.2016.1.67
232. Banks, S. & Dinges, D. F. Behavioral and physiological consequences of sleep restriction. *J Clin Sleep Med* **3**, 519-528 (2007). PMID: 17803017
233. Okun, M. L. & O'Brien, L. M. Concurrent insomnia and habitual snoring are associated with adverse pregnancy outcomes. *Sleep Med* **46**, 12-19 (2018). DOI: 10.1016/j.sleep.2018.03.004
234. Romero, R. & Badr, M. S. A role for sleep disorders in pregnancy complications: Challenges and opportunities. *Am J Obstet Gynecol.* **210**, 3-11 (2014). DOI: 10.1016/j.ajog.2013.11.020
235. Williams, M. A. *et al.* Associations of early pregnancy sleep duration with trimester-specific blood pressures and hypertensive disorders in pregnancy. *Sleep.* **33**, 1363-1371 (2010). DOI: 10.1093/sleep/33.10.1363
236. Haney, A., Buysse, D. J., Rosario, B. L., Chen, Y. F. & Okun, M. L. Sleep disturbance and cardiometabolic risk factors in early pregnancy: a preliminary study. *Sleep Med* **15**, 444-450 (2014). DOI: 10.1016/j.sleep.2014.01.003

237. Facco, F. L. *et al.* Objectively measured short sleep duration and later sleep midpoint in pregnancy are associated with a higher risk of gestational diabetes. *Am J Obstet Gynecol.* **217**, 447.e1-447.e13 (2017). DOI: 10.1016/j.ajog.2017.05.066
238. Facco, F. L. *et al.* Association of adverse pregnancy outcomes with self-reported measures of sleep duration and timing in women who are nulliparous. *J Clin Sleep Med.* **14**, 2047-2056 (2018). DOI: 10.5664/jcsm.7534
239. Begtrup, L. M. *et al.* Night work and miscarriage: A Danish nationwide register-based cohort study. *Occup Environ Med* **76**, 302-308 (2019). DOI: 10.1136/oemed-2018-105592
240. Okun, M. L., Schetter, C. D. & Glynn, L. M. Poor sleep quality is associated with preterm birth. *Sleep* **34**, 1493-1498 (2011). DOI: 10.5665/sleep.1384
241. Li, R. *et al.* Sleep disturbances during pregnancy are associated with cesarean delivery and preterm birth. *J Matern Fetal Neonatal Med* **30**, 733-738 (2017). DOI: 10.1080/14767058.2016.1183637
242. Lee, K. A. & Gay, C. L. Sleep in late pregnancy predicts length of labor and type of delivery. *Am J Obstet Gynecol.* **191**, 2041-2046 (2004). DOI: 10.1016/j.ajog.2004.05.086
243. Torres-Farfan, C. *et al.* Maternal melatonin effects on clock gene expression in a nonhuman primate fetus. *Endocrinology* **147**, 4618-4626 (2006). DOI: 10.1210/en.2006-0628
244. Lanoix, D., Beghdadi, H., Lafond, J. & Vaillancourt, C. Human placental trophoblasts synthesize melatonin and express its receptors. *Journal of Pineal Research* **45**, 50-60 (2008). DOI: 10.1111/j.1600-079x.2008.00555.x
245. Zhang, L. *et al.* Effects of melatonin administration on embryo implantation and offspring growth in mice under different schedules of photoperiodic exposure. *Reprod Biol Endocrinol.* **15**, 78. (2017). DOI: 10.1186/s12958-017-0297-7
246. Voiculescu, S. E., Zygouropoulos, N., Zahiu, C. D. & Zagrean, A. M. Role of melatonin in embryo fetal development. *Journal of medicine and life* **7**, 488-492 (2014). PMID: 25713608
247. Olcese, J. M. Melatonin and female reproduction: An expanding universe. *Front Endocrinol (Lausanne)* **11**, 85 (2020). DOI: 10.3389/fendo.2020.00085
248. Ivanov, D. *et al.* Melatonin, its beneficial effects on embryogenesis from mitigating oxidative stress to regulating gene expression. *Int J Mol Sci* **22**, 5885 (2021). DOI: 10.3390/ijms22115885
249. Unfer, V., Raffone, E., Rizzo, P. & Buffo, S. Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previousin vitrofertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study. *Gynecological Endocrinology* **27**, 857-861 (2011). DOI: 10.3109/09513590.2011.564687
250. Hardeland, R. Melatonin and chromatin. *Melatonin Research* **2**, 67-93 (2019). DOI: 10.32794/mr11250012
251. Hsu, C. N. & Tain, Y. L. Light and circadian signaling pathway in pregnancy: Programming of adult health and disease. *Int J Mol Sci* **21**, E2232 (2020). DOI: 10.3390/ijms21062232
252. Warland, J., Dorrian, J., Morrison, J. L. & O'Brien, L. M. Maternal sleep during pregnancy and poor fetal outcomes: A scoping review of the literature with meta-analysis. *Sleep Med Rev* **41**, 197-219 (2018). DOI: 10.1016/j.smrv.2018.03.004
253. Sateia, M. J. International Classification of Sleep Disorders-Third Edition. *Chest* **146**, 1387-1394 (2014). DOI: 10.1378/chest.14-0970
254. Herring, S. J. *et al.* Do pregnant women accurately report sleep time? A comparison between self-reported and objective measures of sleep duration in pregnancy among a sample of urban mothers. *Sleep Breath* **17**, 1323-1327 (2013). DOI: 10.1007/s11325-013-0835-2
255. Patterson, M. R. *et al.* 40 years of actigraphy in sleep medicine and current state of the art algorithms. *npj Digital Medicine* **6**, 51 (2023). DOI: 10.1038/s41746-023-00802-1

256. Shin, M., Swan, P. & Chow, C. M. The validity of Actiwatch2 and SenseWear armband compared against polysomnography at different ambient temperature conditions. *Sleep Sci* **8**, 9-15 (2015). DOI: 10.1016/j.slsci.2015.02.003
257. Sharif, M. M. & Bahammam, A. S. Sleep estimation using BodyMedia's SenseWear armband in patients with obstructive sleep apnea. *Ann Thorac Med* **8**, 53-57 (2013). DOI: 10.4103/1817-1737.105720
258. Soric, M. *et al.* Validation of a multi-sensor activity monitor for assessing sleep in children and adolescents. *Sleep Med* **14**, 201-205 (2013). DOI: 10.1016/j.sleep.2012.11.003
259. Hedman, C., Pohjasvaara, T., Tolonen, U., Suhonen-Malm, A. S. & Myllylä, V. V. Effects of pregnancy on mothers' sleep. *Sleep Med* **3**, 37-42 (2002). DOI: 10.1016/s1389-9457(01)00130-7
260. Lee, K. A., Zaffke, M. E. & McEnany, G. Parity and sleep patterns during and after pregnancy. *Obstet Gynecol.* **95**, 14-18 (2000). DOI: 10.1016/s0029-7844(99)00486-x
261. Mindell, J. A., Cook, R. A. & Nikolovski, J. Sleep patterns and sleep disturbances across pregnancy. *Sleep Med* **16**, 483-488 (2015). DOI: 10.1016/j.sleep.2014.12.006
262. Tsai, S. Y., Lee, P. L., Lin, J. W. & Lee, C. N. Cross-sectional and longitudinal associations between sleep and health-related quality of life in pregnant women: A prospective observational study. *Int J Nurs Stud.* **56**, 45-53 (2016). DOI: 10.1016/j.ijnurstu.2016.01.001
263. Lof, M. & Forsum, E. Activity pattern and energy expenditure due to physical activity before and during pregnancy in healthy Swedish women. *Br J Nutr* **95**, 296-302 (2006). DOI: 10.1079/bjn20051497
264. Ladyman, C. & Signal, T. L. Sleep Health in Pregnancy: A Scoping Review. *Sleep Med Clin* **13**, 307-333 (2018). DOI: 10.1016/j.jsmc.2018.04.004
265. Hanson, M., Godfrey, K. M., Lillycrop, K. A., Burdge, G. C. & Gluckman, P. D. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. *Prog Biophys Mol Biol* **106**, 272-280 (2011). DOI: 10.1016/j.pbiomolbio.2010.12.008
266. World Health Organisation, Physical activity. (2022). https://www.who.int/news-room/fact-sheets/detail/physical-activity
267. Davenport, M. H. *et al.* 2019 Canadian Guideline for Physical Activity Throughout Pregnancy: Methodology. *J Obstet Gynaecol Can* **40**, 1468-1483 (2018). DOI: 10.1016/j.jogc.2018.09.004
268. UK Chief Medical Officers' physical activity guidelines: pregnancy and after childbirth. 1, *Department of Health and Social Care* (2019). https://www.gov.uk/government/publications/physical-activity-guidelines-pregnancy-and-after-childbirth
269. Committee on Obstetric Practice. Physical Activity and Exercise During Pregnancy and the Postpartum Period: ACOG Committee Opinion, Number 804. *Obstet Gynecol* **135**, e178-e188 (2020). DOI: 10.1097/AOG.0000000000003772
270. Ming, W. K. *et al.* The effect of exercise during pregnancy on gestational diabetes mellitus in normal-weight women: a systematic review and meta-analysis. *BMC Pregnancy Childbirth* **18**, 440 (2018). DOI: 10.1186/s12884-018-2068-7
271. Reyes, L. M. & Davenport, M. H. Exercise as a therapeutic intervention to optimize fetal weight. *Pharmacol Res* **132**, 160-167 (2018). DOI: 10.1016/j.phrs.2018.04.016
272. Mudd, L. M., Owe, K. M., Mottola, M. F. & Pivarnik, J. M. Health benefits of physical activity during pregnancy: an international perspective. *Med Sci Sports Exerc* **45**, 268-277 (2013). DOI: 10.1249/MSS.0b013e31826cebcb

273. Barakat, R., Franco, E., Perales, M., López, C. & Mottola, M. F. Exercise during pregnancy is associated with a shorter duration of labor. A randomized clinical trial. *Eur J Obstet Gynecol Reprod Biol* **224**, 33-40 (2018). DOI: 10.1016/j.ejogrb.2018.03.009
274. Witvrouwen, I., Mannaerts, D., Van Berendoncks, A. M., Jacquemyn, Y. & Van Craenenbroeck, E. M. The Effect of Exercise Training During Pregnancy to Improve Maternal Vascular Health: Focus on Gestational Hypertensive Disorders. *Front Physiol* **11**, 450 (2020). DOI: 10.3389/fphys.2020.00450
275. Russo, L. M., Harvey, M. W., Pekow, P. & Chasan-Taber, L. Physical Activity and Risk of Cesarean Delivery in Hispanic Women. *J Phys Act Health* **16**, 116-124 (2019). DOI: 10.1123/jpah.2018-0072
276. Jackson, M. R., Gott, P., Lye, S. J., Ritchie, J. W. & Clapp, J. F. The effects of maternal aerobic exercise on human placental development: placental volumetric composition and surface areas. *Placenta* **16**, 179-191 (1995). DOI: 10.1016/0143-4004(95)90007-1
277. Bhattacharjee, J., Mohammad, S., Goudreau, A. D. & Adamo, K. B. Physical activity differentially regulates VEGF, PlGF, and their receptors in the human placenta. *Physiol Rep* **9**, e14710 (2021). DOI: 10.14814/phy2.14710 10.1002/14651858.CD009021.pub2 10.4199/C00016ED1V01Y201008ISP009
278. Hardy, D. B., Mu, X., Marchiori, K. S. & Mottola, M. F. Exercise in Pregnancy Increases Placental Angiogenin without Changes in Oxidative or Endoplasmic Reticulum Stress. *Med Sci Sports Exerc* **53**, 1846-1854 (2021). DOI: 10.1249/MSS.0000000000002647
279. Ramírez-Vélez, R., Bustamante, J., Czerniczyniec, A., Aguilar de Plata, A. C. & Lores-Arnaiz, S. Effect of exercise training on eNOS expression, NO production and oxygen metabolism in human placenta. *PLoS One* **8**, e80225 (2013). DOI: 10.1371/journal.pone.0080225
280. Smith, K. M., Lanningham-Foster, L. M., Welk, G. J. & Campbell, C. G. Validity of the SenseWear® Armband to predict energy expenditure in pregnant women. *Med Sci Sports Exerc* **44**, 2001-2008 (2012). DOI: 10.1249/MSS.0b013e31825ce76f
281. Abeysekera, M. V., Morris, J. A. & O'Sullivan, A. J. Techniques to measure free-living energy expenditure during pregnancy ,Äì A guide for clinicians and researchers. *Obstet Med.* **7**, 60-5. Epub 2014 Mar 27 (2014). DOI: 10.1177/1753495X14528324.
282. Smith, K. M., Foster, R. C. & Campbell, C. G. Accuracy of physical activity assessment during pregnancy: an observational study. *BMC Pregnancy Childbirth.* **11,** 86 (2011). DOI: 10.1186/1471-2393
283. Chasan-Taber, L. *et al.* Development and validation of a Pregnancy Physical Activity Questionnaire. *Med Sci Sports Exerc* **36**, 1750-1760 (2004). DOI: 10.1249/01.mss.0000142303.49306.0d
284. Berntsen, S., Stafne, S. N. & Mørkved, S. Physical activity monitor for recording energy expenditure in pregnancy. *Acta Obstet Gynecol Scand* **90**, 903-907 (2011). DOI: 10.1111/j.1600-0412.2011.01172.x
285. Wu, W. J., Yu, H. B., Tai, W. H., Zhang, R. & Hao, W. Y. Validity of Actigraph for Measuring Energy Expenditure in Healthy Adults: A Systematic Review and Meta-Analysis. *Sensors (Basel)* **23**, 8545 (2023). DOI: 10.3390/s23208545
286. Harrison, C. L., Thompson, R. G., Teede, H. J. & Lombard, C. B. Measuring physical activity during pregnancy. *Int J Behav Nutr Phys Act.* **8**, 19 (2011). DOI: 10.1186/1479-5868 (2011).
287. Rousham, E. K., Clarke, P. E. & Gross, H. Significant changes in physical activity among pregnant women in the UK as assessed by accelerometry and self-reported activity. *Eur J Clin Nutr* **60**, 393-400 (2006). DOI: 10.1038/sj.ejcn.1602329

288. Borodulin, K. M., Evenson, K. R., Wen, F., Herring, A. H. & Benson, A. M. Physical activity patterns during pregnancy. *Med Sci Sports Exerc* **40**, 1901-1908 (2008). DOI: 10.1249/MSS.0b013e31817f1957
289. Gaston, A. & Cramp, A. Exercise during pregnancy: a review of patterns and determinants. *J Sci Med Sport* **14**, 299-305 (2011). DOI: 10.1016/j.jsams.2011.02.006
290. Sjögren Forss, K., Ekvall Hansson, E., Troein, M. & Stjernberg, L. Patterns of physical activity among women and men before and during pregnancy. *Public Health* **128**, 814-816 (2014). DOI: 10.1016/j.puhe.2014.06.010
291. Abbasi, M. & van den Akker, O. A systematic review of changes in women's physical activity before and during pregnancy and the postnatal period. *Journal of Reproductive and Infant Psychology* **33**, 325-358 (2015). DOI: 10.1080/02646838.2015.1012710
292. Richardsen, K. R. *et al.* Objectively recorded physical activity in pregnancy and postpartum in a multi-ethnic cohort: association with access to recreational areas in the neighbourhood. *Int J Behav Nutr Phys Act* **13**, 78 (2016). DOI: 10.1186/s12966-016-0401-y
293. Forsdahl, A. Living conditions in childhood and subsequent development of risk factors for arteriosclerotic heart disease. The cardiovascular survey in Finnmark 1974-75. *Journal of Epidemiology & Community Health* **32**, 34-37 (1978). DOI: 10.1136/jech.32.1.34
294. Barker, D. J. P. & Osmond, C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *The Lancet* **327**, 1077-1081 (1986). DOI: 10.1016/S0140-6736(86)91340-1
295. Hoffman, D. J., Reynolds, R. M. & Hardy, D. B. Developmental origins of health and disease: current knowledge and potential mechanisms. *Nutr Rev* **75**, 951-970 (2017). DOI: 10.1093/nutrit/nux053
296. Suzuki, K. The developing world of DOHaD. *J Dev Orig Health Dis* **9**, 266-269 (2018). DOI: 10.1017/S2040174417000691
297. Pařízková, J. Pregnancy, Exercise and Late Effects in the Offspring until Adult Age. *SOJ Gynecology, Obstetrics & Women's Health* **3**, 1-6 (2017). DOI: 10.15226/2381-2915/3/2/00125
298. Bhutta, Z. A. *et al.* Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost. *The Lancet* **382**, 452-477 (2013). DOI: 10.1016/S0140-6736(13)60996-4
299. Wallach, E. E. *et al.* The role of genomic imprinting in implantation. *Fertility and sterility* **62**, 903-910 (1994). DOI: 10.1016/S0015-0282(16)57048-4
300. Moore, G. E. *et al.* Evidence that insulin is imprinted in the human yolk sac. *Diabetes* **50**, 199-203 (2001). DOI: 10.2337/diabetes.50.1.199
301. Ober, C., Loisel, D. A. & Gilad, Y. Sex-specific genetic architecture of human disease. *Nat Rev Genet* **9**, 911-922 (2008). DOI: 10.1038/nrg2415
302. Bermejo-Alvarez, P., Rizos, D., Lonergan, P. & Gutierrez-Adan, A. Transcriptional sexual dimorphism during preimplantation embryo development and its consequences for developmental competence and adult health and disease. *REPRODUCTION* **141**, 563-570 (2011). DOI: 10.1530/rep-10-0482
303. Kobayashi, S. *et al.* Comparison of gene expression in male and female mouse blastocysts revealed imprinting of the X-linked gene, Rhox5/Pem, at preimplantation stages. *Curr Biol* **16**, 166-172 (2006). DOI: 10.1016/j.cub.2005.11.071
304. Bermejo-Alvarez, P., Rizos, D., Rath, D., Lonergan, P. & Gutierrez-Adan, A. Sex determines the expression level of one third of the actively expressed genes in bovine blastocysts. *Proc Natl Acad Sci U S A* **107**, 3394-3399 (2010). DOI: 10.1073/pnas.0913843107

305. Kay, G. F., Barton, S. C., Surani, M. A. & Rastan, S. Imprinting and X chromosome counting mechanisms determine Xist expression in early mouse development. *Cell* **77**, 639-650 (1994). DOI: 10.1016/0092-8674(94)90049-3
306. Hartshorn, C., Rice, J. E. & Wangh, L. J. Developmentally-regulated changes of Xist RNA levels in single preimplantation mouse embryos, as revealed by quantitative real-time PCR. *Mol Reprod Dev* **61**, 425-436 (2002). DOI: 10.1002/mrd.10037
307. Gutiérrez-Adán, A., Oter, M., Martinez-Madrid, B., Pintado, B. & De La Fuente, J. Differential expression of two genes located on the X chromosome between male and female in vitro–produced bovine embryos at the blastocyst stage. *Molecular reproduction and development* **55**, 146-151 (2000). DOI: 10.1002/(SICI)1098-2795(200002)55:2<146::AID-MRD3>3.0.CO;2-F
308. Peippo, J. *et al.* Sex-chromosome linked gene expression in in-vitro produced bovine embryos. *Mol Hum Reprod* **8**, 923-929 (2002). DOI: 10.1093/molehr/8.10.923
309. Wrenzycki, C. *et al.* In vitro production and nuclear transfer affect dosage compensation of the X-linked gene transcripts G6PD, PGK, and Xist in preimplantation bovine embryos. *Biol Reprod* **66**, 127-134 (2002). DOI: 10.1095/biolreprod66.1.127
310. Jiménez, A. *et al.* Hyperglycemia-induced apoptosis affects sex ratio of bovine and murine preimplantation embryos. *Mol Reprod Dev* **65**, 180-187 (2003). DOI: 10.1002/mrd.10286
311. Morton, K. M. *et al.* Altered mRNA expression patterns in bovine blastocysts after fertilisation in vitro using flow-cytometrically sex-sorted sperm. *Mol Reprod Dev* **74**, 931-940 (2007). DOI: 10.1002/mrd.20573
312. Taylor, D. M. *et al.* Quantitative measurement of transcript levels throughout human preimplantation development: analysis of hypoxanthine phosphoribosyl transferase. *Mol Hum Reprod* **7**, 147-154 (2001). DOI: 10.1093/molehr/7.2.147
313. Gutiérrez-Adán, A. *et al.* Developmental consequences of sexual dimorphism during pre-implantation embryonic development. *Reprod Domest Anim* **41 Suppl 2**, 54-62 (2006). DOI: 10.1111/j.1439-0531.2006.00769.x
314. Bredbacka, K. & Bredbacka, P. Glucose controls sex-related growth rate differences of bovine embryos produced in vitro. *J Reprod Fertil* **106**, 169-172 (1996). DOI: 10.1530/jrf.0.1060169
315. Tiffin, G. J., Rieger, D., Betteridge, K. J., Yadav, B. R. & King, W. A. Glucose and glutamine metabolism in pre-attachment cattle embryos in relation to sex and stage of development. *J Reprod Fertil* **93**, 125-132 (1991). DOI: 10.1530/jrf.0.0930125
316. Ray, P. F., Conaghan, J., Winston, R. M. & Handyside, A. H. Increased number of cells and metabolic activity in male human preimplantation embryos following in vitro fertilization. *J Reprod Fertil* **104**, 165-171 (1995). DOI: 10.1530/jrf.0.1040165
317. Mak, W. *et al.* Reactivation of the paternal X chromosome in early mouse embryos. *Science* **303**, 666-669 (2004). DOI: 10.1126/science.1092674
318. Okamoto, I., Otte, A. P., Allis, C. D., Reinberg, D. & Heard, E. Epigenetic dynamics of imprinted X inactivation during early mouse development. *Science* **303**, 644-649 (2004). DOI: 10.1126/science.1092727
319. Engel, N. Sex Differences in Early Embryogenesis: Inter-Chromosomal Regulation Sets the Stage for Sex-Biased Gene Networks: The dialogue between the sex chromosomes and autosomes imposes sexual identity soon after fertilization. *Bioessays* **40**, e1800073 (2018). DOI: 10.1002/bies.201800073
320. van Duijn, L. *et al.* Higher preconceptional maternal body mass index is associated with faster early preimplantation embryonic development: the Rotterdam periconception cohort. *Reprod Biol Endocrinol* **19**, 145 (2021). DOI: 10.1186/s12958-021-00822-0
321. Wilcox, A. J., Baird, D. D. & Weinberg, C. R. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med* **340**, 1796-1799 (1999). DOI: 10.1056/NEJM199906103402304

322. Wilcox, A. J., Dunson, D. & Baird, D. D. The timing of the “fertile window” in the menstrual cycle: day specific estimates from a prospective study. *BMJ* **321**, 1259-1262 (2000). DOI: 10.1136/bmj.321.7271.1259
323. Park, S. J., Goldsmith, L. T., Skurnick, J. H., Wojtczuk, A. & Weiss, G. Characteristics of the urinary luteinizing hormone surge in young ovulatory women. *Fertil Steril* **88**, 684-690 (2007). DOI: 10.1016/j.fertnstert.2007.01.045
324. Vietheer, A., Kiserud, T., Lie, R. T., Haaland, Ø. A. & Kessler, J. Sleep and physical activity from before conception to the end of pregnancy in healthy women: A longitudinal actigraphy study. *Sleep Medicine* **83**, 89-98 (2021). DOI: 10.1016/j.sleep.2021.04.028
325. Vietheer, A., Kiserud, T., Lie, R. T., Haaland, Ø. A. & Kessler, J. Effect of maternal sleep on embryonic development. *Scientific reports* **12**, 17099 (2022). DOI: 10.1038/s41598-022-21516-6
326. Lohman, T. *Anthropometric Standardization Reference Manual* (Champaign, IL : Human kinetics book, 1988). ISBN: 9780873223317
327. *Body Composition Analyser BC-418-MA. Instruction Manual* (Tanita Corporation of America. https://www.tanita.com/es/.downloads/download/?file=855638086&fl=en_US,
328. Lukaski, H. C., Bolonchuk, W. W., Hall, C. B. & Siders, W. A. Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J Appl Physiol (1985)* **60**, 1327-1332 (1986). DOI: 10.1152/jappl.1986.60.4.1327
329. Sadeh, A. The role and validity of actigraphy in sleep medicine: an update. *Sleep medicine reviews* **15**, 259-267 (2011). DOI: 10.1016/j.smrv.2010.10.001
330. Andre, D. Assessing resting metabolic rate using a multi-sensor armband. *Obesity (Silver Spring)* **15**, 1337; author reply 1337-8 (2007). DOI: 10.1038/oby.2007.156
331. Matthews, C. E., Ainsworth, B. E., Thompson, R. W. & Bassett, D. R. Sources of variance in daily physical activity levels as measured by an accelerometer. *Med Sci Sports Exerc* **34**, 1376-1381 (2002). DOI: 10.1097/00005768-200208000-00021
332. Ainsworth, B. E. *et al.* 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc* **43**, 1575-1581 (2011). DOI: 10.1249/MSS.0b013e31821ece12
333. Tremblay, M. S. *et al.* Sedentary Behavior Research Network (SBRN) - Terminology Consensus Project process and outcome. *Int J Behav Nutr Phys Act* **14**, 75 (2017). DOI: 10.1186/s12966-017-0525-8
334. Thompson, P. D. in *ACSM'Guidelines for Exercise Testing and Prescription* (eds Pescatello, L. S., Arena, R., Riebe, D. & Thompson, P. D.) 2-14 (Lippincott Williams&Wilkins, 2014). ISBN: 978-1609136055
335. McParlin, C. *et al.* Objectively measured physical activity during pregnancy: a study in obese and overweight women. *BMC Pregnancy Childbirth* **10**, 76 (2010). DOI: 10.1186/1471-2393-10-76
336. McVeigh, J. A. *et al.* Objectively measured patterns of sedentary time and physical activity in young adults of the Raine study cohort. *Int J Behav Nutr Phys Act.* (2016) **13**, 41. DOI: 10.1186/s12966-016.
337. Sunseri, M. *et al.* The SenseWear armband as a sleep detection device. *White Papers Body Media* 1-9. https://pdfs.semanticscholar.org/e36b/f00cac00e094eb605bbf2bcc5a384e96874c.pdf
338. Shin, M., Swan, P. & Chow, C. M. The validity of Actiwatch2 and SenseWear armband compared against polysomnography at different ambient temperature conditions. *Sleep Sci.* **8**, 9-15 (2015). DOI: 10.1016/j.slsci.2015.02.003

339. Zhu, B. *et al.* Objective sleep in pregnant women: a comparison of actigraphy and polysomnography. *Sleep Health.* **4**, 390-396 (2018). DOI: 10.1016/j.sleh.2018.07.011
340. Donald, I., Macvicar, J. & Brown, T. G. Investigation of abdominal masses by pulsed ultrasound. *The Lancet* **271**, 1188-1195 (1958). DOI: 10.1016/s0140-6736(58)91905-6.
341. Bree, R. L. *et al.* Transvaginal sonography in the evaluation of normal early pregnancy: correlation with HCG level. *AJR Am J Roentgenol* **153**, 75-79 (1989). DOI: 10.2214/ajr.153.1.75
342. Salvesen, K. A. EFSUMB: safety tutorial: epidemiology of diagnostic ultrasound exposure during pregnancy-European committee for medical ultrasound safety (ECMUS). *Eur J Ultrasound* **15**, 165-171 (2002). DOI: 10.1016/s0929-8266(02)00038-1
343. Pexsters, A. *et al.* Clinical implications of intra- and interobserver reproducibility of transvaginal sonographic measurement of gestational sac and crown-rump length at 6-9 weeks' gestation. *Ultrasound Obstet Gynecol* **38**, 510-515 (2011). DOI: 10.1002/uog.8884
344. Sande, R. K., Matre, K., Eide, G. E. & Kiserud, T. Ultrasound safety in early pregnancy: reduced energy setting does not compromise obstetric Doppler measurements. *Ultrasound Obstet Gynecol* **39**, 438-443 (2012). DOI: 10.1002/uog.10148
345. Bhide, A. *et al.* ISUOG practice guidelines: use of Doppler ultrasonography in obstetrics. **41**, 233-239 (2013). DOI: 10.1002/uog.12371
346. Salvesen, K. *et al.* ISUOG statement on the safe use of Doppler for fetal ultrasound examination in the first 13 + 6 weeks of pregnancy (updated). *Ultrasound in Obstetrics & Gynecology* **57**, 1020-1020 (2021). DOI: 10.1002/uog.23610
347. Sande, R. *et al.* Safety Aspects of Perinatal Ultrasound. *Ultraschall Med* **42**, 580-598 (2021). DOI: 10.1055/a-1538-6295
348. Preisler, J. *et al.* Defining safe criteria to diagnose miscarriage: prospective observational multicentre study. *BMJ* **351**, h4579 (2015). DOI: 10.1136/bmj.h4579
349. Napolitano, R. *et al.* Pregnancy dating by fetal crown-rump length: a systematic review of charts. *BJOG* **121**, 556-565 (2014). DOI: 10.1111/1471-0528.12478
350. Popović, Z. B. & Thomas, J. D. Assessing observer variability: a user's guide. *Cardiovasc Diagn Ther* **7**, 317-324 (2017). DOI: 10.21037/cdt.2017.03.12
351. Robinson, H. P. & Fleming, J. E. A critical evaluation of sonar “crown-rump length” measurements. *Br J Obstet Gynaecol* **82**, 702-710 (1975). DOI: 10.1111/j.1471-0528.1975.tb00710.x
352. Signal, T. L. *et al.* Sleep duration and quality in healthy nulliparous and multiparous women across pregnancy and post-partum. *Aust N Z J Obstet Gynaecol.* **47**, 16-22 (2007). DOI: 10.1111/j.1479-828X.2006.00672.x
353. Little, S. E., McNamara, C. J. & Miller, R. C. Sleep changes in normal pregnancy. *Obstetrics & Gynecology* **123**, 153S (2014). DOI: 10.1097/01.AOG.0000447145.52005.ac
354. Ebert, R. M., Wood, A. & Okun, M. L. Minimal Effect of Daytime Napping Behavior on Nocturnal Sleep in Pregnant Women. *J Clin Sleep Med* **11**, 635-643 (2015). DOI: 10.5664/jcsm.4774
355. Berntsen, S. *et al.* Objectively recorded physical activity in early pregnancy: a multiethnic population-based study. *Scand J Med Sci Sports* **24**, 594-601 (2014). DOI: 10.1111/sms.12034
356. Brunner, D. P. *et al.* Changes in sleep and sleep electroencephalogram during pregnancy. *Sleep.* **17**, 576-582. (1994). DOI: 10.1093/sleep/17.7.576
357. Aili, K., Åström-Paulsson, S., Stoetzer, U., Svartengren, M. & Hillert, L. Reliability of Actigraphy and Subjective Sleep Measurements in Adults: The Design of Sleep Assessments. *J Clin Sleep Med* **13**, 39-47 (2017). DOI: 10.5664/jcsm.6384

358. Phillips, S. M. *et al.* The Validity, Reliability, and Feasibility of Measurement Tools Used to Assess Sleep of Pre-school Aged Children: A Systematic Rapid Review. *Front Pediatr* **9**, 770262 (2021). DOI: 10.3389/fped.2021.770262
359. Tsai, S. Y., Kuo, L. T., Lee, C. N., Lee, Y. L. & Landis, C. A. Reduced sleep duration and daytime naps in pregnant women in Taiwan. *Nurs Res.* **62**, 99-105 (2013). DOI: 10.1097/NNR.0b013e3182830d87
360. Okun, M. L. & Coussons-Read, M. E. Sleep disruption during pregnancy: how does it influence serum cytokines. *J Reprod Immunol* **73**, 158-165 (2007). DOI: 10.1016/j.jri.2006.06.006
361. Izci Balserak, B., Jackson, N., Ratcliffe, S. A., Pack, A. I. & Pien, G. W. Sleep-disordered breathing and daytime napping are associated with maternal hyperglycemia. *Sleep Breath* **17**, 1093-1102 (2013). DOI: 10.1007/s11325-013-0809-4
362. Tsai, S. Y., Lin, J. W., Kuo, L. T., Lee, C. N. & Landis, C. A. Nighttime sleep, daytime napping, and labor outcomes in healthy pregnant women in Taiwan. *Res Nurs Health* **36**, 612-622 (2013). DOI: 10.1002/nur.21568
363. Song, L. *et al.* Afternoon napping during pregnancy and low birth weight: the Healthy Baby Cohort study. *Sleep Med* **48**, 35-41 (2018). DOI: 10.1016/j.sleep.2018.03.029
364. Zheng, X. *et al.* Maternal Habitual Midday Napping Duration and Frequency are Associated with High Birthweight. *Sci Rep* **7**, 10564 (2017). DOI: 10.1038/s41598-017-09683-3
365. Rawal, S., Hinkle, S. N., Zhu, Y., Albert, P. S. & Zhang, C. A longitudinal study of sleep duration in pregnancy and subsequent risk of gestational diabetes: findings from a prospective, multiracial cohort. *Am J Obstet Gynecol* **216**, 399.e1-399.e8 (2017). DOI: 10.1016/j.ajog.2016.11.1051
366. Izci Balserak, B. & Lee, K. A. in *Principles and Practice of Sleep Medicine* (ed Kryger, M., Roth, T. & Dement, W. C.) pp1525.e5-1539.e5 (Elsevier, 2017).
367. Lagadec, N. *et al.* Factors influencing the quality of life of pregnant women: a systematic review. *BMC Pregnancy Childbirth* **18**, 455 (2018). DOI: 10.1186/s12884-018-2087-4
368. Mikec, M. *et al.* Influence of environmental and nutritional stressors on yolk sac utilization, development of chicken gastrointestinal system and its immune status. *World's Poultry Science Journal* **62**, 31-40 (2006). DOI: 10.1079/wps200582
369. Fiksen, Ø. & Folkvord, A. Maternal effects and the benefit of yolk supply in cod larvae in different environments—a simulation model. **ICES Council Meeting**, 1-6 (1999).
370. Watkins, A. J. *et al.* Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. *Biol Reprod* **78**, 299-306 (2008). DOI: 10.1095/biolreprod.107.064220
371. Lara, R. A. & Vasconcelos, R. O. Impact of noise on development, physiological stress and behavioural patterns in larval zebrafish. *Sci Rep* **11**, 6615 (2021). DOI: 10.1038/s41598-021-85296-1
372. Carter, A. M. Unique aspects of human placentation. *Int J Mol Sci* **22**, 8099 (2021). DOI: 10.3390/ijms22158099
373. Jauniaux, E., Jurkovic, D. & Campbell, S. Current topic: in vivo investigation of the placental circulations by Doppler echography. *Placenta* **16**, 323-331 (1995). DOI: 10.1016/0143-4004(95)90089-6
374. Jaffe, R., Jauniaux, E. & Hustin, J. Maternal circulation in the first-trimester human placenta--myth or reality. *Am J Obstet Gynecol* **176**, 695-705 (1997). DOI: 10.1016/s0002-9378(97)70572-6

375. Jauniaux, E., Watson, A. & Burton, G. Evaluation of respiratory gases and acid-base gradients in human fetal fluids and uteroplacental tissue between 7 and 16 weeks' gestation. *Am J Obstet Gynecol* **184**, 998-1003 (2001). DOI: 10.1067/mob.2001.111935
376. New, D. A. Whole-embryo culture and the study of mammalian embryos during organogenesis. *Biol Rev Camb Philos Soc* **53**, 81-122 (1978). DOI: 10.1111/j.1469-185x.1978.tb00993.x
377. Eriksson, U. J. Oxidative DNA damage and embryo development. *Nat Med* **5**, 715 (1999). DOI: 10.1038/10420
378. Chen, E. Y., Fujinaga, M. & Giaccia, A. J. Hypoxic microenvironment within an embryo induces apoptosis and is essential for proper morphological development. *Teratology* **60**, 215-225 (1999). DOI: 10.1002/(SICI)1096-9926(199910)60:4<215::AID-TERA6>3.0.CO;2-2
379. Morrison, S. J. *et al.* Culture in reduced levels of oxygen promotes clonogenic sympathoadrenal differentiation by isolated neural crest stem cells. *J Neurosci* **20**, 7370-7376 (2000). DOI: 10.1523/JNEUROSCI.20-19-07370.2000
380. Studer, L. *et al.* Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen. *J Neurosci* **20**, 7377-7383 (2000). DOI: 10.1523/JNEUROSCI.20-19-07377.2000
381. Beroukhim, G., Esencan, E. & Seifer, D. B. Impact of sleep patterns upon female neuroendocrinology and reproductive outcomes: a comprehensive review. *Reprod Biol Endocrinol* **20**, 16 (2022). DOI: 10.1186/s12958-022-00889-3
382. Stocker, L. J., Macklon, N. S., Cheong, Y. C. & Bewley, S. J. Influence of shift work on early reproductive outcomes: a systematic review and meta-analysis. *Obstet Gynecol* **124**, 99-110 (2014). DOI: 10.1097/AOG.0000000000000321
383. Bonde, J. P., Jørgensen, K. T., Bonzini, M. & Palmer, K. T. Miscarriage and occupational activity: a systematic review and meta-analysis regarding shift work, working hours, lifting, standing, and physical workload. *Scand J Work Environ Health* **39**, 325-334 (2013). DOI: 10.5271/sjweh.3337
384. He, C. *et al.* Melatonin-related genes expressed in the mouse uterus during early gestation promote embryo implantation. *J Pineal Res* **58**, 300-309 (2015). DOI: 10.1111/jpi.12216
385. Hardeland, R., Pandi-Perumal, S. R. & Cardinali, D. P. Melatonin. *Int J Biochem Cell Biol* **38**, 313-316 (2006). DOI: 10.1016/j.biocel.2005.08.020
386. Reiter, R. J. The melatonin rhythm: both a clock and a calendar. *Experientia* **49**, 654-664 (1993). DOI: 10.1007/BF01923947
387. Zhao, D. *et al.* Melatonin Synthesis and Function: Evolutionary History in Animals and Plants. *Front Endocrinol (Lausanne)* **10**, 249 (2019). DOI: 10.3389/fendo.2019.00249
388. Weaver, D. R. The suprachiasmatic nucleus: a 25-year retrospective. *J Biol Rhythms* **13**, 100-112 (1998). DOI: 10.1177/074873098128999952
389. Levi, F. & Schibler, U. Circadian rhythms: mechanisms and therapeutic implications. *Annu Rev Pharmacol Toxicol* **47**, 593-628 (2007). DOI: 10.1146/annurev.pharmtox.47.120505.105208
390. Weaver, D. R. & Emery, P. Chapter 39 - Circadian Timekeeping Fundamental Neuroscience (Fourth Edition) (eds Squire, L. R. *et al.*) 819-845 (*Academic Press*, San Diego, 2013). https://www.sciencedirect.com/science/article/pii/B9780123858702000391
391. Reiter, R. J., Sharma, R., Ma, Q., Rorsales-Corral, S. & de Almeida Chuffa, L. G. Melatonin inhibits Warburg-dependent cancer by redirecting glucose oxidation to the mitochondria: a mechanistic hypothesis. *Cell Mol Life Sci* **77**, 2527-2542 (2020). DOI: 10.1007/s00018-019-03438-1

392. Paria, B. C., Reese, J., Das, S. K. & Dey, S. K. Deciphering the cross-talk of implantation: advances and challenges. *Science* **296**, 2185-2188 (2002). DOI: 10.1126/science.1071601
393. Lane, D. P. Cancer. p53, guardian of the genome. *Nature* **358**, 15-16 (1992). DOI: 10.1038/358015a0
394. Vousden, K. H. & Prives, C. Blinded by the Light: The Growing Complexity of p53. *Cell* **137**, 413-431 (2009). DOI: 10.1016/j.cell.2009.04.037
395. Feng, Z. & Levine, A. J. The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein. *Trends Cell Biol* **20**, 427-434 (2010). DOI: 10.1016/j.tcb.2010.03.004
396. Kung, C. P. & Murphy, M. E. The role of the p53 tumor suppressor in metabolism and diabetes. *J Endocrinol* **231**, R61-R75 (2016). DOI: 10.1530/JOE-16-0324
397. Hu, W., Feng, Z., Teresky, A. K. & Levine, A. J. p53 regulates maternal reproduction through LIF. *Nature* **450**, 721-724 (2007). DOI: 10.1038/nature05993
398. Stewart, C. L. *et al.* Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* **359**, 76-79 (1992). DOI: 10.1038/359076a0
399. Zhao, J. *et al.* Melatonin protect the development of preimplantation mouse embryos from sodium fluoride-induced oxidative injury. *Environ Toxicol Pharmacol* **54**, 133-141 (2017). DOI: 10.1016/j.etap.2017.06.014
400. Ma, W. G., Song, H., Das, S. K., Paria, B. C. & Dey, S. K. Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. *Proc Natl Acad Sci U S A* **100**, 2963-2968 (2003). DOI: 10.1073/pnas.0530162100
401. Richter, H. G., Hansell, J. A., Raut, S. & Giussani, D. A. Melatonin improves placental efficiency and birth weight and increases the placental expression of antioxidant enzymes in undernourished pregnancy. *J Pineal Res* **46**, 357-364 (2009). DOI: 10.1111/j.1600-079X.2009.00671.x
402. Tamura, H. *et al.* The role of melatonin as an antioxidant in the follicle. *J Ovarian Res* **5**, 5 (2012). DOI: 10.1186/1757-2215-5-5
403. Asgari, Z., Ghasemian, F., Ramezani, M. & Bahadori, M. H. The effect of melatonin on the developmental potential and implantation rate of mouse embryos. *Cell J* **14**, 203-208 (2012). PMID 23508820
404. Vgontzas, A. N. *et al.* Chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal axis: Clinical implications. *J Clin Endocrinol Metab* **86**, 3787-3794 (2001). DOI: 10.1210/jcem.86.8.7778
405. Van Cauter, E., Plat, L. & Copinschi, G. Interrelations between sleep and the somatotropic axis. *Sleep* **21**, 553-566 (1998). PMID: 9779515
406. Li, J., Chen, Q., Wang, J., Huang, G. & Ye, H. Does growth hormone supplementation improve oocyte competence and IVF outcomes in patients with poor embryonic development? A randomized controlled trial. *BMC Pregnancy and Childbirth* **20**, 310 (2020). DOI: 10.1186/s12884-020-03004-9
407. Favetta, L. A., St John, E. J., King, W. A. & Betts, D. H. High levels of p66shc and intracellular ROS in permanently arrested early embryos. *Free Radic Biol Med* **42**, 1201-1210 (2007). DOI: 10.1016/j.freeradbiomed.2007.01.018
408. Lysiak, J. J., Zheng, S., Woodson, R. & Turner, T. T. Caspase-9-dependent pathway to murine germ cell apoptosis: mediation by oxidative stress, BAX, and caspase 2. *Cell Tissue Res* **328**, 411-419 (2007). DOI: 10.1007/s00441-006-0341-y
409. Chen, S., Yang, J., Wei, Y. & Wei, X. Epigenetic regulation of macrophages: from homeostasis maintenance to host defense. *Cell Mol Immunol* **17**, 36-49 (2020). DOI: 10.1038/s41423-019-0315-0

410. Turpaev, T. M. & Nechaeva, M. V. Extraembryonic motor activity during the embryogenesis of higher vertebrates. *Neurosci Behav Physiol* **30**, 9-14 (2000). DOI: 10.1007/BF02461386
411. Morgan, M. A. *et al.* Different patterns of myometrial activity and 24-h rhythms in myometrial contractility in the gravid baboon during the second half of pregnancy. *Biol Reprod* **46**, 1158-1164 (1992). DOI: 10.1095/biolreprod46.6.1158
412. Olcese, J. & Beesley, S. Clinical significance of melatonin receptors in the human myometrium. *Fertility and Sterility* **102**, 329-335 (2014). DOI: 10.1016/j.fertnstert.2014.06.020
413. Constant, M., Tran, V. C., Benoit, B. & Vasseur, F. New first-trimester crown-rump length equations from a French general population. *Fetal Diagn Ther* **32**, 277-287 (2012). DOI: 10.1159/000339272
414. von Wolff, M. *et al.* Tumour necrosis factor-alpha (TNF-alpha) in human endometrium and uterine secretion: an evaluation by immunohistochemistry, ELISA and semiquantitative RT-PCR. *Mol Hum Reprod* **5**, 146-152 (1999). DOI: 10.1093/molehr/5.2.146
415. Müller-Schöttle, F. *et al.* Expression of uteroglobin in the human endometrium. *Mol Hum Reprod* **5**, 1155-1161 (1999). DOI: 10.1093/molehr/5.12.1155
416. Kundu, G. C., Mantile, G., Miele, L., Cordella-Miele, E. & Mukherjee, A. B. Recombinant human uteroglobin suppresses cellular invasiveness via a novel class of high-affinity cell surface binding site. *Proc Natl Acad Sci U S A* **93**, 2915-2919 (1996). DOI: 10.1073/pnas.93.7.2915
417. Lala, P. K., Hamilton, G. S. & Athanassiades, A. Role of growth factors and other placental signals in extravillous trophoblast cell function: A review. *Placenta* **19, Suppl. 2**, 327-339 (1998). DOI: 10.1016/S0143-4004(98)80052-0
418. Mahendru, A. A. *et al.* Impact of ovulation and implantation timing on first-trimester crown-rump length and gestational age. *Ultrasound Obstet Gynecol* **40**, 630-635 (2012). DOI: 10.1002/uog.12277
419. Baker, F. C. & Driver, H. S. Circadian rhythms, sleep, and the menstrual cycle. *Sleep Med* **8**, 613-622 (2007). DOI: 10.1016/j.sleep.2006.09.011
420. Lawson, C. C. *et al.* Rotating shift work and menstrual cycle characteristics. *Epidemiology* **22**, 305-312 (2011). DOI: 10.1097/EDE.0b013e3182130016
421. Kloss, J. D., Perlis, M. L., Zamzow, J. A., Culnan, E. J. & Gracia, C. R. Sleep, sleep disturbance, and fertility in women. *Sleep Med Rev* **22**, 78-87 (2015). DOI: 10.1016/j.smrv.2014.10.005
422. Baumgartner, A. *et al.* Influence of partial sleep deprivation on the secretion of thyrotropin, thyroid hormones, growth hormone, prolactin, luteinizing hormone, follicle stimulating hormone, and estradiol in healthy young women. *Psychiatry Res* **48**, 153-178 (1993). DOI: 10.1016/0165-1781(93)90039-j
423. Hall, J. E., Sullivan, J. P. & Richardson, G. S. Brief wake episodes modulate sleep-inhibited luteinizing hormone secretion in the early follicular phase. *J Clin Endocrinol Metab* **90**, 2050-2055 (2005). DOI: 10.1210/jc.2004-2033
424. Iervolino, M. *et al.* Natural Molecules in the Management of Polycystic Ovary Syndrome (PCOS): An Analytical Review. *Nutrients* **13**, 1677 (2021). DOI: 10.3390/nu13051677
425. Voordouw, B. C. *et al.* Melatonin and melatonin-progestin combinations alter pituitary-ovarian function in women and can inhibit ovulation. *J Clin Endocrinol Metab* **74**, 108-117 (1992). DOI: 10.1210/jcem.74.1.1727807
426. Morgan, M. A. *et al.* Different patterns of myometrial activity and 24-h rhythms in myometrial contractility in the gravid baboon during the second half of pregnancy. *Biol Reprod* **46**, 1158-1164 (1992). DOI: 10.1095/biolreprod46.6.1158

427. Delp, M. D. Differential effects of training on the control of skeletal muscle perfusion. *Med Sci Sports Exerc* **30**, 361-374 (1998). DOI: 10.1097/00005768-199803000-00005
428. Bergmann, A., Zygmunt, M. & Clapp, J. F. Running throughout pregnancy: effect on placental villous vascular volume and cell proliferation. *Placenta* **25**, 694-698 (2004). DOI: 10.1016/j.placenta.2004.02.005
429. Ennour-Idrissi, K., Maunsell, E. & Diorio, C. Effect of physical activity on sex hormones in women: a systematic review and meta-analysis of randomized controlled trials. *Breast Cancer Res* **17**, 139 (2015). DOI: 10.1186/s13058-015-0647-3
430. Stampone, C., Nicotra, M., Muttinelli, C. & Cosmi, E. V. Transvaginal sonography of the yolk sac in normal and abnormal pregnancy. *J Clin Ultrasound* **24**, 3-9 (1996). DOI: 10.1002/(SICI)1097-0096(199601)24:1<3::AID-JCU1>3.0.CO;2-N
431. Brent, R. L., Beckman, D. A., Jensen, M. & Koszalka, T. R. Experimental yolk sac dysfunction as a model for studying nutritional disturbances in the embryo during early organogenesis. *Teratology* **41**, 405-413 (1990). DOI: 10.1002/tera.1420410406
432. Wiznitzer, A., Furman, B., Mazor, M. & Reece, E. A. The role of prostanoids in the development of diabetic embryopathy. *Semin Reprod Endocrinol* **17**, 175-181 (1999). DOI: 10.1055/s-2007-1016224
433. Reece, E. A., Ma, X.-D., Wu, Y.-K. & Dhanasekaran, D. Aberrant patterns of cellular communication in diabetes-induced embryopathy I. Membrane signalling. *The Journal of Maternal-Fetal & Neonatal Medicine* **11**, 249-253 (2002). DOI: 10.1080/jmf.11.4.249.253
434. Moley, K. H. Hyperglycemia and apoptosis: mechanisms for congenital malformations and pregnancy loss in diabetic women. *Trends Endocrinol Metab* **12**, 78-82 (2001). DOI: 10.1016/s1043-2760(00)00341-6
435. Zhao, Z. & Reece, E. A. Intracellular metabolism in diabetic embryopathy. *Am J Biomed Sci & Res.* **13**, 418-426 (2021). DOI: 10.34297/AJBSR.2021.13.001891
436. Wentzel, P., Wentzel, C. R., Gäreskog, M. B. & Eriksson, U. J. Induction of embryonic dysmorphogenesis by high glucose concentration, disturbed inositol metabolism, and inhibited protein kinase C activity. *Teratology* **63**, 193-201 (2001). DOI: 10.1002/tera.1034
437. Yang, X., Borg, L. A. & Eriksson, U. J. Altered mitochondrial morphology of rat embryos in diabetic pregnancy. *Anat Rec* **241**, 255-267 (1995). DOI: 10.1002/ar.1092410212
438. Ornoy, A., Zaken, V. & Kohen, R. Role of reactive oxygen species (ROS) in the diabetes-induced anomalies in rat embryos in vitro: reduction in antioxidant enzymes and low-molecular-weight antioxidants (LMWA) may be the causative factor for increased anomalies. *Teratology* **60**, 376-386 (1999). DOI: 10.1002/(SICI)1096-9926(199912)60:6<376::AID-TERA10>3.0.CO;2-Q
439. Willcox, J. K., Ash, S. L. & Catignani, G. L. Antioxidants and prevention of chronic disease. *Crit Rev Food Sci Nutr* **44**, 275-295 (2004). DOI: 10.1080/10408690490468489
440. Weinberg, C. R., Baird, D. D. & Wilcox, A. J. The sex of the baby may be related to the length of the follicular phase in the conception cycle. *Hum Reprod* **10**, 304-307 (1995). DOI: 10.1093/oxfordjournals.humrep.a135932
441. Deegan, D. F. & Engel, N. Sexual dimorphism in the age of genomics: How, when, where. *Front Cell Dev Biol* **7**, 186 (2019). DOI: 10.3389/fcell.2019.00186
442. Goldstein, C. A. & Depner, C. Miles to go before we sleep…a step toward transparent evaluation of consumer sleep tracking devices. *Sleep* **44**, (2021). DOI: 10.1093/sleep/zsab020
443. Detry, M. A. & Ma, Y. Analyzing Repeated Measurements Using Mixed Models. *JAMA* **315**, 407-408 (2016). DOI: 10.1001/jama.2015.19394

9. Appendix

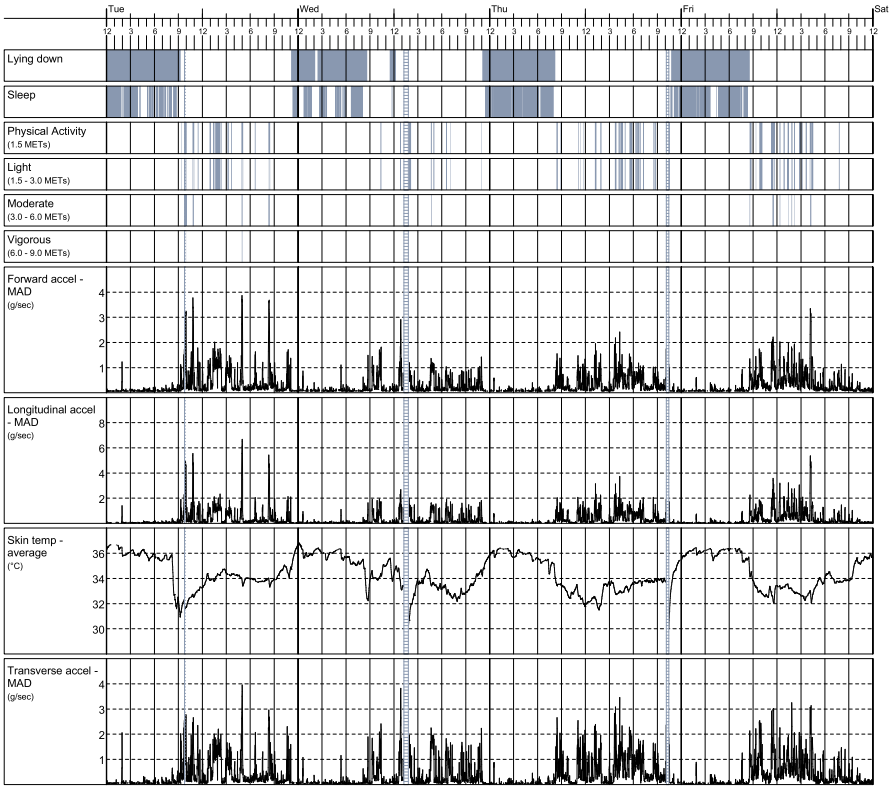
Example for the SenseWear actigraphy registration

SenseWear Report Created Fri 26 Mar 2021 Page 3 of 3 enabled by BODYMEDIA

Clinician / Physician	Hospital / Organization	Practice / Department

Subject	Date of Birth	Gender	Weight	Height	Handed	Smoker	BMI	BSA	WHO RMR
Løp. nr 268 uke 13	Oct 27, 1989 (29)	Female	63.5 kg	170.2 cm	Right	No	21.9	1.7 m²	1,448.0 kcal/day

Start Time	End Time	Duration of View	Duration on-body
Tue 6 Nov 2018 00:00	Sat 10 Nov 2018 00:00	4 days	3 days 23 hrs 7 min (99.1%)



The information contained within this report is not to be used for diagnostic purposes.

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Original Article

Sleep and physical activity from before conception to the end of pregnancy in healthy women: a longitudinal actigraphy study

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ABSTRACT

Background: Sleep and physical activity changes are common in pregnancy, but longitudinal data starting before conception are scarce. Our aim was to determine the changes of the daily total sleep time (TST) and physical activity duration (PAD) from before conception to end of pregnancies in respect of pregestational maternal factors.

Methods: This longitudinal observational study formed part of the CONIMPREG research project and recruited healthy women planning to become pregnant. Sleep and physical activity were recorded around-the-clock for ≥4 days via actigraphy before conception and during each trimester of pregnancy. Data were adjusted according to pregestational maternal body composition, parity and age.

Results: Among 123 women with eligible data, the unadjusted mean (95% confidence interval) TST increased from 415.3 min (405.5–425.2 min) before conception to 458.0 min (445.4–470.6 min) in the 1st trimester, remaining high through the 2nd and 3rd trimesters. Variation was substantial before conception (±2SD range: 307–523 min). The unadjusted mean PAD before conception was 363.7 min (±2SD range: 120–608 min), decreasing sharply to 262.1 min in the first trimester and more gradually thereafter. Vigorous and moderate activity decreased more than light activity. TST and PAD were significantly associated with age, parity, and pregestational body fat percentage; lean body mass was negatively correlated with TST. Results were generally unaffected by seasonal variations.

Conclusion: Marked variations were found in pregestational TST and PAD. Healthy women slept ≥30 min longer during pregnancy, while PAD decreased by ≥ 90 min in early pregnancy and continued to decrease thereafter.

1. Introduction

Sleep and physical activity are essential and interrelated modes of life [1–6]. There have been few longitudinal studies of healthy pregnant women with combined sleep and activity measurements from before conception [7]. Pregestational assessments are important since major physiological changes in pregnancy are already present during the first trimester [8].

Changes in sleep patterns are a well-known phenomenon of normal pregnancy, and are often characterized by frequent awakenings and insomnia [9,10]. This lack of sleep might be partially compensated by daytime napping, indicating the importance of performing 24-h recording of sleep [11]. While poor sleep has been associated with an increase in pregnancy complications [12–18], physical activity can prevent certain complications. This situation has resulted in several recommendations on physical activity in pregnancy being published [19–23].

The maternal body composition changes during pregnancy [24]. As early as the first trimester, overweight (body mass index [BMI] 25–29.9 kg/m^2) is associated with more frequent snoring, insomnia, excessive daytime sleepiness, and short TST, even in otherwise healthy women [25]. During the second trimester there

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are higher risks of obstructive sleep apnea (OSA) syndrome and poor sleep quality [26]. The higher maternal fat mass associated with gestational weight gain [27–29] may also be related to sleep and physical activity in pregnancy. Furthermore, the family situation, number of children, and maternal profession have been shown to influence the circadian rhythm, sleep, and physical activity in healthy pregnancies [30,31]. Although some sleep studies in pregnancy accounted for BMI [18], more-specific and longitudinal analyses of body composition parameters and their influence are not available. In addition, sleep and physical activity have yet to be quantified simultaneously starting from before conception.

The aim of the present study was to determine the changes of simultaneously recorded 24-h sleep and activity duration in healthy women from before conception to late gestation. We hypothesized that there are pregnancy associated changes in sleep and activity, independent of maternal body composition, parity, and age. Further, we assumed that women with a higher percentage of body fat would generally sleep more and be less active over a 24-h period, and conversely, that those with a higher lean body mass would sleep less and be more physically active.

2. Materials and methods

This longitudinal observational study of sleep and physical activity is embedded in the ongoing CONIMPREG (conception-implantation interval and later development) research program. This is a composite research program collecting the data of healthy women from before conception, through pregnancy, and until the child reaches the age of 5 years.

Participants in the present study were recruited during the period 2014–2018 with the aid of social media (targeted Facebook® advertisements) and posters. Healthy nonsmoking women aged 20–35 years and with a BMI of 18–30 kg/m^2 were eligible provided they had an uncomplicated obstetric history and no chronic diseases or fertility problems. Women with appropriate substitution therapy for hypothyroidism were eligible. The participants did not use contraceptives at study entry, including the preceding month. Any participant who did not conceive after a maximum of six sampling cycles was excluded from the study. Written informed consent was obtained from all participants. The study was approved by the Regional Committee for Medical and Health Research Ethics in Norway [REK ref. 2013/856a].

At the first study visit (ie, before conception), height was measured manually using a wall-mounted stadiometer in a standardized way [32]. Weight was measured digitally using hand-to-foot multifrequency bioelectrical impedance analysis (model BC-418, Tanita, Tokyo, Japan). The percentage of body fat was estimated using the instrument's computer software, and the lean body mass was calculated by subtracting the body fat mass from the total body weight. Measurements were performed as recommended by the manufacturer [33] and immediately followed by physical activity and sleep recording.

Based on the last menstrual period, women were scheduled for a first-trimester ultrasound, and gestational age was adjusted according to crown-rump length [34]. Sleep and activity measurements were conducted using the SenseWear® Mini Armband actigraph (model MF-SW, BodyMedia, Pittsburgh, PA, USA) during the following periods (gestational age in weeks + days): 12 + 0–14 + 0, 23 + 0–25 + 0, and 35 + 0–37 + 0 (referred to here as first, second, and third trimesters, respectively; Fig. 1). The participants were instructed to wear the armband continuously on the upper posterior aspect of the nondominant arm for 4 days or more [35] and the recording of each day started at midnight. The SenseWear® actigraph is a wireless, noninvasive sleep and activity monitor that provides estimates of energy expenditure by recording daily life and lifestyle activities. It incorporates triaxial accelerometry, heat flux, galvanic skin response, skin temperature, and near-body temperature as well as demographic characteristics (sex, age, height, and weight) into propriety algorithms [36]. Sleep and wakefulness are discriminated based on motion and skin temperature. The model used in this study has a sampling rate of 32/sec in order to predict sleep versus wakefulness and physical activity with energy expenditure in 1-min epochs. In accordance with the SBRN (sedentary behavior research network) consensus [37] and ACSM (American college of sports medicine) guidelines, the activity was classified as light when the level of metabolic equivalents (MET's) was ≥1.5, moderate at ≥3.0, and vigorous at ≥6.0 [38]. SenseWear® actigraphy has shown good agreement when compared with other standard techniques such as double-labeled water or indirect calorimetry in humans of different ages [39–41]. The validity of this version of the actigraph (as well as older versions) for assessing activity during pregnancy and postpartum has been demonstrated [42–44]. SenseWear® actigraphy has also been validated for the measurement of sleep in other clinical and nonclinical, experimental settings with good agreement for estimating the total sleep time (TST) compared with polysomnography and other actigraphy sleep recordings [45–50]. The software calculated sleep efficiency (SE) as TST proportion (%) of the total duration lying down.

2.1. Statistics

The number of participants was limited by the allocated study period (2014–2018). Raw data were processed and summarized using the SenseWear® Pro analysis software (SenseWear® Professional, version 8.0.0.2903, Body Media) and exported into Excel workbooks (Microsoft Office, Excel version 2016, Redmond, WA, USA). Statistical analysis was performed using IBM SPSS® (version 24, Armonk, NY, USA), R (Foundation for Statistical Computing, version 3.6.1, Vienna, Austria), and (R-studio: Integrated development for R, Boston, MA, USA) software. Sampling days were excluded from the statistical analyses when the data loss exceeded 6% of a single day. This cutoff was chosen pragmatically as a compromise between recording quality aiming for precise 24-h sleep and activity measurements, and recording quantity with respect to the number of recorded days used for mean calculations.

Mean, standard deviation (SD), and range values were calculated for each continuous variable, and frequencies and proportions were calculated for categorical variables to describe the cohort. In addition, 95% confidence intervals (CIs) were calculated for the mean values of sleep and activity duration.

Random intercept models were used to analyze the repeated measurements of TST and physical activity duration (PAD). The pregnancy trimesters were defined as time categories and included as fixed effects in our models. The regression models were adjusted for age, parity, height, lean body mass, and fat percentage. We also tested whether the addition of a random slope by pregnancy trimester significantly improved the models.

As a measure of fit, the Akaike information criterion was calculated and compared against standard regression models using the method of least squares. Differences in the estimated model performance were tested using analysis of variance. Linearity assumptions and normal distribution of the residuals were ascertained, and calculations were carried out with and without outliers (>2SD). The variance inflation factor was calculated to identify possible problems with multicollinearity. Subanalyses were conducted using paired and unpaired Student's *t*-test or nonparametric tests. For all statistical analyses the criterion for statistical significance was set at $p < 0.05$.

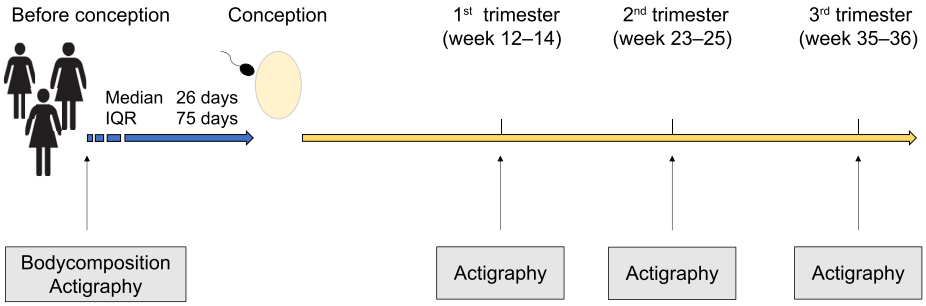

Fig. 1. Timing of sleep and physical activity measurements. The first assessment, performed before conception, was carried out at a median of 26 days before the last menstrual period.

3. Results

Of 249 eligible participants, 123 conceived with a successful pregnancy and were included in the present study (Fig. 2). Demographic information on the study cohort is provided in Table 1. All participants provided sufficient data for analysis at all measurement time points; however, due to some data loss during recording, 99 of the 1579 recorded days (5.9%) were excluded, resulting in a mean duration per recording of 3.6 days (95% CI 3.56–3.69 days). The median initial assessment was 7 weeks + 5 days prior to conception and followed up at gestational age 13 + 2, 24 + 3, and 36 + 1 (median number of weeks + days), respectively. The corresponding interquartile ranges (IQR) were 10 weeks +5 days, 5 days, 8 days, and 8 days.

3.1. Total sleep time

There was a notable normal variation in TST both before conception and during pregnancy (Appendix A), with a ±2SD span of 307–523 min before conception and 320–596 min at first trimester. The unadjusted mean daily TST increased from 415.3 min before conception (Table 2) to 458.0 min in early pregnancy, and remained increased for the remainder of the pregnancy. The same pattern was found after adjustments for maternal age, parity, and body composition factors, with an increase in TST of about 15 min over unadjusted levels at each time point (Table 2).

Compared with measurements made before conception, the adjusted mean TST (Fig. 3) was significantly longer (≥30 min) in all three trimesters ($p < 0.001$). Appendix B presents a comprehensive overview of the entire regression model with estimated effects of age, parity, and pregestational body composition parameters (height, lean body mass, and fat percentage).

3.2. Sleep efficiency

The adjusted mean 24-h sleep efficiency was before pregnancy 88.7% and significantly lower in the first and third trimesters (ie, by 1.4% and 3.1%, respectively). Apart from a negative correlation of 0.44% per 1-kg increment of lean body mass, demographic parameters and body composition variation did not affect sleep efficiency (Table 2 and Appendix C).

3.3. Physical activity duration

The unadjusted mean daily PAD, which was 363.7 min before conception, decreased to 262.1 min at the first trimester and continued to decrease during the remainder of the pregnancy (Table 2). The individual variation in PAD was large, with ±2SD ranging between 119.7 and 607.7 min before conception and between 44.7 and 479.5 min in the first trimester (Appendix A).

Adjustment for maternal age, parity, and body composition parameters did not alter this pattern (Table 2). The adjusted estimated reduction in PAD between the first and third trimester and between the second and third trimesters was 52.4 min (95% CI 73.6–31.3 min, $p < 0.001$) and 39.8 min (95% CI 61.1–18.5 min, $p < 0.001$), respectively (Fig. 3).

Comparison of the different unadjusted physical activity levels revealed that vigorous or moderate exercise constituted 27% of the total physical activity before conception, with the remainder being light activities (73%) (Table 2). In the first trimester (at gestational weeks 12–14) there was a shift toward lighter activities, with reductions in the proportions of vigorous or moderate, and light activity of 42% and 23%, respectively. The unadjusted overall duration of activity was reduced by 28%. At the end of pregnancy, the PAD had reduced by 41% compared with pregestational levels, but that of vigorous or moderate activity had reduced by 56%.

3.4. The influence of maternal pregestational characteristics

Both maternal age and parity were negatively associated with TST. For every 1-year increase in maternal age there was a reduction in daily TST of 3.1 min (95% CI 5.8–0.3 min, $p = 0.03$). Compared with nulliparous participants, those with parity ≥2 experienced a reduction in TST of 41.6 min (95% CI 70.9–12.4 min, $p = 0.01$). A positive association was found between pregestational body fat percentage and TST, with an increase of 1.9 min with each 1% increment in maternal body fat (95% CI 0.2–3.6 min, $p = 0.03$), while a negative association was found for pregestational lean body mass, with a reduction in TST of 3.7 min for every 1-kg increment in maternal lean body mass (95% CI 6.8–0.6 min, $p = 0.02$).

A negative association was also found between maternal age and PAD, with a reduction of 5.7 min for every 1-year increment in maternal age (95% CI 10.2–1.1 min, $p = 0.02$). In contrast to the negative association with TST, parity had a positive impact on PAD; significant effects were already seen from the first child, with an increase in PAD of 44.9 min (40.4–75.4 min, $p < 0.001$) for parity 1 and an increase of 75.0 min (95% CI 26.7–123.4 min, $p < 0.001$) for those with parity ≥2.

Pregestational body fat percentage was negatively associated with PAD, with a reduction of 4.8 min for every 1% increment in body fat (95% CI 7.6–2.0 min, $p < 0.001$). There was no relationship between lean body mass and PAD. An overview of the estimates can be found in Appendices B and C.

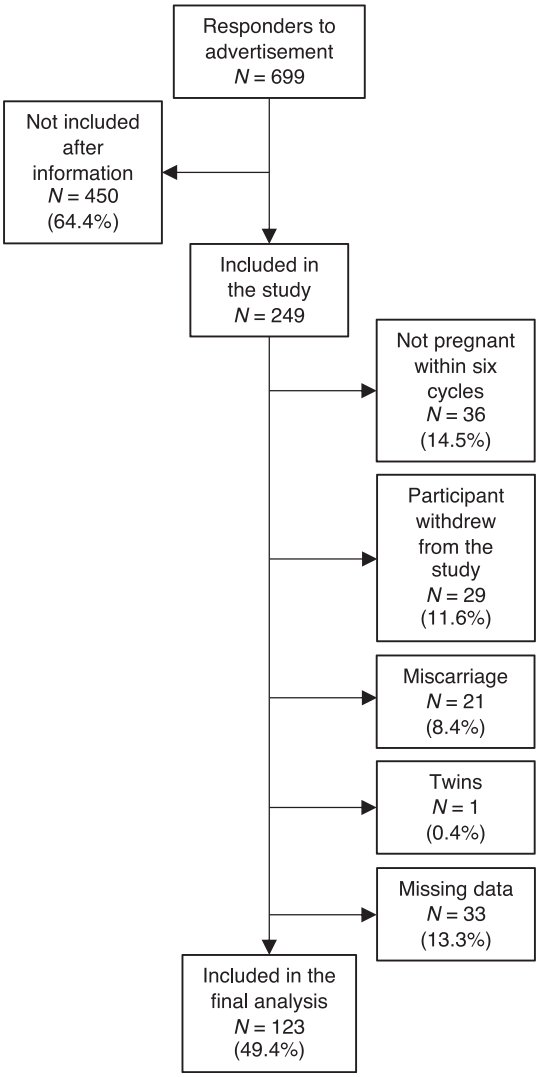

Fig. 2. Participant selection process.

3.5. Seasonal and weekday variations

Comparison of data obtained during the winter and summer seasons revealed no difference in TST at any measurement time point. Conversely, pregestational PAD was longer during the summer; however, there was no seasonal effect on PAD during pregnancy. Individual participant entry to the study was equally distributed over the year. The reduction in mean PAD was independent of season and statistically significant for both the winter and summer seasons (Appendix E).

The data were not confounded by weekday variation. Despite a slightly higher frequency of weekend recordings during the first trimester, recordings with a higher proportion of weekend days did not differ from those recordings with a lower proportion of weekdays (Appendix F).

Table 1
Demographic and anthropometric characteristics at study entry.

Characteristic	N	Mean±SD[a]	Range
Age, years	123	28.9 ± 3.3	20.0–35.0
Height, cm	123	167.9 ± 6.5	149.0–185.0
Weight, kg	123	64.7 ± 8.4	47.1–89.8
BMI[b], kg/m^2	123	22.9 ± 2.6	18.1–29.9
Lean body mass, kg	123	45.5 ± 3.7	36.0–54.9
Body fat, %	123	29.1 ± 5.4	15.9–41.9
Parity	123		
Para 0	60 (48.8%)		
Para 1	51 (41.5%)		
Para ≥2	12 (9.8%)		
Training habits[c]	123		
None	3 (2.3%)		
Effortless walking	33 (26.8%)		
<3 times/week	54 (43.9%)		
≥3 times/week	33 (26.8%)		

[a] SD, standard deviation.
[b] BMI, body mass index.
[c] Established using a non-validated questionnaire that each participant completed at study entry.

4. Discussion

This study found that in healthy women, TST was ≥30 min longer in early pregnancy compared with before conception, and it remained approximately at the same level for the remainder of the pregnancy. Conversely, PAD was reduced by ≥ 90 min in early pregnancy compared with before conception, and continued to decrease for the remainder of the pregnancy, with vigorous or moderate activity being impacted more than light activity. Variations in these patterns were significantly linked to parity, age, and pregestational body composition. Given that the study included healthy women with low-risk pregnancies, we believe that these patterns of changes in sleep are likely to be physiological adaptations to pregnancy.

Increases in TST and reductions in physical activity in pregnancy have been reported before, but they have only rarely been studied longitudinally from before conception [6,9,18,51-58], and seldom in combined sleep and activity assessments (ie, simultaneous recording of sleep and physical activity over 24-h periods) [59,60]. One study conducted using that combination and starting before pregnancy did not have sufficient statistical power to show significant changes in early pregnancy [7], while other studies were based on questionnaires, which are limited by their reliance on recall ability [61–67]. The present study had the advantage of higher statistical power and utilizing a technology that allowed continuous, around-the-clock recording of both sleep and physical activity.

4.1. Sleep

A previous study using nocturnal polysomnography recording in 33 pregnant women in their homes found that TST had increased by 34 min in the first trimester compared with prepregnancy [59]. Although polysomnography is considered the standard for nocturnal sleep assessment, it is not possible to record daytime sleep (ie, napping). A Swedish study that used 24-h heart-rate recording to assess PAD and TST in 12 women found no increase in TST compared with levels recorded before conception [7]. However, analyses of questionnaire data from 23 women in the same study demonstrated a significantly (5 min) longer TST at

Table 2
Unadjusted mean total sleep time (TST), total physical activity duration (PAD), sleep efficiency (SE), and durations of vigorous or moderate and light physical activity (min/day) before conception and through the pregnancy (ie, at 12–14, 23–25, and 35–37 gestational weeks). The adjusted mean was calculated for the main outcomes (TST and total PAD) in a linear regression model with a random intercept at the participant level. Adjustments were made based on cohort medians for age, parity, and body composition parameters (ie, for a 29-year-old nulliparous woman with height 167 cm, lean body mass 45 kg, and body fat 30%).

Time point	N[a]	Mean±SD[b]	Range	Adj.[c] mean	Adj. 95% CI[d]
Daily total sleep time, min					
Before conception	117	415.3 ± 54.0	269.8–542.0	429.1	414.5–443.7
1st trimester	117	458.0 ± 68.8	280.7–709.3	471.6	457.0–486.2
2nd trimester	115	450.9 ± 68.2	253.2–714.7	463.4	448.7–478.1
3rd trimester	113	446.1 ± 70.4	184.7–636.8	459.8	444.9–474.6
Daily sleep efficiency, %					
Before conception	117	82.71 ± 6.34	64.87–94.30	88.69	61.22–116.15
1st trimester	117	81.17 ± 6.51	61.49–95.23	87.29	59.83–114.75
2nd trimester	115	82.44 ± 7.07	52.62–96.41	88.44	61.0–115.89
3rd trimester	113	79.73 ± 8.31	50.23–95.61	85.55	58.09–113.01
Daily total physical activity duration, min					
Before conception	117	363.7 ± 122.0	119.0–829.5	331.7	307.6–355.8
1st trimester	117	262.1 ± 108.7	7.3–568.5	231.6	207.4–255.7
2nd trimester	115	250.6 ± 114.2	17.8–772.0	218.9	194.6–243.2
3rd trimester	113	214.8 ± 95.4	38.2–509.0	179.1	154.7–203.6
Daily light activity duration, min					
Before conception	117	98.6 ± 58.4	17.2–356.2		
1st trimester	117	56.4 ± 37.5	0.0–218.8		
2nd trimester	115	54.3 ± 47.9	0.0–324.0		
3rd trimester	113	43.5 ± 29.2	0.0–123.8		
Daily vigorous or moderate activity duration, min					
Before conception	117	267.2 ± 90.6	94.3–530.8		
1st trimester	117	207.7 ± 87.0	7.2–436.0		
2nd trimester	115	197.5 ± 86.6	18.2–448.0		
3rd trimester	113	172.7 ± 81.0	33.2–439.0		

[a] Missing data were due to periods of shortness of available actigraphs, technical problems, or incompatible family plans of the participants.
[b] SD, standard deviation.
[c] Adj., adjusted.
[d] CI, confidence interval.

gestational week 14 than before conception. Although the studies differ in their methods and results, they corroborate the pattern of sleep development in early pregnancy reported herein—a notable increase in TST compared with the immediate pregestational period.

Contrary to previous research, the present actigraphy study had the power to adjust for maternal factors; this adjustment did not significantly alter the change in TST from before conception (unadjusted 42.7 min, adjusted 42.5 min; Table 2). Accounting for the specific effects of age, lean body mass, fat percentage, and parity, we believe these results represent changes that are more specifically related to pregnancy (Fig. 3 and Appendix B).

During the second and third trimesters, neither the unadjusted nor adjusted TST changed significantly from that recorded in the first trimester, and thus remained higher than before pregnancy. The unadjusted mean TSTs of the second and third trimesters in the present study of 451 and 446 min, respectively, are longer than those reported by Tsai and colleagues in another actigraphy study: 433 min (95% CI 426–439 min) and 424 min (95% CI 417–431 min), respectively [54]. This difference may be attributable to demographic, cultural, and social differences in the study participants [68].

The negative association between TST in pregnancy and parity in the current study corroborates previous data [59]. The effect of parity persisted even after controlling for maternal age, which strengthens its significance. This is in line with another study showing that multiparous women had 10 min shorter TST than their nulliparous counterparts [58]. However, the difference was larger in the present study, 41.6 min. A larger number of participants and different parity subgrouping in the present study might explain some of the variations between the studies.

Fewer opportunities for daytime naps in parous women could be a reason for the difference in TST between women with and without children at the start of this study. It has been reported that the number of parous pregnant women having at least four naps per day is one-third of that in nulliparous women [58]. Daytime naps are common and already present in the first trimester of pregnancy, contributing to the daily TST [11,69,70]. While not necessarily influencing the duration of nighttime sleep, it has been suggested that napping compensates for the possible reduction in sleep quality during pregnancy [53]. Its importance for health during pregnancy is supported by reports on associations between napping and pregnancy outcome [71–75]. We found a reduced sleep efficiency in the first and third trimester, a possible sign of lower sleep quality, supporting an earlier questionnaire study [55]. We speculate that both the extended TST and daytime napping are part of the physiological adaptation to a normal pregnancy.

In our study, 10% more body fat before pregnancy was associated with an increase in TST of 19 min in pregnancy, while a 10-kg higher pregestational lean body mass was associated with a reduction in TST of 37 min. Intuitively, this pattern seems to fit with the notion that a physically active woman sleeps less and acquires relatively more lean mass and less fat. It is noteworthy that sleep deprivation in the general nonpregnant population is linked to an unfavorable body composition (ie, more fat and a lower lean body mass) [76–78]. This is not necessarily a contradiction since no direct comparisons can be drawn with the healthy women in the present study. However, another actigraphy study of early healthy pregnancies found no association between TST and BMI [18]. Little is known about the more-specific associations between lean body mass, body fat percentage, and sleep in healthy pregnancies. Whether or not sleep is a physiological adaption to pregnancy, it seems that women with a higher pregestational lean body mass combined with a lower body fat percentage might better tolerate the pregnancy-related changes, with a lower urge to rest or sleep during the day. Animal experiments have demonstrated

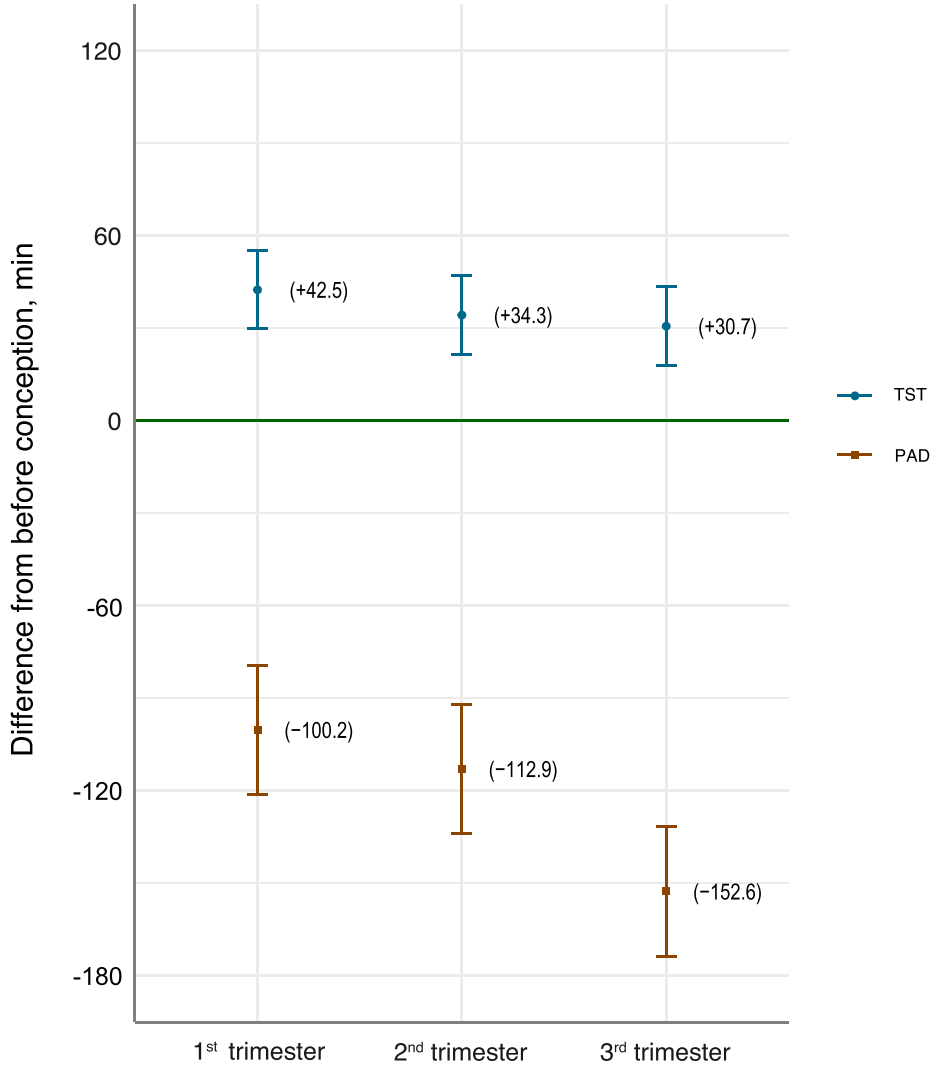

Fig. 3. Changes in mean total sleep time (TST) and total physical activity duration (PAD, min/24 h) in the first, second, and third trimesters of pregnancy over pregestational levels (set to zero in this graph). Data are mean and standard-deviation values for the increases (+) in TST and decreases (−) in PAD relative to pregestational levels, adjusted for pregestational maternal age, parity, height, lean body mass, and body fat percentage.

that gonadal steroids exert a circadian influence on sleep [79]. Conversely, the effects of sleep deprivation have structural and functional consequences for the neurodevelopment of the offspring and its viability [80]. It was shown recently that sleep deprivation in human pregnancies is associated with a higher BMI and higher diastolic blood pressure in the offspring [81], findings that should prompt further research.

4.2. Physical activity

This study assessed all levels of physical activity, including light activities such as housework and shopping. The health effects of vigorous or moderate activities are well known and included in activity guidelines for pregnancy [23,82]; however, there is also evidence supporting the health benefits of light activity [83]. In a study of adults with type 2 diabetes, light activities were more beneficial than structured exercise for glucose control and insulin sensitivity [83]. The previously reported shift from structured exercise to lighter activities during pregnancy [31,61] is confirmed by our results. This justifies the special interest in changes in PAD in our study. While previous SenseWear® actigraphy studies assessed the times spent at different activity levels in pregnancy [56,57,84,85], they neither included assessment of changes from before conception nor considered separate recordings from each trimester. One report on activity at gestational week 15 [57] corresponds well with ours at week 12–14 (Table 2). A couple of

prospective studies using other actigraphy models have shown a similar trend of decreasing physical activity in low-risk pregnancies [86] and obese women [87]. However, pregestational observations remain lacking.

It has been suggested that increasing weight, cardiac load, abdominal expansion, edema, joint pain, and altered sleep pattern impair the quality of life during pregnancy [88] and contribute to the altered physical activity described herein. The 24-h TST is affected by parity and age, but physical activity is negatively correlated with age and positively correlated with parity, suggesting independent effects (Appendix B). It is known that women with children and who are older, are more likely attending to housework and caregiving with less time to exercise [89]. In another Norwegian study, Berntsen et al. found a negative correlation between parity and vigorous or moderate activity in early pregnancy for an ethnically Western subgroup of women [56]. In a subanalysis of our data restricted to vigorous or moderate exercise, the influence of parity was no longer evident suggesting that the association between parity and PAD was dependent upon the level of activity and based mainly on light activities, consistent with previous questionnaire data [67,90].

In contrast to other studies, the present study also controlled for pregestational height, lean body mass, and body fat percentage (Appendix C). Body fat percentage was lower for a higher PAD, which we believe to be plausible [91,92]. We had hypothesized that lean body mass is positively related to physical activity, as shown for nonpregnant populations [93,94]; however, we found that this was not the case for either PAD or vigorous or moderate activity. This could be due to the high contribution of light (habitual) activity and may not be associated with body composition [95].

4.3. Strengths and weaknesses

The study's strengths were the large number of subjects and its prospective longitudinal quantification of sleep and physical activity that commenced before pregnancy. This design makes it possible to measure the effects of the onset of pregnancy on sleep and physical activity and eliminates the potentially confounding impact of recall bias that commonly hampers studies that start in early pregnancy or use questionnaires. Another strength is the current regression model calculating the average of the individual difference in sleep and physical activity from before conception through the entire pregnancy (Table 2 and Fig. 3). The model took the individual variation into account and provided reliable estimates in spite of missing data [96]. TST and PAD were never included in the same regression model since their measurement was based on recordings from the same device and on movement count during the same 24-h period. In this setting, TST and PAD are highly inversely correlated. In addition to age and parity, the models were adjusted for pregestational body composition (height, lean body mass, and body fat percentage) since the body composition in a nonpregnant population is known to be related to physical activity [97] and sleep [76]. Others have shown that sleep disturbances associated with preexisting body composition can be aggravated in pregnancy [98], supporting adjustment of data for those parameters, even in healthy pregnancies. Our analysis demonstrated that those adjustments have a high impact and should be considered when the amount of daily sleep and activity is assessed.

One strength of actigraphy is the ability to measure sleep and activity both simultaneously and continuously in a natural environment for several days. The reliability and validity of actigraphy have been studied extensively using sleep diaries and polysomnography, which are standard methods for sleep assessment [99]. In healthy populations, actigraphy is considered a valid and reliable alternative for recording sleep [46,50].

A weakness of actigraphy compared with polysomnography, is its algorithm which uses motion and temperature to discriminate between sleep and awakeness [100]. This makes the sleep estimates less specific or not appropriate for assessments of other sleep qualities such as efficiency, satisfaction, or alertness [48,99,101]. Our software did not compute sleep onset latency (SOL) and wake after sleep onset (WASO). Further, our protocol did not include a sleep diary that would allow assessment of precise bedtime, wake-up, and voluntary daytime napping. Correspondingly, we do not report these classic measures. The reported sleep efficiency should be interpreted with some caution since our values are not based on exclusively nocturnal recordings, but a continuous 24-h registration over several days, as for all our measurements.

In addition to body movements, skin temperature recordings are incorporated into the SenseWear® actigraphy algorithm to distinguish between the states of sleep and awake, and the SenseWear® actigraph is not validated for sleep recording in pregnancy. The physiological temperature changes that accompany pregnancy may thus result in a systematic bias; however, this limitation is mitigated in the present study by the same technique being used consistently for all repeated measurements. The actigraph used in the present study has also been tested for sleep-time measurements under different ambient temperature conditions [50], yielding no significant difference from polysomnography under comparable conditions [50]. Further, it was found to be comparable with polysomnography for measuring TST in the setting of sleep disturbance in patients with OSA and non-OSA healthy controls [48,49]. The correlation with polysomnography in OSA and controls was high, at 0.92 and 0.91, respectively [49]. An earlier version of our actigraph was also validated for physical activity measurements in pregnancy compared with indirect calorimetry [43].

Weekday and seasonal variations are known confounders for the type of study described here [102,103]. Seasonal differences are especially important in regions where daylight hours are substantially lower during winter, such as in the present study. We found the start of pregnancy to be evenly distributed during the seasons, and with the exception of a higher level of prepregnancy physical activity during summer, there was no significant effect of season on sleep or physical activity (Appendix E). Furthermore, the varying numbers of weekend day recordings included in the statistical analyses did not significantly affect either the TST or PAD data (Appendix F).

We acknowledge that not considering sick leave, daily working hours, or working times as confounding factors may be a limitation of this study. However, the longitudinal changes in a large and healthy cohort were assessed, with recordings made over several days including weekends. None of the women were sick before conception, and sick leave or reduced weekly working hours may rather be attributable to normal pregnancy-related changes, since none of the women whose data were analyzed were admitted to a hospital due to serious pathology.

4.4. Summary

With the aid of around-the-clock actigraphy recording, this longitudinal study demonstrated that healthy women sleep for nearly 7 h per day, but with substantial variation (±2SD range >3.5 h) in the weeks before becoming pregnant. In early pregnancy, total sleep time increased by $\geq$ 30 min and remained so for the remainder of the pregnancy. Conversely, total physical activity duration decreased by < 90 min in early pregnancy, followed by a continued decrease throughout the remainder of the pregnancy.

The decrease was more pronounced for vigorous or moderate exercise than for light activity. Age, parity, body fat percentage, and lean body mass influenced the results in these healthy women during their naturally conceived pregnancies. We consider these patterns of changes in sleep and physical activity to be normal adaptations to pregnancy.

CRediT authorship contribution statement

Alexander Viethee, MD: Methodology, investigation, software, data curation, visualization, validation, formal analysis, writing - original draft preparation, review and editing.

Torvid Kiserud, MD, Professor: Project administration, conceptualization, methodology, investigation, data curation, formal analysis, validation, visualization, writing - review & editing.

Rolv Terje Lie, Professor: Methodology, formal analysis, validation, visualization, writing - review & editing.

Øystein Ariansen Haaland, Professor: Methodology, software, formal analysis, validation, visualization, writing, review and editing.

Jörg Kessler, MD, Professor: Project administration, methodology, investigation, formal analysis, validation, visualization, writing - review & editing.

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Conflict of interest

The authors have no competing interest to declare.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2021.04.028

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2021.04.028.

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References

[1] Hawkins M, et al. Physical activity and sleep quality and duration during pregnancy among hispanic women: estudio PARTO. Behav Sleep Med 2019;17:804–17. https://doi.org/10.1080/15402002.2018.1518225.
[2] Borodulin K, et al. Physical activity and sleep among pregnant women. Paediatr Perinat Epidemiol 2010;24:45–52. https://doi.org/10.1111/j.1365-3016.2009.01081.x.
[3] Sherrill DL, Kotchou K, Quan SF. Association of physical activity and human sleep disorders. Arch Intern Med 1998;158:1894–8. https://doi.org/10.1001/archinte.158.17.1894.
[4] Hartescu I, Morgan K, Stevinson CD. Increased physical activity improves sleep and mood outcomes in inactive people with insomnia: a randomized controlled trial. J Sleep Res 2015;24:526–34. https://doi.org/10.1111/jsr.12297.
[5] Youngstedt SD. Effects of exercise on sleep. Clin Sports Med 2005;24(xi):355–65. https://doi.org/10.1016/j.csm.2004.12.003.
[6] Baker JH, Rothenberger SD, Kline CE, et al. Exercise during early pregnancy is associated with greater sleep continuity. Behav Sleep Med 2018;16:482–93. https://doi.org/10.1080/15402002.2016.1228649.
[7] Lof M, Forsum E. Activity pattern and energy expenditure due to physical activity before and during pregnancy in healthy Swedish women. Br J Nutr 2006;95:296–302. https://doi.org/10.1079/bjn20051497.
[8] Moya J, et al. A review of physiological and behavioral changes during pregnancy and lactation: potential exposure factors and data gaps. J Expo Sci Environ Epidemiol 2014;24:449–58. https://doi.org/10.1038/jes.2013.92.
[9] Izci-Balserak B, et al. Changes in sleep characteristics and breathing parameters during sleep in early and late pregnancy. J Clin Sleep Med 2018;14:1161–8. https://doi.org/10.5664/jcsm.7216.
[10] Driver HS, Shapiro CM. A longitudinal study of sleep stages in young women during pregnancy and postpartum. Sleep 1992;15:449–53. https://doi.org/10.1093/sleep/15.5.449.
[11] Tsai SY, Kuo LT, Lee CN, et al. Reduced sleep duration and daytime naps in pregnant women in Taiwan. Nurs Res 2013;62:99–105. https://doi.org/10.1097/NNR.0b013e3182830d87.
[12] Warland J, Dorrian J, Morrison JL, et al. Maternal sleep during pregnancy and poor fetal outcomes: a scoping review of the literature with meta-analysis. Sleep Med Rev 2018;41:197–219. https://doi.org/10.1016/j.smrv.2018.03.004.
[13] Lee KA, Gay CL. Sleep in late pregnancy predicts length of labor and type of delivery. Am J Obstet Gynecol 2004;191:2041–6. https://doi.org/10.1016/j.ajog.2004.05.086.
[14] Okun ML, Schetter CD, Glynn LM. Poor sleep quality is associated with preterm birth. Sleep 2011;34:1493–8. https://doi.org/10.5665/sleep.1384.
[15] Eichler J, Schmidt R, Hiemisch A, et al. Gestational weight gain, physical activity, sleep problems, substance use, and food intake as proximal risk factors of stress and depressive symptoms during pregnancy. BMC Pregnancy Childbirth 2019;19:175. https://doi.org/10.1186/s12884-019-2328-1.
[16] Facco FL, et al. Objectively measured short sleep duration and later sleep midpoint in pregnancy are associated with a higher risk of gestational diabetes. Am J Obstet Gynecol 2017;217:447.e1. https://doi.org/10.1016/j.ajog.2017.05.066. 447447.e13.
[17] Facco FL, et al. Association of adverse pregnancy outcomes with self-reported measures of sleep duration and timing in women who are nulliparous. J Clin Sleep Med 2018;14:2047–56. https://doi.org/10.5664/jcsm.7534.
[18] Haney A, Buysse DJ, Rosario BL, et al. Sleep disturbance and cardiometabolic risk factors in early pregnancy: a preliminary study. Sleep Med 2014;15:444–50. https://doi.org/10.1016/j.sleep.2014.01.003.
[19] Davenport MH, et al. Impact of prenatal exercise on maternal harms, labour and delivery outcomes: a systematic review and meta-analysis. Br J Sports Med 2019;53:99–107. https://doi.org/10.1136/bjsports-2018-099821.
[20] Davenport MH, et al. Prenatal exercise for the prevention of gestational diabetes mellitus and hypertensive disorders of pregnancy: a systematic review and meta-analysis. Br J Sports Med 2018;52:1367–75. https://doi.org/10.1136/bjsports-2018-099355.
[21] Davenport MH, et al. Impact of prenatal exercise on neonatal and childhood outcomes: a systematic review and meta-analysis. Br J Sports Med 2018;52:1386–96. https://doi.org/10.1136/bjsports-2018-099836.
[22] Davenport MH, et al. Impact of prenatal exercise on both prenatal and postnatal anxiety and depressive symptoms: a systematic review and meta-analysis. Br J Sports Med 2018;52:1376–85. https://doi.org/10.1136/bjsports-2018-099697.
[23] Evenson KR, et al. Guidelines for physical activity during pregnancy: comparisons from around the world. Am J Lifestyle Med 2014;8:102–21. https://doi.org/10.1177/1559827613498204.
[24] Most J, Marlatt KL, Altazan AD, et al. Advances in assessing body composition during pregnancy. Eur J Clin Nutr 2018;72:645–56. https://doi.org/10.1038/s41430-018-0152-8.
[25] Rice JR, et al. High risk for obstructive sleep apnea and other sleep disorders among overweight and obese pregnant women. BMC Pregnancy Childbirth 2015;15:198. https://doi.org/10.1186/s12884-015-0633-x.
[26] Facco FL, Kramer J, Ho KH, et al. Sleep disturbances in pregnancy. Obstet Gynecol 2010;115:77–83. https://doi.org/10.1097/AOG.0b013e3181c4f8ec.
[27] Lederman SA, et al. Body fat and water changes during pregnancy in women with different body weight and weight gain. Obstet Gynecol 1997;90:483–8. https://doi.org/10.1016/s0029-7844(97)00355-4.
[28] Butte NF, Ellis KJ, Wong WW, et al. Composition of gestational weight gain impacts maternal fat retention and infant birth weight. Am J Obstet Gynecol 2003;189:1423–32. https://doi.org/10.1067/s0002-9378(03)00596-9.
[29] Lederman SA, et al. Maternal body fat and water during pregnancy: do they raise infant birth weight. Am J Obstet Gynecol 1999;180:235–40. https://doi.org/10.1016/s0002-9378(99)70181-x.
[30] Ladyman C, Signal TL. Sleep health in pregnancy: a scoping review. Sleep Med Clin 2018;13:307–33. https://doi.org/10.1016/j.jsmc.2018.04.004.
[31] Abbasi M, Van Den Akker O. A systematic review of changes in women's physical activity before and during pregnancy and the postnatal period. J Reprod Infant Psychol 2015;33:325–58. https://doi.org/10.1080/02646838.2015.1012710.
[32] Lohman T. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Book; 1988.

[33] Body composition analyser BC-418-MA. Instruction Manual (Tanita Corporation of America).
[34] Robinson HP. Sonar measurement of fetal crown-rump length as means of assessing maturity in first trimester of pregnancy. Br Med J 1973;4:28–31. https://doi.org/10.1136/bmj.4.5883.28.
[35] Matthews CE, Ainsworth BE, Thompson RW, et al. Sources of variance in daily physical activity levels as measured by an accelerometer. Med Sci Sports Exerc 2002;34:1376–81. https://doi.org/10.1097/00005768-200208000-00021.
[36] Liden CB, et al. Benefits of the Sensewear®armband over other physical activity and energy expenditure measurement techniques. White Papers Body Media 2001;1:1–14.
[37] Tremblay MS, et al. Sedentary behavior research network (SBRN) - terminology consensus project process and outcome. Int J Behav Nutr Phys Activ 2017;14:75. https://doi.org/10.1186/s12966-017-0525-8.
[38] Thompson PD. In: Pescatello LS, Arena R, Riebe D, et al., editors. Benefits and risks associated with physical activity in ACSM'Guidelines for Exercise Testing and Prescription, vols. 2–14. Lippincott Williams&Wilkins; 2014.
[39] Johannsen DL, et al. Accuracy of armband monitors for measuring daily energy expenditure in healthy adults. Med Sci Sports Exerc 2010;42:2134–40. https://doi.org/10.1249/MSS.0b013e3181e0b3ff.
[40] Bhammar DM, Sawyer BJ, Tucker WJ, et al. Validity of Sensewear® armband v5.2 and v2.2 for estimating energy expenditure. J Sports Sci 2016;34:1830–8. https://doi.org/10.1080/02640414.2016.1140220.
[41] Calabró MA, Stewart JM, Welk GJ. Validation of pattern-recognition monitors in children using doubly labeled water. Med Sci Sports Exerc 2013;45:1313–22. https://doi.org/10.1249/MSS.0b013e31828579c3.
[42] Smith KM, Lanningham-Foster LM, Welk GJ, et al. Validity of the Sensewear® armband to predict energy expenditure in pregnant women. Med Sci Sports Exerc 2012;44:2001–8. https://doi.org/10.1249/MSS.0b013e31825ce76f.
[43] Berntsen S, Stafne SN, Mørkved S. Physical activity monitor for recording energy expenditure in pregnancy. Acta Obstet Gynecol Scand 2011;90:903–7. https://doi.org/10.1111/j.1600-0412.2011.01172.x.
[44] Slinde F, et al. Energy expenditure by multisensor armband in overweight and obese lactating women validated by doubly labeled water. Obesity 2013;21:2231–5. https://doi.org/10.1002/oby.20363.
[45] Sunseri M, et al. The sensewear® armband as a sleep detection device. White Papers Body Media 2009:1–9. http://www.integratedfitnesssystems.com/SenseWearAsSleepDetectionDevice.pdf.
[46] Peterson BT, et al. Comparison of actigraphy and polysomnography to assess effects of zolpidem in a clinical research unit. Sleep Med 2012;13:419–24. https://doi.org/10.1016/j.sleep.2011.12.003.
[47] Soric M, et al. Validation of a multi-sensor activity monitor for assessing sleep in children and adolescents. Sleep Med 2013;14:201–5. https://doi.org/10.1016/j.sleep.2012.11.003.
[48] O'Driscol DM, Turton AR, Copland JM, et al. Energy expenditure in obstructive sleep apnea: validation of a multiple physiological sensor for determination of sleep and wake. Sleep Breath 2013;17:139–46. https://doi.org/10.1007/s11325-012-0662-x.
[49] Sharif MM, Bahammam AS. Sleep estimation using bodymedia's sensewear® armband in patients with obstructive sleep apnea. Ann Thorac Med 2013;8:53–7. https://doi.org/10.4103/1817-1737.105720.
[50] Shin M, Swan P, Chow CM. The validity of actiwatch 2 and sensewear®armband compared against polysomnography at different ambient temperature conditions. Sleep Sci 2015;8:9–15. https://doi.org/10.1016/j.slsci.2015.02.003.
[51] Brunner DP, et al. Changes in sleep and sleep electroencephalogram during pregnancy. Sleep 1994;17:576–82. https://doi.org/10.1093/sleep/17.7.576.
[52] Little SE, Mcnamara CJ, Miller RC. Sleep changes in normal pregnancy. Obstet Gynecol 2014;123:153S. https://doi.org/10.1097/01.AOG.0000447145.52005.ac.
[53] Ebert RM, Wood A, Okun ML. Minimal effect of daytime napping behavior on nocturnal sleep in pregnant women. J Clin Sleep Med 2015;11:635–43. https://doi.org/10.5664/jcsm.4774.
[54] Tsai SY, Lee PL, Lin JW, et al. Cross-sectional and longitudinal associations between sleep and health-related quality of life in pregnant women: a prospective observational study. Int J Nurs Stud 2016;56:45–53. https://doi.org/10.1016/j.ijnurstu.2016.01.001.
[55] Hedman C, Pohjasvaara T, Tolonen U, et al. Effects of pregnancy on mothers' sleep. Sleep Med 2002;3:37–42. https://doi.org/10.1016/s1389-9457(01)00130-7.
[56] Berntsen S, et al. Objectively recorded physical activity in early pregnancy: a multiethnic population-based study. Scand J Med Sci Sports 2014;24:594–601. https://doi.org/10.1111/sms.12034.
[57] Richardsen KR, et al. Objectively recorded physical activity in pregnancy and postpartum in a multi-ethnic cohort: association with access to recreational areas in the neighbourhood. Int J Behav Nutr Phys Activ 2016;13:78. https://doi.org/10.1186/s12966-016-0401-y.
[58] Signal TL, et al. Sleep duration and quality in healthy nulliparous and multiparous women across pregnancy and post-partum. Aust N Z J Obstet Gynaecol 2007;47:16–22. https://doi.org/10.1111/j.1479-828X.2006.00672.x.
[59] Lee KA, Zaffke ME, Mcenany G. Parity and sleep patterns during and after pregnancy. Obstet Gynecol 2000;95:14–8. https://doi.org/10.1016/s0029-7844(99)00486-x.
[60] Poudevigne MS, O'connor PJ. Physical activity and mood during pregnancy. Med Sci Sports Exerc 2005;37:1374–80. https://doi.org/10.1249/01.mss.0000174907.27818.ff.
[61] Sjögren Forss K, Ekvall Hansson E, Troein M, et al. Patterns of physical activity among women and men before and during pregnancy. Publ Health 2014;128:814–6. https://doi.org/10.1016/j.puhe.2014.06.010.
[62] Herring SJ, et al. Do pregnant women accurately report sleep time? A comparison between self-reported and objective measures of sleep duration in pregnancy among a sample of urban mothers. Sleep Breath 2013;17:1323–7. https://doi.org/10.1007/s11325-013-0835-2.
[63] Wilson DL, et al. Decreased sleep efficiency, increased wake after sleep onset and increased cortical arousals in late pregnancy. Aust N Z J Obstet Gynaecol 2011;51:38–46. https://doi.org/10.1111/j.1479-828X.2010.01252.x.
[64] Clarke PE, Rousham EK, Gross H, et al. Activity patterns and time allocation during pregnancy: a longitudinal study of British women. Ann Hum Biol 2005;32:247–58. https://doi.org/10.1080/03014460500049915.
[65] Mottola MF, Campbell MK. Activity patterns during pregnancy. Can J Appl Physiol 2003;28:642–53. https://doi.org/10.1139/h03-049.
[66] Weallens E, Clark A, Maclntyre P, et al. A survey of exercise patterns in primigravidae at a Scottish NHS trust: more consistent support and advice required for women and their families. Health Educ J 2003;62:234–45. https://doi.org/10.1177/001789690306200305.
[67] Chasan-Taber L, et al. Correlates of physical activity in pregnancy among Latina women. Matern Child Health J 2007;11:353–63. https://doi.org/10.1007/s10995-007-0201-8.
[68] Reid KJ, et al. Sleep during pregnancy: the numom2b pregnancy and sleep duration and continuity study. Sleep 2017;40:3089705. https://doi.org/10.1093/sleep/zsx045.
[69] Okun ML, Coussons-Read ME. Sleep disruption during pregnancy: how does it influence serum cytokines. J Reprod Immunol 2007;73:158–65. https://doi.org/10.1016/j.jri.2006.06.006.
[70] Mindell JA, Cook RA, Nikolovski J. Sleep patterns and sleep disturbances across pregnancy. Sleep Med 2015;16:483–8. https://doi.org/10.1016/j.sleep.2014.12.006.
[71] Izci Balserak B, Jackson N, Ratcliffe SA, et al. Sleep-disordered breathing and daytime napping are associated with maternal hyperglycemia. Sleep Breath 2013;17:1093–102. https://doi.org/10.1007/s11325-013-0809-4.
[72] Tsai SY, Lin JW, Kuo LT, et al. Nighttime sleep, daytime napping, and labor outcomes in healthy pregnant women in Taiwan. Res Nurs Health 2013;36:612–22. https://doi.org/10.1002/nur.21568.
[73] Song L, et al. Afternoon napping during pregnancy and low birth weight: the healthy baby cohort study. Sleep Med 2018;48:35–41. https://doi.org/10.1016/j.sleep.2018.03.029.
[74] Zheng X, et al. Maternal habitual midday napping duration and frequency are associated with high birthweight. Sci Rep 2017;7:10564. https://doi.org/10.1038/s41598-017-09683-3.
[75] Rawal S, Hinkle SN, Zhu Y, et al. A longitudinal study of sleep duration in pregnancy and subsequent risk of gestational diabetes: findings from a prospective, multiracial cohort. Am J Obstet Gynecol 2017;216:399.e1–8. https://doi.org/10.1016/j.ajog.2016.11.1051.
[76] Ogilvie RP, Patel SR. The epidemiology of sleep and obesity. Sleep Health 2017;3:383–8. https://doi.org/10.1016/j.sleh.2017.07.013.
[77] Ogilvie RP, et al. Sex and race differences in the association between sleep duration and adiposity: the Bogalusa heart study. Sleep Health 2019;5:84–90. https://doi.org/10.1016/j.sleh.2018.10.010.
[78] Kim K, Shin D, Jung GU, et al. Association between sleep duration, fat mass, lean mass and obesity in Korean adults: the fourth and fifth Korea national health and nutrition examination surveys. J Sleep Res 2017;26:453–60. https://doi.org/10.1111/jsr.12504.
[79] Mong JA, Cusmano DM. Sex differences in sleep: impact of biological sex and sex steroids. Philos Trans R Soc Lond B Biol Sci 2016;371. https://doi.org/10.1098/rstb.2015.0110. 20150110.
[80] Pires GN, et al. Effects of sleep modulation during pregnancy in the mother and offspring: evidences from preclinical research. J Sleep Res 2020:e13135. https://doi.org/10.1111/jsr.13135. EPub ahead of print.
[81] Harskamp-Van Ginkel MW, et al. Gestational sleep deprivation is associated with higher offspring bmi and blood pressure. Sleep 2020;43:zsaa110. https://doi.org/10.1093/sleep/zsaa110/33345023/zsaa110.pdf.
[82] Mottola MF, et al. No. 367-2019 Canadian guideline for physical activity throughout pregnancy. J Obstet Gynaecol Can 2018;40:1528–37. https://doi.org/10.1016/j.jogc.2018.07.001.
[83] Duvivier BM, et al. Breaking sitting with light activities vs structured exercise: a randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. Diabetologia 2017;60:490–8. https://doi.org/10.1007/s00125-016-4161-7.
[84] Mørkrid K, et al. Objectively recorded physical activity and the association with gestational diabetes. Scand J Med Sci Sports 2014;24:e389–97. https://doi.org/10.1111/sms.12183.
[85] Richardsen KR, et al. Predicting who fails to meet the physical activity guideline in pregnancy: a prospective study of objectively recorded physical activity in a population-based multi-ethnic cohort. BMC Pregnancy Childbirth 2016;16:186. https://doi.org/10.1186/s12884-016-0985-x.
[86] Rousham EK, Clarke PE, Gross H. Significant changes in physical activity among pregnant women in the UK as assessed by accelerometry and self-reported activity. Eur J Clin Nutr 2006;60:393–400. https://doi.org/10.1038/sj.ejcn.1602329.

[87] McParlin C, et al. Objectively measured physical activity during pregnancy: a study in obese and overweight women. BMC Pregnancy Childbirth 2010;10:76. https://doi.org/10.1186/1471-2393-10-76.
[88] Lagadec N, et al. Factors influencing the quality of life of pregnant women: a systematic review. BMC Pregnancy Childbirth 2018;18:455. https://doi.org/10.1186/s12884-018-2087-4.
[89] Sternfeld B, Ainsworth BE, Quesenberry Jr CP. Physical activity patterns in a diverse population of women. Prev Med 1999;28:313–23. https://doi.org/10.1006/pmed.1998.0470.
[90] Borodulin KM, Evenson KR, Wen F, et al. Physical activity patterns during pregnancy. Med Sci Sports Exerc 2008;40:1901–8. https://doi.org/10.1249/MSS.0b013e31817f1957.
[91] Esparza J, et al. Daily energy expenditure in Mexican and USA Pima Indians: low physical activity as a possible cause of obesity. Int J Obes Relat Metab Disord 2000;24:55–9. https://doi.org/10.1038/sj.ijo.0801085.
[92] Pratt M, Macera CA, Blanton C. Levels of physical activity and inactivity in children and adults in the United States: current evidence and research issues. Med Sci Sports Exerc 1999;31:S526–33. https://doi.org/10.1097/00005768-199911001-00007.
[93] Deere K, Sayers A, Davey Smith G, et al. High impact activity is related to lean but not fat mass: findings from a population-based study in adolescents. Int J Epidemiol 2012;41:1124–31. https://doi.org/10.1093/ije/dys073.
[94] Vainionpää A, et al. Intensity of exercise is associated with bone density change in premenopausal women. Osteoporos Int 2006;17:455–63. https://doi.org/10.1007/s00198-005-0005-x.
[95] Speakman JR, Westerterp KR. Associations between energy demands, physical activity, and body composition in adult humans between 18 and 96 y of age. Am J Clin Nutr 2010;92:826–34. https://doi.org/10.3945/ajcn.2009.28540.
[96] R-Core-team, Bates D, Debroy S, et al. Linear mixed-effect models (lme); package nlme, version 3.1-152. RDokumentation; 2021.
[97] Westerterp KR. Changes in physical activity over the lifespan: impact on body composition and sarcopenic obesity. Obes Rev 2018;19(Suppl 1):8–13. https://doi.org/10.1111/obr.12781.
[98] Izci Balserak B, Lee KA. Sleep and sleep disorders associated with pregnancy in Principles and Practice of sleep medicine. In: Kryger M, Roth T, Dement WC, editors. Elsevier; 2017. 1525.e5. https://doi.org/10.1016/B978-0-323-24288-2.00156-2. 1539.e5.
[99] Sadeh A. The role and validity of actigraphy in sleep medicine: an update. Sleep Med Rev 2011;15:259–67. https://doi.org/10.1016/j.smrv.2010.10.001.
[100] Zhu B, et al. Objective sleep in pregnant women: a comparison of actigraphy and polysomnography. Sleep Health 2018;4:390–6. https://doi.org/10.1016/j.sleh.2018.07.011.
[101] Buysse DJ. Sleep health: can we define it? Does it matter. Sleep 2014;37:9–17. https://doi.org/10.5665/sleep.3298.
[102] Aili K, Åström-Paulsson S, Stoetzer U, et al. Reliability of actigraphy and subjective sleep measurements in adults: the design of sleep assessments. J Clin Sleep Med 2017;13:39–47. https://doi.org/10.5664/jcsm.6384.
[103] Lehnkering H, Siegmund R. Influence of chronotype, season, and sex of subject on sleep behavior of young adults. Chronobiol Int 2007;24:875–88. https://doi.org/10.1080/07420520701648259.

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Corrigendum to "Sleep and physical activity from before conception to the end of pregnancy in healthy women: A longitudinal actigraphy study" [Sleep Med 83 (2021) 89–98]

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The authors regret to point out that two sub-headings in Table 2 (i.e., ***'Daily vigorous or moderate activity duration, min'*** and ***'Daily light activity duration, min'***) have been swapped. The heading in row 17 should be in the position of the heading in row 22, and conversely, the heading in row 22 should be in the position of the one in row 17 *(please find the revised Table 2 below)*.

The authors would like to apologise for any inconvenience caused.

Revised Table 2:

Table 2

Time point	*N*	Mean ± SD[a]	Range	Adj.[b] mean	Adj. 95% CI[c]
Daily total sleep time, min					
Before conception	117	415.3 ± 54.0	269.8–542.0	429.1	414.5–443.7
1st trimester	117	458.0 ± 68.8	280.7–709.3	471.6	457.0–486.2
2nd trimester	115	450.9 ± 68.2	253.2–714.7	463.4	448.7–478.1
3rd trimester	113	446.1 ± 70.4	184.7–636.8	459.8	444.9–474.6
Daily sleep efficiency, %					
Before conception	117	82.71 ± 6.34	64.87–94.30	88.69	61.22–116.15
1st trimester	117	81.17 ± 6.51	61.49–95.23	87.29	59.83–114.75
2nd trimester	115	82.44 ± 7.07	52.62–96.41	88.44	61.0–115.89
3rd trimester	113	79.73 ± 8.31	50.23–95.61	85.55	58.09–113.01

(*continued on next column*)

Table 2 (*continued*)

Time point	*N*	Mean ± SD[a]	Range	Adj.[b] mean	Adj. 95% CI[c]
Daily total physical activity duration, min					
Before conception	117	363.7 ± 122.0	119.0–829.5	331.7	307.6–355.8
1st trimester	117	262.1 ± 108.7	7.3–568.5	231.6	207.4–255.7
2nd trimester	115	250.6 ± 114.2	17.8–772.0	218.9	194.6–243.2
3rd trimester	113	214.8 ± 95.4	38.2–509.0	179.1	154.7–203.6
Daily light activity duration, min					
Before conception	117	267.2 ± 90.6	94.3–530.8		
1st trimester	117	207.7 ± 87.0	7.2–436.0		
2nd trimester	115	197.5 ± 86.6	18.2–448.0		
3rd trimester	113	172.7 ± 81.0	33.2–439.0		
Daily vigorous or moderate activity duration, min					
Before conception	117	98.6 ± 58.4	17.2–356.2		
1st trimester	117	56.4 ± 37.5	0.0–218.8		
2nd trimester	115	54.3 ± 47.9	0.0–324.0		
3rd trimester	113	43.5 ± 29.2	0.0–123.8		

[a] SD, standard deviation.
[b] Adj., adjusted.
[c] CI, confidence interval.

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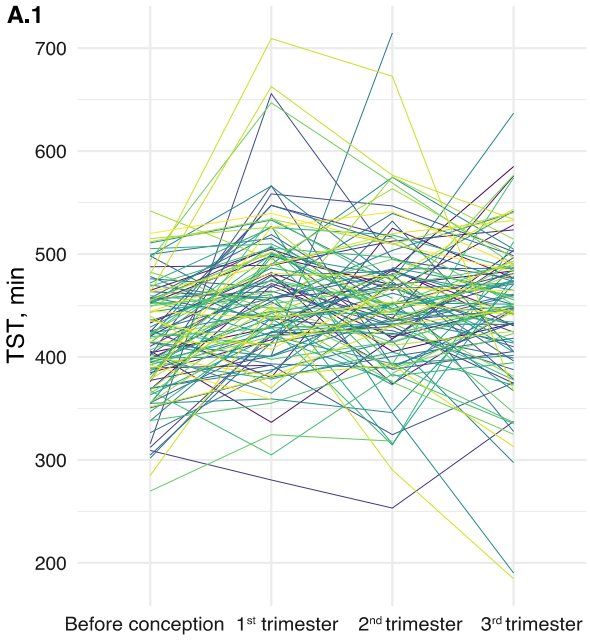
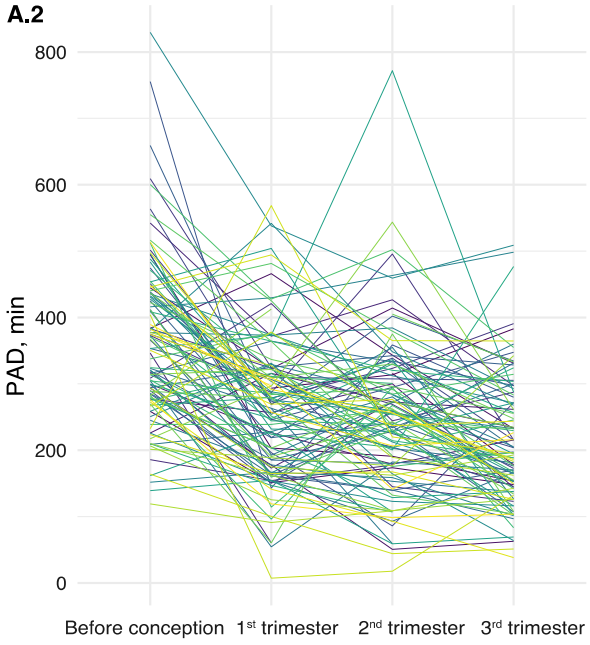
Appendix A Total sleep time (TST) (**Figure A.1)** and total physical activity duration (PAD) **(Figure A.2)** in individual subjects from before conception to the end of pregnancy in 123 healthy women. Each point represents the individual mean of the actigraphy recordings.





Appendix B TST changes from before conception to the end of pregnancy, and the influence of maternal pregestational covariates. The mean estimate for the before-conception category (reference) was calculated for a 29-year-old nulliparous woman with height 167 cm, lean body mass 45 kg, and body fat 30%. There was a significant variation in TST across the participants. Minus signs reflect a reduction in TST.

Table B.1

Changes in TST compared with before conception, min

	Estimate (mean)	Adj. 95% CI*	*p*
Before conception	429.1	(414.5–443.7)	
(reference)			
1st trimester	42.5	(29.8–55.2)	<0.001
2nd trimester	34.3	(21.5–47.1)	<0.001
3rd trimester	30.7	(17.8–43.5)	<0.001
Maternal pregestational factors			
Age (per 1-year increment)	–3.1	(–5.8 to –0.3)	0.02
Para 1 (relative to para 0)	–14.8	(–33.3 to 3.6)	<0.001
Para 2+ (relative to para 0)	–41.6	(–70.9 to –12.4)	<0.001
Height (per 1-cm increment)	1.0	(–0.7 to 2.7)	0.32
Lean body mass (per 1-kg increment)	–3.7	(–6.8 to –0.6)	0.55
Fat percentage (per 1% increment)	1.9	(0.2 to 3.6)	<0.001

* Adj. 95% CI, adjusted 95% confidence interval

Appendix C

Table C.1 Sleep efficiency (SE) changes from before conception to the end of pregnancy, and the influence of maternal pregestational covariates. The mean estimate for the before-conception category (reference) was calculated for a 29-year-old nulliparous woman with height 167 cm, lean body mass 45 kg, and body fat 30%. There was a significant variation in SE across the participants. Minus signs reflect a reduction in SE.

Changes in SEF compared with before conception, %			
	Estimate (mean)	Adj. 95% CI*	*p*
Before conception	88.69	(61.22–116.15)	
(reference)			
1st trimester	–1.396	(–2.702 to –0.090)	<0.001
2nd trimester	–0.240	(–1.554 to 1.073)	0.7
3rd trimester	–3.134	(–4.453 to –1.816)	<0.001
Maternal pregestational factors			
Age (per 1-year increment)	0.065	(–0.257 to 0.388)	0.7
Para 1 (relative to para 0)	0.452	(–1.699 to 2.604)	0.7
Para 2+ (relative to para 0)	–1.035	(–4.446 to –2.376)	0.6
Height (per 1-cm increment)	–0.093	(–0.100 to 0.286)	0.3
Lean body mass (per 1-kg increment)	–0.442	(–0.803 to –0.081)	<0.018
Fat percentage (per 1% increment)	–0.116	(-0.311 to 0.078)	0.2

* Adj. 95% CI, adjusted 95% confidence interval

Appendix D Total PAD changes from before conception and the influence of maternal pregestational covariates. The mean estimate for the before-conception category (reference) was calculated for a 29-year-old nulliparous woman with height 167 cm, lean body mass 45 kg, and body fat 30%. There was significant variation of the total PAD across the participants. Minus signs reflect a reduction in PAD.

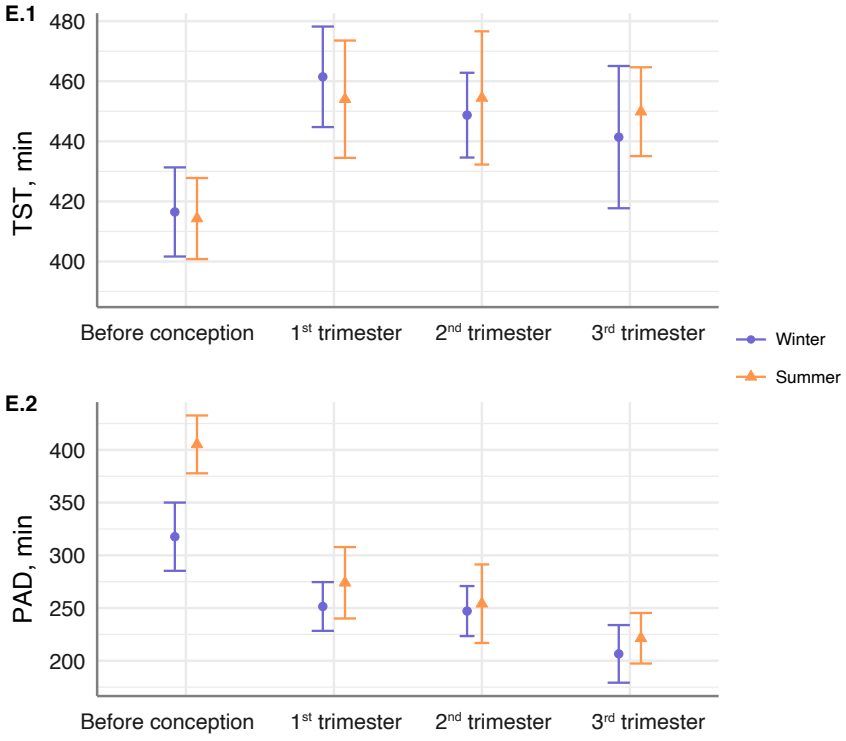
Table D.1

Changes in PAD from before conception, min

	Estimate (mean)	Adj. 95% CI*	*p*
Before conception (reference)	331.7	307.6–355.8	
1st trimester	–100.1	–121.0 to –79.2	<0.001
2nd trimester	–112.8	–133.8 to –91.8	<0.001
3rd trimester	–152.6	–173.7 to –131.5	<0.001
Maternal pregestational factors			
Age (per 1-year increment)	–5.7	–10.2 to –1.1	0.02
Para 1 (relative to para 0)	44.9	14.4–75.4	<0.001
Para ≥2 (relative to para 0)	75.0	26.7–123.4	<0.001
Height (per 1-cm increment)	1.4	–1.4 to 4.1	0.32
Lean body mass (per 1-kg increment)	–1.6	–6.7 to 3.6	0.55
Fat percentage (per 1% increment)	–4.8	–7.6 to –2.0	<0.001

* Adj. 95% CI, adjusted 95% confidence interval

Appendix E Season did not significantly influence 24-hour TST for the measurements carried out before or during pregnancy (**E.1**), but there was a significant seasonal influence on the PAD (**E.2**). PAD was significantly longer during summer before pregnancy, but did not differ significantly between summer and winter during pregnancy. The month when pregnancy started was reasonably evenly distributed through the year. Data are mean and 95% CI values.



Appendix F Influence of including weekend days in the 24-hour actigraphy recordings. There was a slightly higher proportion of weekend days in 1st trimester recordings than before conception. The Kruskal-Wallis test did not reveal any significant differences in the TST and PAD between the groups with the highest and lowest proportions of weekend days. These findings suggest that including weekend days did not confound the results.

Table F.1

Proportion of actigraphy recordings done on workdays	*N*	Mean rank (TST 1st trimester)	Mean rank (PAD 1st trimester)
0–33%	9	53.61	58.44
34–50%	65	59.42	56.99
51–83%	19	54.03	52.11
84–100%	22	59.07	65.89
Total	115		
Kruskal-Wallis *H*		0.564	1.886
p		0.905	0.569

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Effect of maternal sleep on embryonic development

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The concept of developmental origin of health and disease has ignited a search for mechanisms and health factors influencing normal intrauterine development. Sleep is a basic health factor with substantial individual variation, but its implication for early prenatal development remains unclear. During the embryonic period, the yolk sac is involved in embryonic nutrition, growth, hematopoiesis, and likely in fetal programming. Maternal body measures seem to influence its size in human female embryos. In this prospective, longitudinal observational study of 190 healthy women recruited before natural conception, we assessed the effect of prepregnant sleep duration (actigraphy) on the fetal crown-rump-length (CRL) and yolk sac size (ultrasound). All women gave birth to a live child. The prepregnancy daily sleep duration had an effect on the male yolk sac and CRL at the earliest measurement only (7 weeks). I.e., the yolk sac diameter decreased with increasing sleep duration (0.22 mm·h^{-1}d^{-1}, 95%CI [0.35-0.09], *P* < *0.01*), and CRL decreased (0.92 mm·h^{-1}d^{-1}, 95%CI [1.77-0.08], *P* = 0.03). Since there was no association at the second measurement (10 weeks), and in the group of female fetuses at any measure point, we suggest a sex- and time-dependent embryonic adaptation to sleep generated differences in the intrauterine environment in normal pregnancies.

It is widely accepted that sleep is essential for our health and physiologic homeostasis. This is certainly the case for pregnancy, as sleep disturbances are associated with pregnancy complications[1], such as preeclampsia, gestational diabetes, preterm birth, and prolonged birth duration[2–6]. Ethical reasons restrict in vivo experiments, so studies of sleep effects on human embryonic physiology and development are scarce[1,7]. This is a dilemma, since physiology and its normal variation lay the foundation for the understanding of pathology and disease. Why is, for example, more than one nightshift per week associated with a 30% higher risk of early pregnancy loss?[8] What are the pathophysiologic mechanisms? And, how much of regular sleep is needed in pregnancy?

In vitro experiments in assisted reproduction and animal studies demonstrate a possible circadian rhythm link to embryonic development and epigenetic programming via melatonin, cortisol, and sex steroids[9–17]. Further, deep sleep influences the growth hormone secretion in humans[18], which in turn has notable effects on fertility and the intra-uterine environment[19]. Likewise, sleep disturbances (such as insomnia) are linked to HPA-axis activation[20] and rise in cortisol levels[21]—a stress response with capability to impair fertility during critical periods, ovulation, implantation, and placental growth and development[22]. Nevertheless, animal studies, in vitro studies, and studies on infertile cohorts seeking health services cannot necessarily reflect the normal physiology of healthy women; this is where we can employ ultrasound as a non-invasive method.

In pregnancy, the human yolk sac is one of the first structures that can be assessed by ultrasound[23]. It has a complex surface structure that ensures embryonal nutrition and growth before the placenta is sufficiently developed[24,25], and its size is associated with fetal anomalies and unfavorable pregnancy outcomes[26–28]. Previous animal studies have demonstrated yolk sac size variation related to environmental factors, e.g., noise, temperature, and nutrition[27,29,30]. Further, women's body composition, i.e., lower weight and height have been linked to larger yolk sac[31], while human sleep with its large-scale physiologic variation has not been a focus of research[32,33].

Thus, we hypothesized that maternal sleep has an effect on embryonic development in humans, and aimed at assessing the effect of maternal sleep recordings on measurements of yolk sac size and crown-rump-length (CRL).

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Method

The present study is a prospective, longitudinal observational study of the relation of actigraphy measured sleep in healthy women with ultrasound assessed early fetal development. It is embedded in the ongoing CONIMPREG research program which collects data of healthy women from before conception, through pregnancy, and until the child reaches the age of 5 years.

Study cohort

The participants were recruited during the period 2014–2020 by means of social media (targeted Facebook® advertisements) and posters. Healthy non-smoking women aged 20–35 years with a BMI of 18–30 kg/m^2 were eligible provided they had an uncomplicated obstetric history and no chronic diseases or fertility problems. The participants did not use contraceptives at study entry, including the preceding month. The participants who did not conceive during any of the 6 monitored cycles allotted to them, discontinued the participation (Fig. 1). The study was approved by the Regional Committee for Medical Research Ethics South East Norway (REK South East, ref. 2013/856a). Written informed consent was obtained from all participants and all research was performed in accordance with relevant guidelines and regulations.

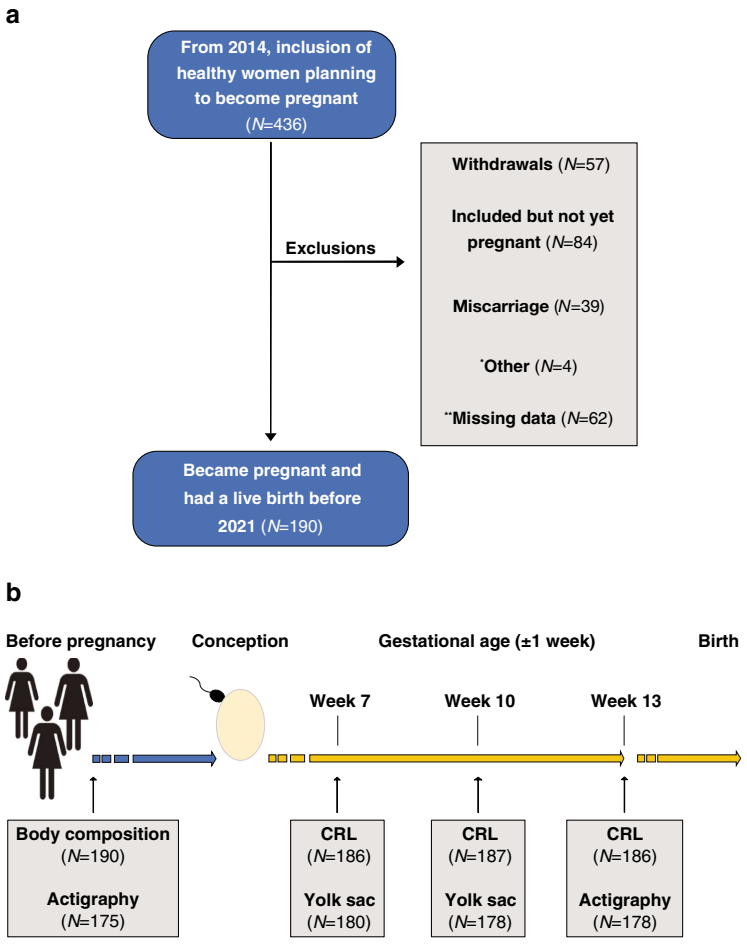

Figure 1. (**a**) Flowchart of the study population with the participant exclusions—*other: 1 twin pregnancy, 2 abortions > 18 weeks (fetal anomaly), and 1 irregular menstrual cycle; **missing data: Pregnancies with incomplete entry data ($N = 54$) and pregnant < 13 weeks at time of data extraction ($N = 8$). (**b**) Timing of measurements and recordings.

Data collection

The current study includes data from four consecutive study visits (Fig. 1). At the first visit (i.e., at inclusion and before the participants attempted to conceive), prepregnant height was measured manually using a wall-mounted stadiometer[34] and weight was measured digitally using hand-to-foot bioelectrical impedance analysis (model BC-418, Tanita, Tokyo, Japan). The body fat percentage was estimated using the instrument's computer software, and the lean body mass was calculated by subtracting the body fat mass from the total body weight. Measurements were performed as recommended by the manufacturer[35] and immediately followed by actigraphy recording. Following inclusion, the woman monitored her menstrual cycles by recording events, such as bleeding, sexual intercourse, and pregnancy tests. Once she had a positive pregnancy test, she reported to the study office and was scheduled for the second visit at gestational age (GA) 7 ± 1 weeks calculated from her last menstrual period (LMP). At that second visit, the gestational age was confirmed using transvaginal ultrasound scanning. The embryonic CRL and secondary yolk sac were assessed[36]. Later in pregnancy, at the third visit, 10 ± 1 weeks, the ultrasound measurements were repeated and at the fourth visit, 13 ± 1 weeks (end of the 1st trimester), maternal body composition and sleep duration were re-assessed along with the sonographic measurement of the CRL (Fig. 1).

Actigraphy

Sleep duration was recorded with the SenseWear Mini Armband, model MF-SW, BodyMedia, Pittsburgh, PA, USA. The monitor was worn on the upper posterior aspect of the nondominant arm for 4 days[37] and the recording started at midnight. The SenseWear actigraph is a wireless, noninvasive monitor with a sampling rate of 32/sec and incorporates triaxial accelerometry, heat flux, galvanic skin response, skin temperature, and near-body temperature, and takes into account information on sex, age, height, and weight[38]. Sleep and wakefulness are discriminated based on motion and skin temperature in 1-min epochs. Sleep raw data were processed and summarized using the manufacturer's analysis software (SenseWear Professional, version 8.0.0.2903, Body Media) and exported into Excel (Microsoft Office, Excel version 2016, Redmond, WA, USA). This actigraph has been compared with other models and standard measurement methods in clinical and experimental settings[39–44]. Recently, our group used this monitor to measure sleep in pregnancy with results in good agreement with other actigraphy studies[33]. The sleep efficiency (SE) was calculated by the software as sleep proportion of the time lying down.

Embryonic and fetal assessment

The fetal crown-rump-length and yolk sac size were examined by seven obstetricians and one certified midwife using a transvaginal ultrasound transducer, 6–12 MHz, Voluson Expert E8; (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). The yolk sac diameter was determined as the average of two perpendicular diameters repeated thrice[31]. Yolk sac growth rate was calculated as the difference in yolk sac diameter divided by the number of days between the two measurements.

Statistics

Statistical analysis was performed using SPSS (version 24, Armonk, NY, USA), R (Foundation for Statistical Computing, version 4.1, Vienna, Austria), and R-studio (Integrated development for R, Boston, MA, USA) software. Sampling days were excluded from the statistical analyses when the data loss exceeded 6% of a single day. Mean, standard deviation (SD) with minimum and maximum value were calculated for each continuous variable, and frequencies and proportions were calculated for categorical variables. When the distribution was asymmetric the median and interquartile range were reported. In addition, the 95% confidence intervals (CIs) of the mean were calculated for sleep parameters, the number of recorded days, the frequency of weekend days, and the CRL and yolk sac size with their GA at the measurements.

Linearity assumptions and normal distribution of the residuals were ascertained, and ordinary least square linear and quantile regression models were used to analyze the yolk sac size association with the sleep duration before pregnancy and the sleep duration at the end of the first trimester (week 13). Results were compared with results from robust regression methods (iterated reweighted least squares by Huber weights and bi-square weighting) and heteroskedastic methods (sandwich variance estimators). Calculations were carried out with and without model outliers (Cooks distance > 4 times). Mixed models were used for the repeated measurements analysis. The regression models were calculated with and without fetal sex stratification and stratification from lower to higher quartiles of the sleep duration. In sub analysis, we controlled for GA, maternal age, parity, physical activity level before conception, and maternal body composition parameters (i.e., height, weight, body mass index, lean body mass, and body fat percent). Those factors were added one by one to the primary model (yolk sac or CRL size by total sleep duration) and were included if they were altering the effect-size of the association notably. As measures of fit, the adjusted R–squared and Akaike information criterion were calculated. Differences between the regression models were tested using analysis of variance methods. The different strata were also compared with paired and unpaired Student's t-test or nonparametric tests.

Ethical declaration and informed consent

The study was approved by: Regional Comittee for Medical Research Ethics South East Norway (REK South East, ref. 2013/856a). E-mail: rek-sorost@medisin.uio.no. Written informed consent was obtained from all participants and all research was performed in accordance with relevant guidelines and regulations.

Results

Of 436 eligible participants, 190 (43.6%) conceived with a successful pregnancy and provided sufficient data to be included in the present study (Fig. 1). Their demographic information and obstetric outcomes are provided in Tables 1 and 2.

During actigraphy, 92.1% of all days before conception and 93.7% at the end of the first trimester were successfully recorded. The yolk sac was measured totally 358 times, and the CRL 559 times (Fig. 2). The summary statistics of the actigraphy recording, yolk sac diameter, and CRL are shown in Tables 3 and 4.

The median time from the first sleep recording to estimated day of conception (i.e., 14 days after LMP) was 36 days (IQR 10–75). The majority conceived within the two first menstrual cycles. The total sleep duration before conception was 38 min shorter than at the end of the first trimester (95%CI [28.6–47.2], $P < 0.01$), the total length of actigraphy and the frequency of recorded weekend days did not differ before and after conception.

Prepregnant sleep duration, but not the other sleep parameters before pregnancy had an effect on the yolk sac size (Supplementary Table S1). Generally, the effect on the yolk sac and CRL was weaker when the relation to measurement time was not taken into account (Supplementary Tables S1 and S2). The following analyses were stratified, all grouped according to time of the measurement at week 7, week 10, and week 13 for the CRL (Table 5 and 6).

Relation between daily sleep duration and yolk sac size

At the first ultrasound measurement (week 7), but not at the second (week 10), we found a sex-dependent negative effect of the prepregnant sleep duration on the yolk sac of male embryos (Fig. 3 and Table 5), i.e., longer sleep before pregnancy was associated with smaller yolk sac sizes.

It was noted that the sleep duration recorded later, at the end of the first trimester (week 13), was similarly linked to the yolk sac size at 7 weeks, but here, the effect was weaker (Fig. 3 and Table 5).

N = 190	Frequency	Nmiss	Mean	SD	Min	Max
Age (years)		None	29.0	3.1	20.0	35.0
Height (cm)		None	167.7	6.2	149.0	185.0
Weight (kg)		None	64.7	8.3	47.1	89.8
BMI		None	23.0	2.6	17.8	29.9
Lean body mass (kg)		None	45.7	3.8	36.0	55.6
Body fat (%)		None	28.8	5.5	15.9	41.9
Cycle length (days)		None	28.5	1.7	24	35
Parity		None				
0	89 (46.8%)					
1	79 (41.6%)					
≥ 2	22 (11.6%)					
Training efforts		None				
None	3 (1.6%)					
Effortless walk	46 (24.2%)					
< 3 times·week^{-1}	90 (47.4%)					
≥ 3 times·week^{-1}	51 (26.8%)					

Table 1. Descriptive statistics of the participants—missing values (Nmiss); mean; standard deviation (SD); range (Min, Max). Training efforts established using a non-validated questionnaire that each participant completed at study entry.

N = 190	Frequency	Nmiss	Mean	SD	Min	Max
Hypertension| PE	6 (3.2%)	None				
Gestational diabetes	7 (3.7%)	None				
Pregnancy length (days)		None	276.7	10.7	214	297
Preterm birth	6 (3.2%)	None				
Birthweight (g)		None	3566	491	1230	4910
Neonatal Sex (female)	94 (49.5%)	None				
Apgar < 7 (5 min)	2 (1.1%)	1				

Table 2. Selected descriptive statistics of pregnancy, common gestational diseases (i.e., hypertension, preeclampsia (PE), diabetes), and newborns—missing values (Nmiss); mean; standard deviation (SD); range (Min, Max).

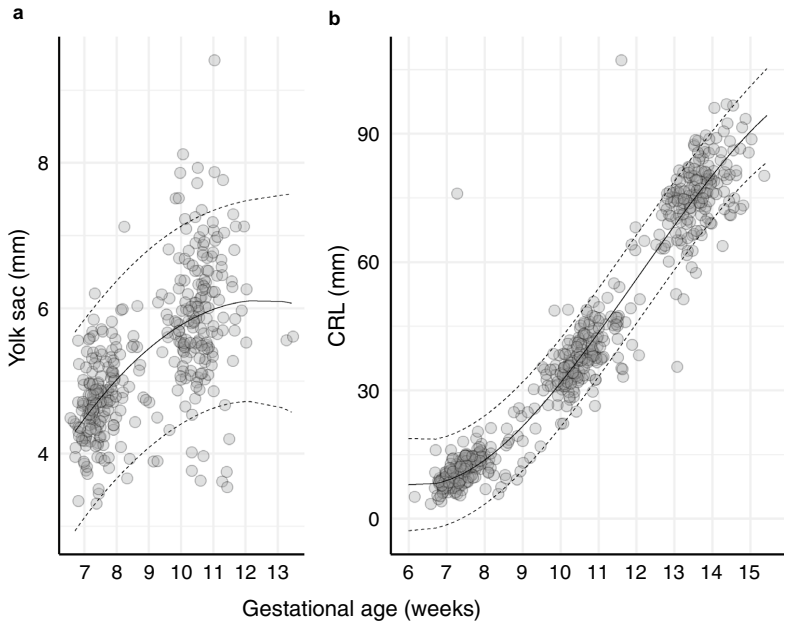

Figure 2. (**a**) First and second yolk sac measurements (total $N = 358$), and (**b**) the three serial crown-rump-length (CRL) measurements (total $N = 559$), presented with predicted mean (mixed model with random intercept) and 95% prediction band. Gestational age was based on last menstrual period.

Term	*N*	Mean	SD	95%CI
Sleep before pregnancy: Days before conception	178	51.7	53.1	(43.8–59.5)
Sleep before pregnancy: Number of recorded days	177	3.7	0.7	(3.6–3.8)
Sleep before pregnancy: Daily sleep time (min)	175	423.1	54.1	(415.0–431.2)
Sleep efficiency before pregnancy (%)	175	83.0	6.3	(82.0–83.9)
Week 13: GA at 2nd sleep recording (weeks)	181	13.2	0.8	(13.1–13.3)
Week 13: Sleep measurement (recorded days)	178	3.7	0.6	(3.6–3.8)
Week 13: Daily sleep time (min)	178	460.5	66.6	(450.6–470.3)
Week 13: Sleep efficiency (%)	178	81.6	7.0	(80.6–82.7)
Week 13–prepregnancy: Sleep difference (min)	172	37.9	61.7	(28.6–47.2)

Table 3. Summary statistics of the actigraphy data from 190 low-risk pregnancies before pregnancy and at 13 gestational weeks—number of measurements (*N*); mean; standard deviation (SD); 95% confidence interval (95%CI). The sleep difference was calculated from the two sleep measurements; time to conception was calculated as number of days from actigraphy recording at inclusion to day 14 of the menstrual cycle when the woman conceived.

There was a considerable variation in the yolk sac growth rate between the two measurement time points (Supplementary Fig. S1), but no association between the average growth rate and sleep duration (Supplementary Table S3).

Grouping of the sleep duration in quartiles, however, suggested that the yolk sac size was larger for the lowest sleep quartile than for the highest (Fig. 4); for these quartiles—lowest and highest—the sleep-yolk sac association also tended to be stronger.

Further, the relation between sleep duration before pregnancy and the embryonic yolk sac at week 7 was consistent across the yolk sac deciles (Supplementary Figs. S2 and S3), and still present after adjustment for maternal characteristics and GA (Supplementary Tables S4 and S5). In sub-analyses, these adjustments were made for all models (i.e., yolk sac at 7 and 10 weeks), with preconception sleep duration and sleep duration at the end of

Term	*N*	Mean	SD	95% CI
Week 7: 1st measurement; GA (weeks)	186	7.5	0.9	(7.4–7.7)
Week 7: 1st crown-rump-length (mm)	186	11.4	3.8	(10.8–11.9)
Week 7: 1st yolk sac diameter (mm)	180	4.7	0.6	(4.7–4.8)
Week 10: 2nd measurement; GA (weeks)	187	10.6	0.9	(10.5–10.7)
Week 10: 2nd crown-rump-length (mm)	187	37.8	6.9	(36.8–38.8)
Week 10: 2nd yolk sac diameter (mm)	178	5.9	0.9	(5.8–6.1)
Week 7–10: Yolk sac growth rate(mm·week^{-1})	170	0.038	0.033	(0.033–0.043)
Week 13: 3rd measurement; GA (weeks)	185	13.6	1.0	(13.4–13.7)
Week 13: 3rd crown-rump-length (mm)	186	76.4	8.1	(75.2–77.6)

Table 4. Summary statistics of the ultrasound data with number of measurements (*N*), mean, standard deviation (SD), and 95% confidence interval (95%CI) from 190 low-risk pregnancies—gestational age by LMP (GA) at the measurement; yolk sac size; crown-rump-length (CRL).

Group	*N*	Effect	95% CI	Adj.*r2*	AIC	*p*
Yolk sac at week 7 by sleep duration before pregnancy						
All	165	−0.12 mm·h^{-1}·d^{-1}	(−0.22 to −0.03)	0.03	291.4	**0.01**
Male	84	−0.22 mm·h^{-1}·d^{-1}	(−0.34 to −0.09)	0.11	133.6	**<0.01**
Female	81	−0.04 mm·h^{-1}·d^{-1}	(−0.19–0.10)	−0.01	157.4	0.53
Yolk sac at week 7 by sleep duration at the end of first trimester						
All	168	−0.06 mm·h^{-1}·d^{-1}	(−0.14–0.02)	0.012	300.3	0.15
Male	84	−0.13 mm·h^{-1}·d^{-1}	(−0.25 to −0.01)	0.056	142.1	**0.03**
Female	83	−0.01 mm·h^{-1}·d^{-1}	(−0.12–0.09)	0.001	159.9	0.79
Yolk sac at week 10 by sleep duration before pregnancy						
All	164	−0.07 mm·h^{-1}·d^{-1}	(−0.22–0.09)	0.004	444.3	0.40
Male	82	−0.07 mm·h^{-1}·d^{-1}	(−0.29–0.14)	0.005	213.6	0.51
Female	81	−0.06 mm·h^{-1}·d^{-1}	(−0.29–0.14)	0.004	234.9	0.59
Yolk sac at week 10 by sleep duration at the end of first trimester						
All	167	−0.04 mm·h^{-1}·d^{-1}	(−0.17–0.08)	0.003	450.7	0.48
Male	83	−0.10 mm·h^{-1}·d^{-1}	(−0.28–0.09)	0.013	215.8	0.29
Female	83	−0.01 mm·h^{-1}·d^{-1}	(−0.18–0.17)	0.000	238.8	0.79

Table 5. Prediction of the yolk sac diameter (all, males, and females) at 7 weeks and 10 weeks of gestation by total daily sleep duration before pregnancy and at 13 weeks of gestation. Calculated using ordinary least square regression models—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion). Significant are in bold.

Group	*N*	Effect	95% CI	Adj.*r2*	AIC	*p*
CRL at week 7 by sleep duration before pregnancy						
All	171	−0.69 mm·h^{-1}·d^{-1}	(−1.32 to −0.05)	0.02	950.9	**0.03**
Male	87	−0.92 mm·h^{-1}·d^{-1}	(−1.77 to −0.08)	0.04	472.5	**0.03**
Female	83	−0.53 mm·h^{-1}·d^{-1}	(−1.48–0.42)	0.002	478.9	0.27
CRL at week 10 by sleep duration before pregnancy						
All	173	−0.44 mm·h^{-1}·d^{-1}	(−1.57–0.70)	−0.002	1163.8	0.45
Male	88	−0.30 mm·h^{-1}·d^{-1}	(−1.82–1.21)	−0.009	579.8	0.69
Female	84	−0.58 mm·h^{-1}·d^{-1}	(−2.31–1.15)	−0.006	586.6	0.51
CRL at week 13 by sleep duration before pregnancy						
All	164	0.02 mm·h^{-1}·d^{-1}	(−1.33–1.36)	−0.005	1205.1	0.98
Male	82	0.39 mm·h^{-1}·d^{-1}	(−1.60–2.38)	−0.010	611.4	0.70
Female	81	−0.23 mm·h^{-1}·d^{-1}	(−2.09–1.63)	−0.01	598.3	0.81

Table 6. Prediction of the fetal crown-rump-length (CRL)measured at gestational week 7, 10, and 13 (all, males, and females) by total daily sleep duration before pregnancy and at gestational week 13. Calculated using ordinary least square regression models—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion). Significant are in bold.

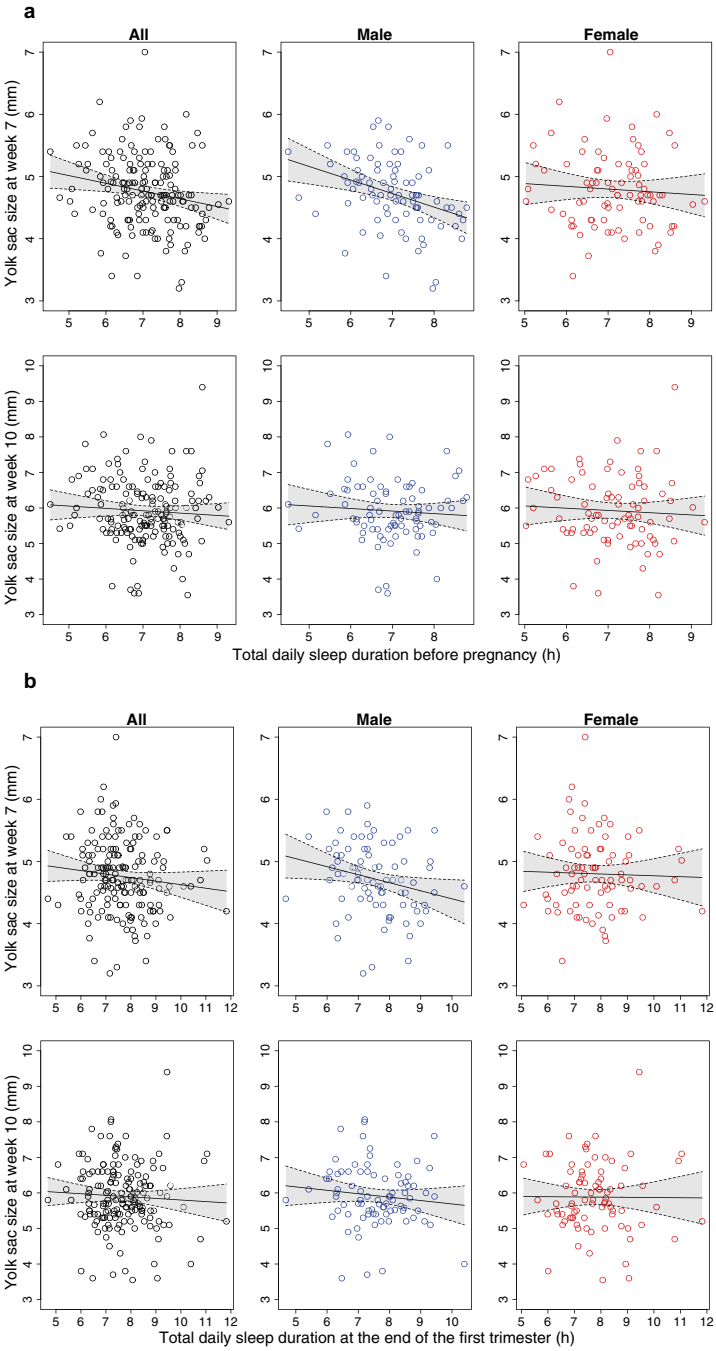

Figure 3. Effect of daily total sleeping time before pregnancy (**a**), and effect of the daily total sleeping time at 13 weeks (**b**) on the first (upper row) and second yolk sac measurement (lower row) in naturally conceived healthy pregnancies presented with regression line and its 95% confidence interval. The first column represents the total dataset and the second and third the analysis according to embryonic sex.

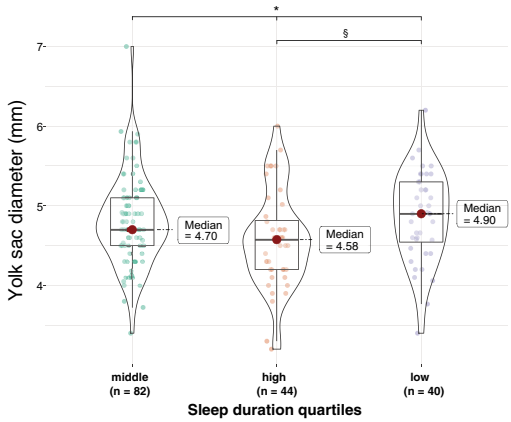

Figure 4. Raw-data-descriptive-inferential-statistics plot of the yolk sac size at week 7 (N = 166) grouped according to prepregnant sleep duration quartiles: Low sleep duration range (4 h 30 min–6 h 28 min); middle sleep duration range (6 h 29 min–7 h 40 min); high sleep duration range (7 h 42 min–9 h 19 min)—* Kruskal–Wallis test ($p = 0.03$; § Post-hoc-test (Dunn's test), $p = 0.038$.

the 1st trimester (13 weeks) as predictor, all stratified and unstratified by fetal sex. In none of the models these adjustments changed our conclusions.

Maternal sleep duration and embryo size through the first trimester

Similar to the association between yolk sac diameter and sleep duration, the embryonic CRL was shorter when the prepregnant sleep duration increased. Stratification by the time of measurement and fetal sex revealed a comparable pattern, i.e., the effect was confined to male embryos at week 7, not later (week 10 or week 13) (Table 6). The sleep duration at the end of the 1st trimester (week 13) was not associated with any of the CRL measurements (males or females) at week 7, week 10, or week 13. Adjustments for maternal age, parity and body composition did not change these results significantly.

Finally, the interval from actigraphy sleep measurement before pregnancy to LMP/estimated conception was neither influencing the CRL-sleep-association nor the yolk sac-sleep-association (i.e., the number of menstrual cycles to achieve pregnancy was irrelevant for these relations).

Discussion

This study demonstrates a sex- and time-dependent relation between prepregnant sleep duration and human embryonic development. The association between a shorter maternal sleep duration before pregnancy and a larger yolk sac and CRL was confined to male embryos at 7 weeks of gestation.

However, this effect on 7-weeks' embryos could be traced later in pregnancy. I.e., although sleep duration assessed at 13 weeks was 38 min longer, it still had a distributional pattern that significantly linked it to the yolk sac size at 7 weeks.

A strength of the study is the prospective longitudinal design to address our study questions. Secondly, our participants were healthy women who conceived naturally without any influence of hormonal treatment commonly used in assisted reproduction. However, our study did not discriminate daytime and nocturnal sleep duration. The total daily sleep duration included daytime naps and does not necessarily reflect the sleep quality and quantity at night. In addition, we cannot be certain that the sleep patterns recorded before pregnancy, although close to conception (Table 2), were continued into early pregnancy. An additional use of sleep diaries would have strengthened the study and provided a deeper insight. On the other hand, the fact that the sleep duration pattern at 13 weeks was similarly related to yolk sac and embryo at 7 weeks as was prepregnant sleep duration, suggests that the sleep pattern was similarly distributed through the entire period.

Stress, physical and psychological, are known confounders with quite similar hormonal responses, but the present study was not designed to disentangle such factors from sleep effects. The study population, however, consisted of healthy women with no history of chronic diseases or risk factors, and the chance for being under chronic physical and psychological stress should therefore be low.

There is some evidence from animal studies supporting an effect of environmental factors on the yolk sac (e.g. temperature, nutrition, and noise)[29,30,45,46], but extrapolation to human conditions may not be warranted as yolk sac development and implantation mechanisms differ between species[25,47,48]. However, a human study found that the yolk sac diameter was influenced by maternal body size[31]. They reported a smaller yolk sac at 8–12 weeks when maternal prepregnant height or weight was high. The relation was sex-dependent, i.e., shown only in female fetuses. Conversely, adjustment for maternal body composition parameters, including height and weight, did not change our estimates of the relation between daily sleep duration and yolk sac size (Supplementary Tables S4 and

S5). We suggest to consider this rather as a concept than contradiction. The first trimester is a developmental rush hour. From conception and implantation to the end of week 13, myriads of developmental steps are taking place with different time windows opening and closing at high frequency. In the current study the sleep effect on the yolk sac was present at week 7, but not 3 weeks later supporting this notion.

Our findings can be explained by at least two different biological models. First, the increase of the yolk sac size could compensate for a less favorable intrauterine environment due to a shorter sleep duration. This concept has been suggested before (in connection with maternal body size) and implies that the human yolk sac can compensate for reduced access to maternal nutrients by means of a larger surface[31]. It is in line with the time-dependent and early effect on the yolk sac at 7 weeks: early nutrition depends foremost on the yolk sac; later, there is a gradual transition to a hemotrophic placental supply[24,25]. The similar association of the embryonic CRL at week 7 (Table 6) may appear inconsistent with such a model of yolk sac compensation, but could be explained by a delay of the compensatory effect.

A second model would be that there is a systematic shift of the embryonic or fetal age related to the measured sleep duration. I.e., both time of ovulation with the fertile window (day 8–15)[49] and time of implantation vary (i.e., day 6–12 after ovulation)[50]. The influence of sleep on the HPA-axis, estradiol, melatonin, and other hormones[20,51] can cause menstrual cycle changes[11,18,20,21,51–54] and earlier ovulation. In addition, the interval from ovulation to implantation is inversely correlated with the first trimester measurement of the fetal CRL[55]. Thus, an association between the prepregnant sleep duration with early ovulation or shorter ovulation–implantation interval would result in a systematically larger yolk sac diameter and fetal CRL at any estimated GA by the LMP-method. This theory provides an explanation why both the yolk sac size and the CRL are sleep-related in early gestation. Later when size variation increases, due to individual embryonic and yolk sac growth or because the yolk sac dissolves[25,56], these sleep-related findings may not be traceable.

The present study also demonstrated a fetal sex-dependent sleep-yolk sac association that was present only for male embryos. Weinberg et al. reported already in 1995 that women with a short follicular phase before conception tended to give birth to males[57]. It is tempting to propose that a similar mechanism could explain the present results. Nevertheless, sexual dimorphism alone could explain sex-specific environmental effects including a sex-specific plasticity that depends on embryonic age[58]. That means, time windows for developmental influence may differ between the sexes as seen in the present and a previous study on yolk sac size[31]. Male embryos at 7 weeks in the present study were sensitive to maternal sleep, while female embryos after 8 weeks in the previous study were sensitive to maternal body height and weight, which could be analogue to the different timelines for development of girls and boys during postnatal life.

The present findings are not derived from extreme or pathologic cases but represent the healthy majority of pregnancies and point out that human development exhibit a measurable sensitivity to environmental factors already during the embryonic period. Note that the current yolk sac changes were associated with the sleep duration before pregnancy. Epigenetic programming has been linked both to sleep[9–17] and immune progenitor cells produced in the yolk sac[59]. This opens the possibility of influencing and conditioning individual health development of the offspring right from the periconceptional period.

The present results made us also realize how rapid the pace of development is during the 1st trimester with sex and GA specific time windows that were susceptible to maternal and environmental effects (i.e., sleep and body composition). Future studies need to consider a correspondingly rapid frequency of observational windows to discern effects.

In addition, the two suggested explanatory models require further investigation; (a) the yolk sac compensation of a less favorable intrauterine environment due to a shorter prepregnant sleep duration and/or (b) an earlier ovulation or implantation due to a shorter prepregnant sleep duration. A precise determination of time of ovulation and implantation would be a good starting point[50].

Conclusion

Normal variation in prepregnant sleep does have an effect on embryonic development in healthy women; a shorter sleep duration corresponds to a larger yolk sac and CRL. Two features stand out; there is a sex-difference with an effect that appears to be exclusively among males, and the effect seems to operate during a short time window—around 7 weeks of pregnancy—underscoring the rapid pace of development that dominates the embryonic period.

Data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files).

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References

1. Romero, R. & Badr, M. S. A role for sleep disorders in pregnancy complications: Challenges and opportunities. *Am. J. Obstet. Gynecol.* **210**, 3–11. https://doi.org/10.1016/j.ajog.2013.11.020 (2014).
2. Williams, M. A. *et al.* Associations of early pregnancy sleep duration with trimester-specific blood pressures and hypertensive disorders in pregnancy. *Sleep* **33**, 1363–1371. https://doi.org/10.1093/sleep/33.10.1363 (2010).
3. Facco, F. L. *et al.* Objectively measured short sleep duration and later sleep midpoint in pregnancy are associated with a higher risk of gestational diabetes. *Am. J. Obstet. Gynecol.* **217**, 447.e1-447.e13. https://doi.org/10.1016/j.ajog.2017.05.066 (2017).
4. Facco, F. L. *et al.* Association of adverse pregnancy outcomes with self-reported measures of sleep duration and timing in women who are nulliparous. *J Clin Sleep Med.* **14**, 2047–2056. https://doi.org/10.5664/jcsm.7534 (2018).

5. Okun, M. L., Schetter, C. D. & Glynn, L. M. Poor sleep quality is associated with preterm birth. *Sleep* **34**, 1493–1498. https://doi.org/10.5665/sleep.1384 (2011).
6. Lee, K. A. & Gay, C. L. Sleep in late pregnancy predicts length of labor and type of delivery. *Am. J. Obstet. Gynecol.* **191**, 2041–2046. https://doi.org/10.1016/j.ajog.2004.05.086 (2004).
7. Warland, J., Dorrian, J., Morrison, J. L. & O'brien, L. M. Maternal sleep during pregnancy and poor fetal outcomes: A scoping review of the literature with meta-analysis. *Sleep Med. Rev.* **41**, 197–219. https://doi.org/10.1016/j.smrv.2018.03.004 (2018).
8. Begtrup, L. M. *et al.* Night work and miscarriage: A danish nationwide register-based cohort study. *Occup. Environ. Med.* **76**, 302–308. https://doi.org/10.1136/oemed-2018-105592 (2019).
9. Torres-Farfan, C. *et al.* Maternal melatonin effects on clock gene expression in a nonhuman primate fetus. *Endocrinology* **147**, 4618–4626. https://doi.org/10.1210/en.2006-0628 (2006).
10. Lanoix, D., Beghdadi, H., Lafond, J. & Vaillancourt, C. Human placental trophoblasts synthesize melatonin and express its receptors. *J. Pineal Res.* **45**, 50–60. https://doi.org/10.1111/j.1600-079x.2008.00555.x (2008).
11. Zhang, L. *et al.* Effects of melatonin administration on embryo implantation and offspring growth in mice under different schedules of photoperiodic exposure. *Reprod. Biol. Endocrinol.* **15**, 78. https://doi.org/10.1186/s12958-017-0297-7 (2017).
12. Voiculescu, S. E., Zygouropoulos, N., Zahiu, C. D. & Zagrean, A. M. Role of melatonin in embryo fetal development. *J. Med. Life* **7**, 488–492 (2014).
13. Olcese, J. M. Melatonin and female reproduction: An expanding universe. *Front. Endocrinol. (Lausanne)* **11**, 85. https://doi.org/10.3389/fendo.2020.00085 (2020).
14. Ivanov, D. *et al.* Melatonin, its beneficial effects on embryogenesis from mitigating oxidative stress to regulating gene expression. *Int. J. Mol. Sci.* **22**, 5885. https://doi.org/10.3390/ijms22115885 (2021).
15. Unfer, V., Raffone, E., Rizzo, P. & Buffo, S. Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previous *in vitro* fertilization cycles for poor oocyte quality: A prospective, longitudinal, cohort study. *Gynecol. Endocrinol.* **27**, 857–861. https://doi.org/10.3109/09513590.2011.564687 (2011).
16. Hardeland, R. Melatonin and chromatin. *Melatonin Res.* **2**, 67–93 (2019).
17. Hsu, C. N. & Tain, Y. L. Light and circadian signaling pathway in pregnancy: Programming of adult health and disease. *Int. J. Mol. Sci.* **21**, E2232. https://doi.org/10.3390/ijms21062232 (2020).
18. Van Cauter, E., Plat, L. & Copinschi, G. Interrelations between sleep and the somatotropic axis. *Sleep* **21**, 553–566 (1998).
19. Li, J., Chen, Q., Wang, J., Huang, G. & Ye, H. Does growth hormone supplementation improve oocyte competence and ivf outcomes in patients with poor embryonic development? A randomized controlled trial. *BMC Pregnancy Childbirth* **20**, 310. https://doi.org/10.1186/s12884-020-03004-9 (2020).
20. Kloss, J. D., Perlis, M. L., Zamzow, J. A., Culnan, E. J. & Gracia, C. R. Sleep, sleep disturbance, and fertility in women. *Sleep Med. Rev.* **22**, 78–87. https://doi.org/10.1016/j.smrv.2014.10.005 (2015).
21. Vgontzas, A. N. *et al.* Chronic insomnia is associated with nyctohemeral activation of the hypothalamic-pituitary-adrenal axis: Clinical implications. *J. Clin. Endocrinol. Metab.* **86**, 3787–3794. https://doi.org/10.1210/jcem.86.8.7778 (2001).
22. Nakamura, K., Sheps, S. & Arck, P. C. Stress and reproductive failure: Past notions, present insights and future directions. *J. Assist. Reprod. Genet.* **25**, 47–62. https://doi.org/10.1007/s10815-008-9206-5 (2008).
23. Mantoni, M. & Pedersen, J. F. Ultrasound visualization of the human yolk sac. *J. Clin. Ultrasound* **7**, 459–460 (1979).
24. Burton, G. J., Hempstock, J. & Jauniaux, E. Nutrition of the human fetus during the first trimester—a review. *Placenta* **22**(Suppl A), S70-7. https://doi.org/10.1053/plac.2001.0639 (2001).
25. Ross, C. & Boroviak, T. E. Origin and function of the yolk sac in primate embryogenesis. *Nat. Commun.* **11**, 3760. https://doi.org/10.1038/s41467-020-17575-w (2020).
26. Lindsay, D. J. *et al.* Yolk sac diameter and shape at endovaginal us: Predictors of pregnancy outcome in the first trimester. *Radiology* **183**, 115–118. https://doi.org/10.1148/radiology.183.1.1549656 (1992).
27. Cepni, I. *et al.* Significance of yolk sac measurements with vaginal sonography in the first trimester in the prediction of pregnancy outcome. *Acta Obstet. Gynecol. Scand.* **76**, 969–972. https://doi.org/10.3109/00016349709034911 (1997).
28. Oh, J. S., Wright, G. & Coulam, C. B. Gestational sac diameter in very early pregnancy as a predictor of fetal outcome. *Ultrasound Obstet. Gynecol.* **20**, 267–269. https://doi.org/10.1046/j.1469-0705.2002.00774.x (2002).
29. Lara, R. A. & Vasconcelos, R. O. Impact of noise on development, physiological stress and behavioural patterns in larval zebrafish. *Sci. Rep.* **11**, 6615. https://doi.org/10.1038/s41598-021-85296-1 (2021).
30. Mikec, M. *et al.* Influence of environmental and nutritional stressors on yolk sac utilization, development of chicken gastrointestinal system and its immune status. *World's Poult. Sci. J.* **62**, 31–40. https://doi.org/10.1079/wps200582 (2006).
31. Odland Karlsen, H. *et al.* The human yolk sac size reflects involvement in embryonic and fetal growth regulation. *Acta Obstet. Gynecol. Scand.* **98**, 176–182. https://doi.org/10.1111/aogs.13466 (2018).
32. Lee, K. A., Zaffke, M. E. & Mcenany, G. Parity and sleep patterns during and after pregnancy. *Obstet Gynecol.* **95**, 14–18. https://doi.org/10.1016/s0029-7844(99)00486-x (2000).
33. Vietheer, A., Kiserud, T., Lie, R. T., Haaland, Ø. A. & Kessler, J. Sleep and physical activity from before conception to the end of pregnancy in healthy women: A longitudinal actigraphy study. *Sleep Med.* https://doi.org/10.1016/j.sleep.2021.04.028 (2021).
34. Lohman, T. *Anthropometric Standardization Reference Manual* (Human kinetics book, 1988).
35. Corporation, T. *Body Composition Analyser BC-418-MA. Instruction Manual*, (Tanita Corporation of America. https://www.tanita.com/es/.downloads/download/?file=855638086&fl=en_US,
36. Robinson, H. P. Sonar measurement of fetal crown-rump length as means of assessing maturity in first trimester of pregnancy. *Br. Med. J.* **4**, 28–31. https://doi.org/10.1136/bmj.4.5883.28 (1973).
37. Matthews, C. E., Ainsworth, B. E., Thompson, R. W. & Bassett, D. R. Sources of variance in daily physical activity levels as measured by an accelerometer. *Med. Sci. Sports Exerc.* **34**, 1376–1381. https://doi.org/10.1097/00005768-200208000-00021 (2002).
38. Liden, C. B. *et al.* Benefits of the sensewear armband over other physical activity and energy expenditure measurement techniques. *White Papers Body Media* **1**, 1–14 (2001).
39. Sunseri, M. et al. The sensewear armband as a sleep detection device. *White Papers Body Media* 1–9
40. Peterson, B. T. *et al.* Comparison of actigraphy and polysomnography to assess effects of zolpidem in a clinical research unit. *Sleep Med.* **13**, 419–424. https://doi.org/10.1016/j.sleep.2011.12.003 (2012).
41. Soric, M. *et al.* Validation of a multi-sensor activity monitor for assessing sleep in children and adolescents. *Sleep Med.* **14**, 201–205. https://doi.org/10.1016/j.sleep.2012.11.003 (2013).
42. O'driscol, D. M., Turton, A. R., Copland, J. M., Strauss, B. J. & Hamilton, G. S. Energy expenditure in obstructive sleep apnea: Validation of a multiple physiological sensor for determination of sleep and wake. *Sleep Breath.* **17**, 139–146. https://doi.org/10.1007/s11325-012-0662-x (2013).
43. Sharif, M. M. & Bahammam, A. S. Sleep estimation using bodymedia's sensewear armband in patients with obstructive sleep apnea. *Ann. Thorac. Med.* **8**, 53–57. https://doi.org/10.4103/1817-1737.105720 (2013).
44. Shin, M., Swan, P. & Chow, C. M. The validity of actiwatch2 and sensewear armband compared against polysomnography at different ambient temperature conditions. *Sleep Sci.* **8**, 9–15. https://doi.org/10.1016/j.slsci.2015.02.003 (2015).
45. Fiksen, Ø. & Folkvord, A. Maternal effects and the benefit of yolk supply in cod larvae in different environments—a simulation model. ICES Council Meeting, 1–6 (1999)

46. Watkins, A. J. *et al.* Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. *Biol. Reprod.* **78**, 299–306. https://doi.org/10.1095/biolreprod.107.064220 (2008).
47. Burton, G. J., Cindrova-Davies, T. & Turco, M. Y. Review: Histotrophic nutrition and the placental-endometrial dialogue during human early pregnancy. *Placenta* **102**, 21–26. https://doi.org/10.1016/j.placenta.2020.02.008 (2020).
48. Carter, A. M. Unique aspects of human placentation. *Int. J. Mol. Sci.* **22**, 8099. https://doi.org/10.3390/ijms22158099 (2021).
49. Wilcox, A. J., Dunson, D. & Baird, D. D. The timing of the "fertile window" in the menstrual cycle: Day specific estimates from a prospective study. *BMJ* **321**, 1259–1262. https://doi.org/10.1136/bmj.321.7271.1259 (2000).
50. Wilcox, A. J., Baird, D. D. & Weinberg, C. R. Time of implantation of the conceptus and loss of pregnancy. *N. Engl. J. Med.* **340**, 1796–1799. https://doi.org/10.1056/NEJM199906103402304 (1999).
51. Carlomagno, G., Minini, M., Tilotta, M. & Unfer, V. From implantation to birth: Insight into molecular melatonin functions. *Int. J. Mol. Sci.* **19**, 2802. https://doi.org/10.3390/ijms19092802 (2018).
52. Baker, F. C. & Driver, H. S. Circadian rhythms, sleep, and the menstrual cycle. *Sleep Med.* **8**, 613–622. https://doi.org/10.1016/j.sleep.2006.09.011 (2007).
53. Lawson, C. C. *et al.* Rotating shift work and menstrual cycle characteristics. *Epidemiology* **22**, 305–312. https://doi.org/10.1097/EDE.0b013e3182130016 (2011).
54. He, C. *et al.* Melatonin-related genes expressed in the mouse uterus during early gestation promote embryo implantation. *J. Pineal Res.* **58**, 300–309. https://doi.org/10.1111/jpi.12216 (2015).
55. Mahendru, A. A. *et al.* Impact of ovulation and implantation timing on first-trimester crown-rump length and gestational age. *Ultrasound Obstet. Gynecol.* **40**, 630–635. https://doi.org/10.1002/uog.12277 (2012).
56. Blaas, H. G., Eik-Nes, S. H. & Bremnes, J. B. The growth of the human embryo. A longitudinal biometric assessment from 7 to 12 weeks of gestation. *Ultrasound Obstet. Gynecol.* **12**, 346–354. https://doi.org/10.1046/j.1469-0705.1998.12050346.x (1998).
57. Weinberg, C. R., Baird, D. D. & Wilcox, A. J. The sex of the baby may be related to the length of the follicular phase in the conception cycle. *Hum. Reprod.* **10**, 304–307. https://doi.org/10.1093/oxfordjournals.humrep.a135932 (1995).
58. Deegan, D. F. & Engel, N. Sexual dimorphism in the age of genomics: How, when, where. *Front Cell Dev. Biol.* **7**, 186. https://doi.org/10.3389/fcell.2019.00186 (2019).
59. Chen, S., Yang, J., Wei, Y. & Wei, X. Epigenetic regulation of macrophages: From homeostasis maintenance to host defense. *Cell Mol. Immunol.* **17**, 36–49. https://doi.org/10.1038/s41423-019-0315-0 (2020).

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Author contributions

A.V.: Conceptualization, methodology, investigation, software, data curation, visualization, validation, formal analysis, interpretation, writing—original draft preparation, review and editing; T.K.: Project administration, conceptualization, methodology, investigation, data curation, formal analysis, interpretation, visualization, writing—review & editing. R.T.L.: Methodology, formal analysis, validation, visualization, writing—review & editing. Ø.A.H.: Methodology, software, formal analysis, validation, visualization, writing, review and editing. J.K.: Project administration, methodology, investigation, formal analysis, validation, visualization, writing—review & editing.

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Competing interests

The authors declare no competing interests.

Additional information

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Author Correction: Effect of maternal sleep on embryonic development

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Alexander Vietheer, Torvid Kiserud, Øystein Ariansen Haaland, Rolv Terje Lie & Jörg Kessler

Correction to: *Scientific Reports* https://doi.org/10.1038/s41598-022-21516-6, published online 12 October 2022

The original version of this Article contained a typo in the Abstract.

"I.e., the yolk sac diameter decreased with increasing sleep duration (0.22 mm·h^{-1}d^{-1}, 95%CI [0.35–0.09], $P < 0.01$), and CRL increased (0.92 mm·h^{-1}d^{-1}, 95%CI [1.77–0.08], $P = 0.03$)."

now reads:

"I.e., the yolk sac diameter decreased with increasing sleep duration (0.22 mm·h^{-1}d^{-1}, 95%CI [0.35–0.09], P < 0.01), and CRL decreased (0.92 mm·h^{-1}d^{-1}, 95%CI [1.77–0.08], P = 0.03)."

The original Article has been corrected.

Table S1 Prediction of the unstratified serial yolk sac measurements at gestational week 7 and 10 (in one group) by various sleep parameters: i.e., duration, difference between prepregnant and 1st trimester sleep duration (Δ-sleep), and sleep efficiency. Estimates calculated with mixed model linear regression accounting for gestational age—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion). Two participants were excluded from the analysis due to uncertain information on LMP dates.

Term	*N*	Effect	95% CI	Adj.*r*2	AIC	*p*
Yolk sac diameter by sleep duration before pregnancy ($mm \cdot h^{-1} \cdot d^{-1}$)	172	-0.09	(-0.18– -0.00)	0.71	711.1	**0.04**
Yolk sac diameter by sleep duration at end of 1st trimester ($mm \cdot h^{-1} \cdot d^{-1}$)	174	-0.05	(-0.12–0.02)	0.71	718.6	0.14
Yolk sac diameter by Δ-sleep ($mm \cdot h^{-1} \cdot d^{-1}$)	169	0.02	(-0.06–0.10)	0.71	698.2	0.64
Yolk sac diameter by sleep efficiency before pregnancy ($mm \cdot \%^{-1}$)	173	-0.00	(-0.01–0.01)	0.71	715.6	0.86
Yolk sac diameter by sleep efficiency at end of 1st trimester ($mm \cdot \%^{-1}$)	174	0.00	(-0.01–0.01)	0.71	720.4	0.57

Table S2 Prediction of the unstratified (all) and sex-stratified serial crown-rump-length measurements in week 7, 10, and 13 (in one group) by total daily sleep duration before pregnancy and at gestational week 13. Estimated using mixed model regression accounting for gestational age—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion).

Group	*N*	*Effect*	95% CI	Adj.*r*2	AIC	*p*
CRL by sleep duration before conception						
All	174	-0.25 $mm \cdot h^{-1} \cdot d^{-1}$	(-1.17–0.66)	0.94	3386.0	0.58
Male	89	-0.56 $mm \cdot h^{-1} \cdot d^{-1}$	(-1.89–0.79)	0.92	1810.2	0.41
Female	84	0.10 $mm \cdot h^{-1} \cdot d^{-1}$	(-1.18–1.39)	0.95	1512.2	0.88
CRL by sleep duration at the end of the 1st trimester						
All	176	-0.40 $mm \cdot h^{-1} \cdot d^{-1}$	(-1.17–0.28)	0.94	3410.5	0.23
Male	90	-0.20 $mm \cdot h^{-1} \cdot d^{-1}$	(-1.36–0.95)	0.92	1823.0	0.72
Female	85	-0.57 $mm \cdot h^{-1} \cdot d^{-1}$	(-1.53–0.38)	0.95	1520.8	0.23

Figure S1 First (week 7) and second (week 10) yolk sac measurements (in total *N*=358) presented as longitudinal observations by gestational age (here based on the fetal crown-rump-length). The individual pregnancies are distinguished by the color gradient.

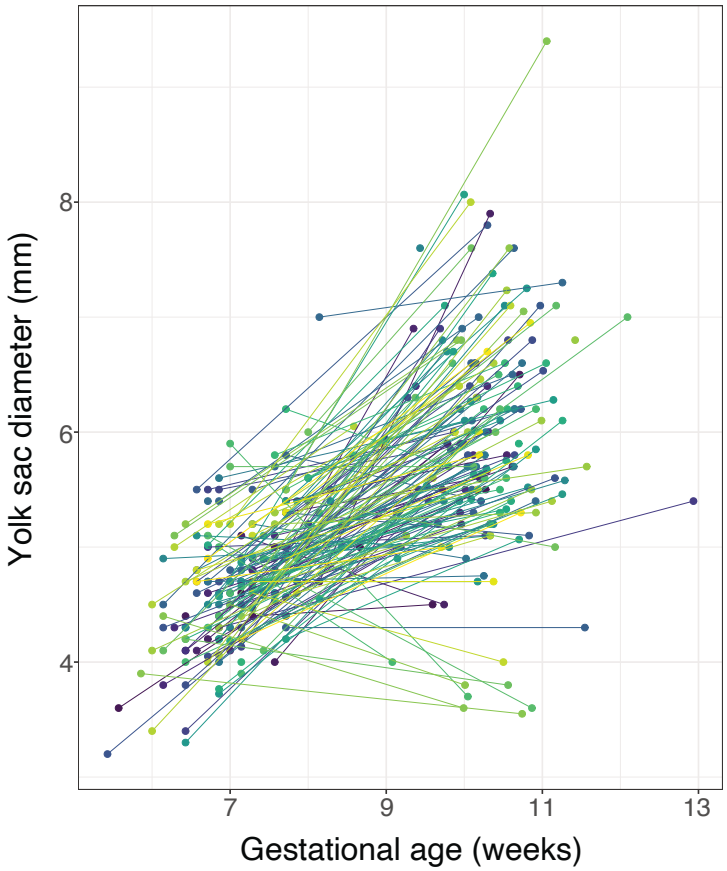

Table S3 Prediction of the yolk sac growth rate in mm/week (all, males, and females) between gestational week 7 and 10 by daily preconception sleep duration (in hours), sleep duration at end of first trimester, and their individual differences (end of 1st trimester–preconception). Calculated by ordinary least square regression models—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion).

Group	*N*	Effect	95% CI	Adj.*r*2	AIC	*p*
Yolk sac growth rate by sleep duration before conception						
All	156	0.002 mm/week·h^{-1}·d^{-1}	(-0.004–0.007)	-0.005	-619.5	0.60
Male	76	0.003 mm/week·h^{-1}·d^{-1}	(-0.004–0.01)	-0.004	-317.6	0.40
Female	79	-0.000 mm/week·h^{-1}·d^{-1}	(-0.008–0.008)	-0.012	-299.4	0.98
Yolk sac growth rate by sleep duration at the end of 1st trimester						
All	159	0.000 mm/week·h^{-1}·d^{-1}	(-0.004–0.005)	-0.006	-640.3	0.85
Male	77	-0.001 mm/week·h^{-1}·d^{-1}	(-0.008–0.005)	-0.011	-322.1	0.66
Female	81	0.002 mm/week·h^{-1}·d^{-1}	(-0.005–0.008)	-0.009	-314.5	0.59
Yolk sac growth rate by Δ-sleep duration						
All	153	0.000 mm/week·h^{-1}·d^{-1}	(-0.005–0.005)	-0.006	-617.6	0.90
Male	75	-0.004 mm/week·h^{-1}·d^{-1}	(-0.012–0.003)	0.007	-313.8	0.22
Female	77	0.004 mm/week·h^{-1}·d^{-1}	(-0.003–0.011)	0.001	-301.8	0.30

Figure S2 Unadjusted quantile regression-lines for the yolk sac size at the first measurement (week 7) by total daily preconception sleeping time in hours (*N*=165); median (thick black line) and 5th, 20th, 30th, 40th, 60th, 70th, 80th, and 95th percentile (grey); individual observations (open circles); ordinary least square regression-line (red).

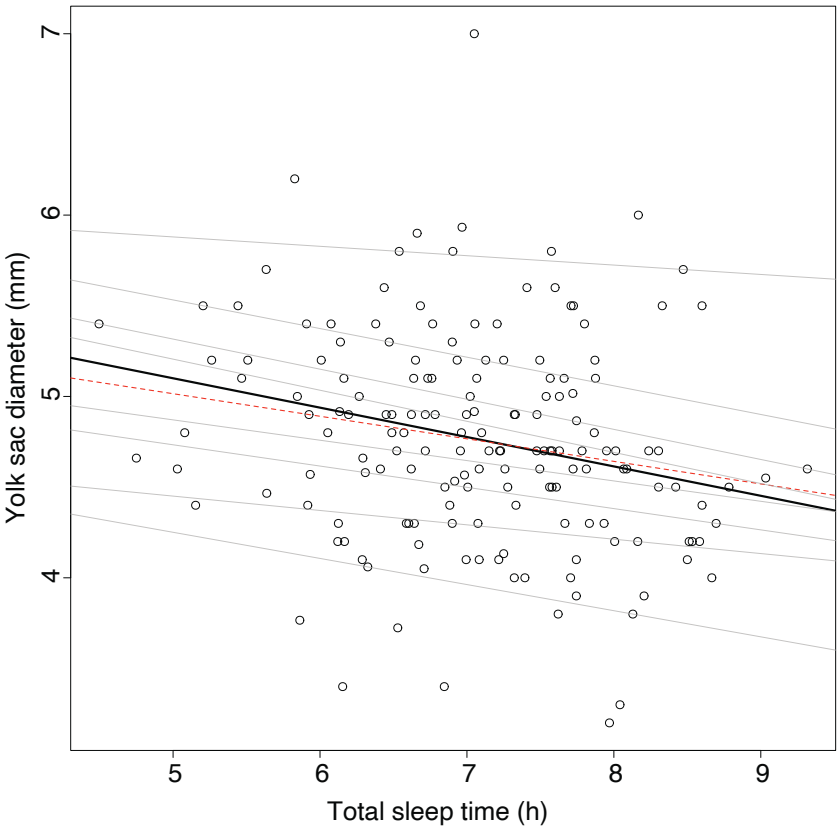

Figure S3 Overview of the results of the quantile regression models: The estimates on the y-axis with 95% confidence intervals (change of yolk sac size in mm per hour of daily sleep duration) were calculated for yolk sac deciles on the x-axis; zero-effect (red line)

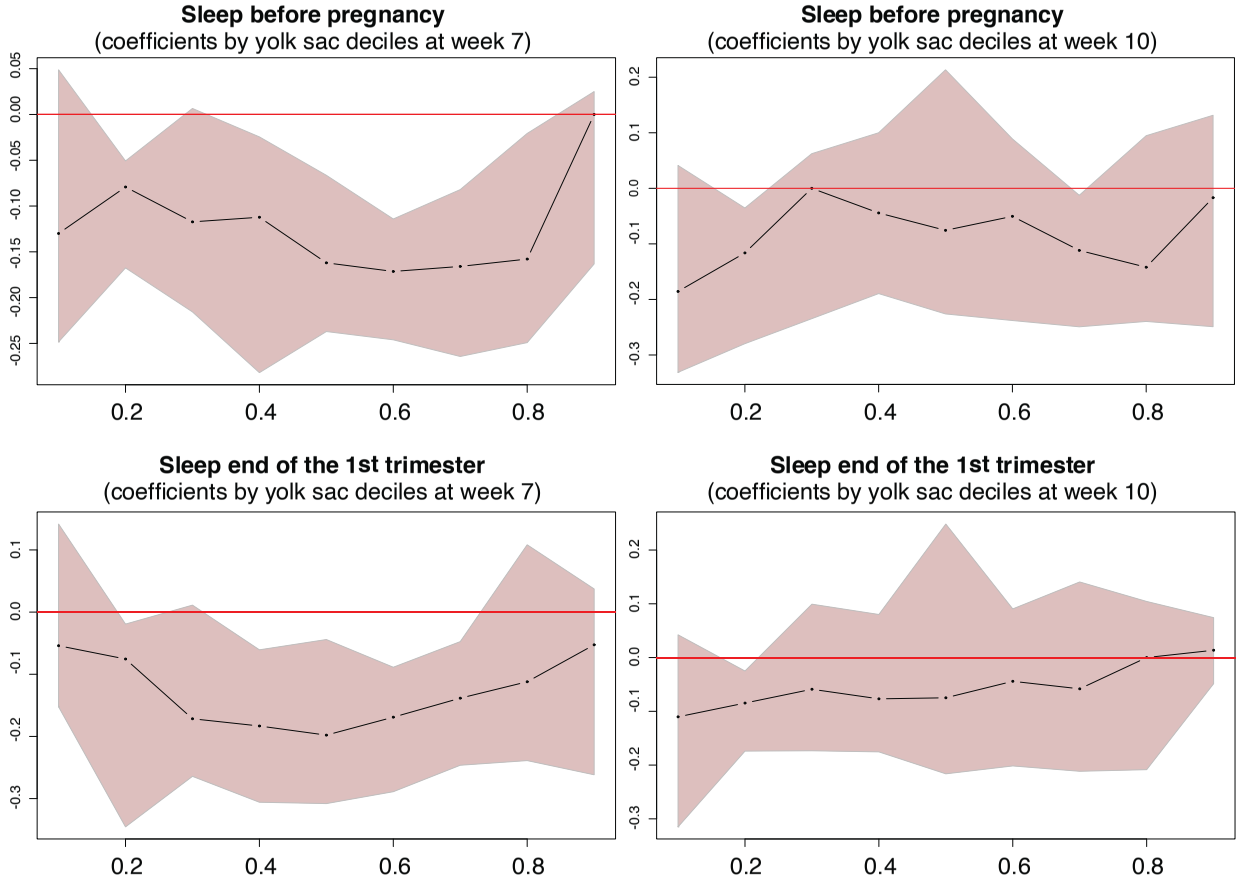

Table S4 Prediction of the yolk sac diameter (males and females) at gestational week 7 by total daily sleep duration before pregnancy. Calculated by ordinary least square regression models—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion). The crude model (grey background) with its estimates and significance level can be compared with the adjusted models listened below, controlling for maternal health parameters: i.e., age, parity, physical activity level before conception (PA), height, weight, BMI, lean body mass (LBM), body fat percent (BFP), and gestational age (GA). The sleep-yolk sac relation remained significant after adjustments that caused minute changes in the estimated effect.

Model	*N*	Effect	95% CI	Adj.*r*2	AIC	*p*
Crude	165	-0.12 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.22 to -0.03)	0.032	291.4	**0.01**
Adjusted by age	165	-0.12 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.22 to -0.03)	0.026	293.4	**0.01**
Adjusted by parity	165	-0.12 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.22 to -0.02)	0.047	296.6	**0.02**
Adjusted by PA	165	-0.12 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.22 to -0.03)	0.022	295.9	**0.01**
Adjusted by height	165	-0.12 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.22 to -0.03)	0.030	292.6	**0.01**
Adjusted by weight	165	-0.13 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.22 to -0.03)	0.026	293.3	**0.01**
Adjusted by BMI	165	-0.11 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.21 to -0.00)	0.027	293.2	**0.01**
Adjusted by LBM	165	-0.12 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.22 to -0.02)	0.032	292.4	**0.01**
Adjusted by BFP	165	-0.12 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.22 to -0.02)	0.017	293.1	**0.02**
Adusted by GA	164	-0.10 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.19 to -0.01)	0.091	281.8	**0.03**

Table S5 Prediction of the yolk sac diameter (males and females) at gestational week 7 by total daily sleep duration before pregnancy. Calculated by ordinary least square regression models simultaneously adjusted for age, number of previous children, physical activity level before conception, height, lean body mass, body fat percent, and gestational age—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion). The effect on male embryos remains significant.

Group	N	Effect	95% CI	Adj.*r*2	AIC	*p*
All	**165**	-0.08 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.18 – 0.01)	0.056	297.7	0.09
Male	**83**	-0.19 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.32 to -0.05)	0.16	138.7	**<0.01**
Female	**81**	-0.01 $mm \cdot h^{-1} \cdot d^{-1}$	(-0.15–0.16)	0.029	163.4	0.95

Table S6 Variable key variables table S7 (dataset)

Id	Study inclusion number
Age	Age of the participant at inclusion
para	Number of previous births
mens.cyc	Average length of menstrual cycle at inclusion
hypert.dis	Hypertensive disease or preeclampsia during the studied pregnancy
gdm	Gestational diabetes mellitus
wt.1	Weight at inclusion
ht	Height at inclusion
bmi.1	BMI at inclusion
bfp.1	Body fat percent at inclusion
lbm.1	Lean body mass at inclusion
physac	Level of weekly physical activity at inclusion
child.wt	Weight of the child at birth
apgar5	APGAR-score after 5 minutes
apgar10	APGAR-score after 10 minutes
childSex	Sex of the child
tot.preg.length.crl.mix	Total pregnancy length calculated from gestational age by earliest available fetal crown-rump-length
preterm	Preterm birth
lmp	First day of the last menstrual period
g_date.1	Date of the first assessment in pregnancy (week 7)
crl.1	Fetal crown-rump-length at the first assessment (week 7)
ys.1	Yolk sac diameter at the first assessment (week 7)
Examininer7	Identification number of the examiner at the first assessment (week 7)
g_date.2	Date of the second assessment in pregnancy (week 10)
crl.2	Fetal crown-rump-length at the second assessment (week 10)
ys.2	Yolk sac diameter at the second assessment (week 10)
Examininer10	Identification number of the examiner at the second assessment (week 10)
g_date.3	Date of the third assessment in pregnancy (week 13)
crl.3	Fetal crown-rump-length at the third assessment (week 13)
Examininer13	Identification number of the examiner at the third assessment (week 13)
sw.0.days.before.concept	Number of days from first actigraphy recording to ovulation (day 14 from LMP)
sw_date.1	Date of the first actigraphy recording (inclusion)
sw.ndays.1	Number of complete days (on body time≥94% per 24 hours) at the first recording
tst.1	Mean total sleep duration per day in minutes at the first recording
se.1	Sleep efficiency in % at the first recording
sw_date.2	Date of the second actigraphy recording (week 13)
sw.ndays.2	Number of complete days (on body time≥94% per 24 hours) at the second recording
tst.2	Mean total sleep duration per day in minutes at the second recording
se.2	Sleep efficiency in % at the second recording
excl	Exclusions: excluded 0; if excluded, reason by number 1–5

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Maternal physical activity affects yolk sac size and growth in early pregnancy, but girls and boys use different strategies

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This longitudinal study investigated the impact of actigraphy-measured maternal physical activity on yolk sac size during early development. The yolk sac, a transient extraembryonic organ, plays a crucial role in embryonic development and is involved in metabolism, nutrition, growth, and hematopoiesis. Prospectively collected data from 190 healthy women indicated that their total daily physical activity, including both light and moderate-vigorous activity, was associated with yolk sac growth dynamics depending on embryonic sex and gestational age. Higher preconception maternal physical activity was linked to a larger yolk sac at 7 weeks (95% CI [0.02–0.13 mm]) and a smaller yolk sac at 10 weeks' gestation (95% CI [− 0.18 to − 0.00]) in male embryos; in female embryos, the yolk sac size was increased at 10 weeks' gestation (95% CI [0.06–0.26]) and was, on average, 24% larger than that in male embryos (95% CI [0.12–0.38]). Considering the pattern of other maternal effects on yolk sac size—e.g., body composition and sleep duration—we suggest that physiological yolk sac adaptations occur in short, sex-specific time windows and can be influenced by various maternal factors.

The ability of an embryo and fetus to adapt to the intrauterine environment, including maternal factors, is considered to diminish over time[1–3]. Even before conception, maternal health factors are relevant for optimal endometrial preparation[4] and implantation[5]. In addition, the response to environmental and maternal factors can vary between the sexes as early as the moment of conception based on their specific genetic and epigenetic potential[2,6–8].

The rapid stages during early gestational development might be accompanied by corresponding rapid shifts in sensitivity to maternal and environmental cues. More detailed insight into the effect of specific factors and the sequence of events may reveal mechanisms of interest beyond fundamental knowledge, such as public health measures and clinical management[1].

The secondary yolk sac is a prominent structure during early human embryonic development that is easily visualized using ultrasound imaging during the first trimester. It is located in the exocoelomic cavity and remains connected to the developing embryo by the vitelline duct and its vessels (Fig. 1a,b). The yolk sac is involved in gastrointestinal tract formation, protein synthesis, stem cell production, and hematopoiesis[9–12]; for example, it is the origin of macrophage subtypes with high plasticity for epigenetic programming[13]. Through surface diffusion and transport proteins, the yolk sac membrane also facilitates gas exchange and provides nutrients until the placenta is sufficiently developed[9–12,14].

Recently, our group found that in a healthy pregnancy, the yolk sac at approximately 8 weeks' gestation is larger when the maternal height and weight are low, suggesting a compensatory adaptation to maintain embryonic growth within an optimal trajectory, and the effect was essentially observed in female embryos[15]. In a

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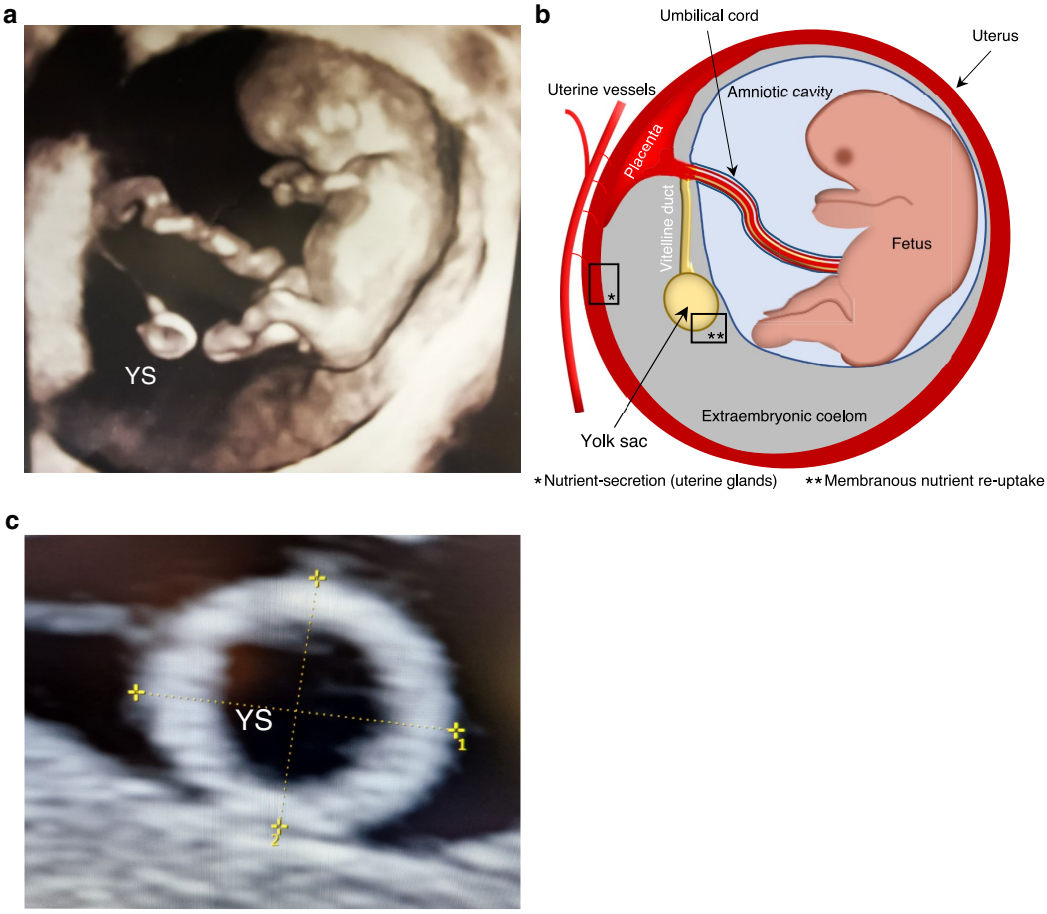

Figure 1. (**a**) 3D ultrasound of a 10-weeks' embryo with a secondary yolk sac (YS). (**b**) Graphical illustration of the embryo-yolk sac connection with the yolk sac localized outside the amniotic cavity in the extraembryonic coelom. * Uterine glands secrete amino acids, ions, carbohydrates (glucose), lipids, proteins (e.g., cytokines, enzymes, hormones, growth factors, proteases and their inhibitors, and transporters)[61]. ** Yolk sac membrane with a vascular plexus envelope is involved in transport or resynthesis and exocytosis of nutrients, either directly into the surrounding blood vessels or the yolk sac cavity[11]. (**c**) Yolk sac size was assessed by two perpendicular outer-to-outer diameters measured thrice, and the mean was entered into the statistics.

second study, a shorter maternal sleep duration was linked to a larger yolk sac at 7 weeks of gestation, but this was essentially limited to male embryos[16].

This brings attention to another maternal factor, physical activity, which is related to healthy weight gain, improved maternal glucose control[17,18], and favorable obstetric outcomes[18–22]. More specifically, maternal physical activity is associated with a larger placental volume, villous surface area and vascular volume[23] and modulates factors related to placental angiogenesis[24,25]. Physical activity also downregulates genes involved in placental fatty acid and insulin transport, upregulates genes involved in amino acid transport across the placenta, and reduces oxidative stress[18,26].

Based on this background, we speculate that physical activity in healthy women before and during early pregnancy affects the intrauterine environment. Thus, we hypothesize that these effects are reflected in the size of the yolk sac, which is involved in embryonic growth regulation, and that the effects are sex specific.

Results

The cohort consisted of 436 eligible participants (Fig. 3 and Table 1), of whom 190 (43.6%) became pregnant and provided sufficient data for inclusion in the present study (all study data with keys are supplied in Supplementary Tables S1 and S2). These 190 women had regular menstrual cycles with a median of 28 days (range

$n = 190$	Frequency	Missing	Mean	SD	Min	Max	IQR
Age (years)		0	29.0	3.1	20.0	35.0	27–31
Height (cm)		0	167.7	6.2	149.0	185.0	164–172
Weight (kg)		0	64.7	8.3	47.1	89.8	58.9–71.2
BMI		0	23.0	2.6	17.8	29.9	21–24.8
Lean body mass (kg)		0	45.7	3.8	36.0	55.6	43.0–48
Body fat (%)		0	28.8	5.5	15.9	41.9	25–32.9
Cycle length (days)		0	28.5	1.7	24	35	28–29
Parity		0					
0	89 (46.8%)						
1	79 (41.6%)						
≥ 2	22 (11.6%)						
Training efforts*		0					
None	3 (1.6%)						
Effortless walking	46 (24.2%)						
< 3 times week^{-1}	90 (47.4%)						
≥ 3 times week^{-1}	51 (26.8%)						

Table 1. Descriptive statistics of the participants[16] are presented as the mean, standard deviation (SD), range (Min, Max), and interquartile range (IQR). *Training efforts established based on a unvalidated questionnaire completed at study entry by each participant.

24–35 days, interquartile range (IQR) 1 day) and successful pregnancies resulting in live-born neonates with a median pregnancy length of 281 days (IQR 12 days) according to the date of the last menstrual period (LMP) or 278.5 days (IQR 11.8 days) based on the embryonic crown-rump length (CRL) in the first trimester[27]. Generally, the rate of pregnancy complications was low, e.g., gestational hypertension (3.2%), gestational diabetes (3.7%), preterm birth (3.2%), and a 5-min Apgar score less than seven (1.1%). The demographic characteristics of the study cohort are provided in Table 1.

Daily physical activity duration

In total, 92.1% of all recorded days before conception and 93.7% of the recorded days at the end of the first trimester fulfilled our eligibility criteria[16], and no participant was excluded from the analysis. The total duration of actigraphy and the frequency of recorded data for weekend days before conception did not differ from those recorded after conception. With notable individual variation, the total daily activity duration was 5 h and 55 min before conception (95% CI [5 h 37 min–6 h 13 min], and the duration was 1 h 36 min shorter at the end of the first trimester (95% CI [1 h 19 min–1 h 55 min]) (Table 2, Supplementary, Fig. S1). This pattern was similar for the different activity intensities (light and moderate-vigorous activity) (Table 2).

Term	n	Mean/Median	SD/IQR	95% CI
1st actigraphy recording (before pregnancy)	176			
Days before estimated conception*		36**	10–75**	(43.8–59.5)
Number of recorded days		3.7	0.7	(3.6–3.8)
Total activity (min day^{-1})		354.7	120.9	(336.7–372.7)
Light activity (min day^{-1})		259.7	94.1	(245.7–273.7)
Moderate or vigorous activity (min day^{-1})		96.8	55.9	(88.5–105.1)
2nd actigraphy recording (week 13)	178			
Gestational age (weeks)		13.2	0.8	(13.1–13.3)
Number of recorded days		3.7	0.6	(3.6–3.8)
Total activity (min day^{-1})		254.5	101.6	(239.5–269.5)
Light activity (min day^{-1})		198.8	83.1	(186.5–211.1)
Moderate or vigorous activity (min day^{-1})		57.5	36.2	(52.1–62.8)

Table 2. Summary statistics of maternal physical activity based on actigraphy data of 190 low-risk pregnant women before pregnancy and at 13 weeks of gestation, presented with the number of measurements (n), mean or median, standard deviation (SD) or interquartile range (IQR), and 95% confidence interval of the mean (95% CI). Daily maternal physical activity duration was classified as total, light, and moderate or vigorous activity. *Time to conception was calculated as the number of days from the start of the maternal actigraphy recording at inclusion to day 14 of the cycle that led to conception. **The median or IQR was calculated.

Yolk sac size

A total of 358 yolk sac measurements were obtained through sonographic assessment at gestational weeks 7 and 10, showing an increase in the mean size from 4.7 mm at week 7 (95% CI [4.7–4.8]) to 5.9 mm at week 10 (95% CI [5.8–6.1]) (Fig. 2a, Table 3); the difference of 1.14 mm was significant (95% CI [0.99–1.29]). However, individual growth rates displayed considerable variation (Fig. 2b).

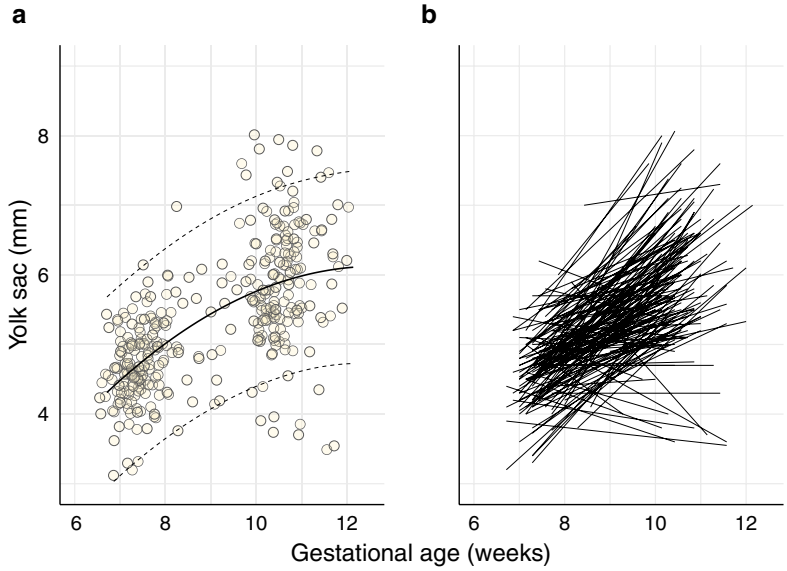

Figure 2. First and second yolk sac measurements by gestational age based on the last menstrual period. (**a**) In total, 358 measurements included the mean and 95% prediction band. (**b**) A line between the first and second yolk sac measurements representing the individual yolk sac growth rate. Line plot demonstrating the variations in yolk sac growth between the two measurements (no line is shown when only one measurement was available, and one participant was not included in this plot as the menstrual age at the time of the second measurement was an outlier beyond 14 gestational weeks).

Term	Sex	*n*	Mean	SD	95% CI	*p**
Week 7: 1st measurement; GA (weeks)		180	7.6	0.7	(7.5–7.7)	
	♂	89	7.5	0.5	(7.4–7.6)	0.08
	♀	91	7.7	0.9	(7.5–7.8)	
Week 7: 1st yolk sac diameter (mm)		180	4.7	0.6	(4.7–4.8)	
	♂	89	4.7	0.6	(4.6–4.8)	0.39
	♀	91	4.8	0.6	(4.7–4.9)	
Week 10: 2nd measurement; GA (weeks)		178	10.6	0.8	(10.5–10.7)	
	♂	87	10.6	0.7	(10.5–10.8)	0.65
	♀	91	10.7	0.9	(10.5–10.9)	
Week 10: 2nd yolk sac diameter (mm)		178	5.9	0.9	(5.8–6.0)	
	♂	87	5.9	0.8	(5.7–6.1)	0.99
	♀	91	5.9	1.0	(5.7–6.1)	
Week 7–10: Yolk sac growth rate (mm week^{-1})		170	0.38	0.33	(0.33–0.43)	
	♂	81	0.37	0.29	(0.30–0.43)	0.67
	♀	89	0.39	0.36	(0.32–0.46)	

Table 3. Summary statistics of the ultrasound data of 190 low-risk pregnant women presented with subgroups (males and females), the number of measurements (*n*), mean, standard deviation (SD), 95% confidence interval of the mean (95% CI), and *p*-value (*p*) for the between-group tests. Gestational age (GA) was based on the last menstrual period. *The unpaired t test was performed for the yolk sac data, and the Mann—Whitney U test (Wilcoxon rank-sum test) was performed for the gestational age data.

Inter- and intraobserver variability of yolk sac sonographic measurements

The reproducibility study of the yolk sac ultrasound measurements showed intraobserver variability of 0.08% and interobserver variability of 0.09%, corresponding to an intraobserver standard error of measurement (SEM) of 0.029 mm, with a 95% confidence interval (CI) of ± 0.056 mm. The interobserver SEM was 0.03 mm, resulting in a minimum detectable difference of 0.08 mm for the applied measurement technique.

Effect of daily maternal physical activity duration on yolk sac size

When gestational age (GA) and embryonic sex were not accounted for, physical activity had no significant effect on yolk sac size, i.e., neither before conception nor at the end of the first trimester (0.03 mm·h^{-1}, 95% CI [− 0.02 to 0.08]; and 0.00 mm·h^{-1}, 95% CI [− 0.06 to 0.06], respectively).

The effect of daily physical activity on yolk sac size is a function of fetal sex and GA

At 7 weeks gestation, the yolk sac diameter of male embryos was larger when the prepregnancy physical activity duration was longer (i.e., 10% larger for the average amount of daily physical activity before pregnancy; $p < 0.01$). At this stage, such an effect was not evident in female embryos ($p = 0.93$). However, at 10 weeks' gestation, maternal physical activity was associated with yolk sac size in both male and female embryos (Fig. 3, Supplementary Table S3). At this stage, the relation between yolk sac size and maternal physical activity became negative for male embryos ($p = 0.04$), while for female embryos, a stronger and positive correlation was shown ($p < 0.01$), similar to the effect observed in male embryos at gestational week 7. Notably, the interaction between embryonic sex and daily maternal physical activity was also highly significant (0.24 mm·h^{-1}; 95% CI [0.12–0.38]), underscoring the differential effect of maternal physical activity on yolk sac size at 10 weeks' gestation, depending on the sex of the embryo (Fig. 4, Supplementary Table S3). When considering the average duration of maternal activity prior to conception, this effect translates to a yolk sac that is 24% larger in female embryos than in male embryos.

For the physical activity recordings at 13 weeks' gestation, a similar relation between maternal physical activity and yolk sac size in male and female embryos at either 7- or 10-weeks' gestation was observed but did not reach significance ($p \geq 0.1$) (Supplementary Table S3; Fig. 3).

Adjusting the analysis for maternal age, parity, and body composition had a negligible impact on the results (Supplementary Tables S4–S7); the same applied to adjustments for GA (Supplementary Tables S4–S7), GA-adjusted yolk sac Z scores (Supplementary Table S8, Eq. 1 and Code C1), and quantile regression results (Supplementary Figs. S2–S5).

In a subanalysis, we stratified by time of inclusion due to the long study period and did not observe any significant effect on our results (Supplementary Tables S4–S7). The same applied for the few women with pregnancy complications in the study; their exclusion from the analysis did not alter our results (Supplementary Tables S4–S7).

Effect of maternal physical activity on yolk sac growth velocity (mm·week^{-1})

In addition to the association between daily maternal physical activity duration and yolk sac size at different GAs (weeks 7 and 10), we also found an effect on yolk sac growth dynamics. In both male and female embryos, the recorded maternal physical activity before pregnancy was associated with variation in yolk sac growth velocity (mm·week^{-1}) but differed by 10% per hour of physical activity between the sexes, and this was highly significant ($p < 0.01$). In contrast to male embryos, where yolk sac growth was lower at higher activity durations ($p < 0.01$), female embryos showed higher growth between 7 and 10 weeks' gestation ($p = 0.01$) (Supplementary Table S9).

Effect of maternal physical activity intensity on yolk sac size

The total daily duration of maternal physical activity was classified based on intensity, as light versus moderate-vigorous physical activity. Even within these subcategories, the impact of maternal physical activity, dependent on sex and GA, was similar to that observed for the total physical activity duration (Fig. 5).

Discussion

This study of low-risk human pregnancies demonstrated that maternal physical activity before and during early pregnancy affects embryonic development, in this case, yolk sac size. A graded yolk sac response based on maternal physical activity duration was observed across all activity levels, encompassing both light and moderate-vigorous activities. Notably, we found that the effect was sex dependent, with different time windows and directions of impact (Fig. 3b, 4). Additionally, the sex-specific effect on yolk sac growth rate between gestational weeks 7 and 10 revealed the dimension of an inverse effect on yolk sac growth dynamics for male and female embryos. Therefore, we hypothesize that high physical activity levels may strain the intrauterine environment and cause compensatory enlargement of the yolk sac surface at different GAs to ensure adequate nutritional support for embryonic growth, determined by embryonic sex.

Based on the two previous studies[15,16] and the present study, a distinct pattern emerges: sensitive windows in embryonic development seem short, and the timing and effects are sex-specific (confer overview in Fig. 6). For example, at 8 weeks of gestation, a larger yolk sac size in female embryos develops when the maternal height and weight are low[15]. On the other hand, at 7 weeks' gestation, a larger yolk sac is seen in male embryos when the maternal sleep duration is short[16]. The present study showed that an extended maternal physical activity leads to a larger yolk sac in male embryos at 7 weeks' gestation, while in female embryos, an extended physical activity is associated with a smaller yolk sac at 10 weeks' gestation. This figure illustrates not only the sex-specific modifications of the observed effects in terms of timing and direction but also underscores the importance of precise and frequent observations—during a phase of rapid progression through consecutive developmental stages—to

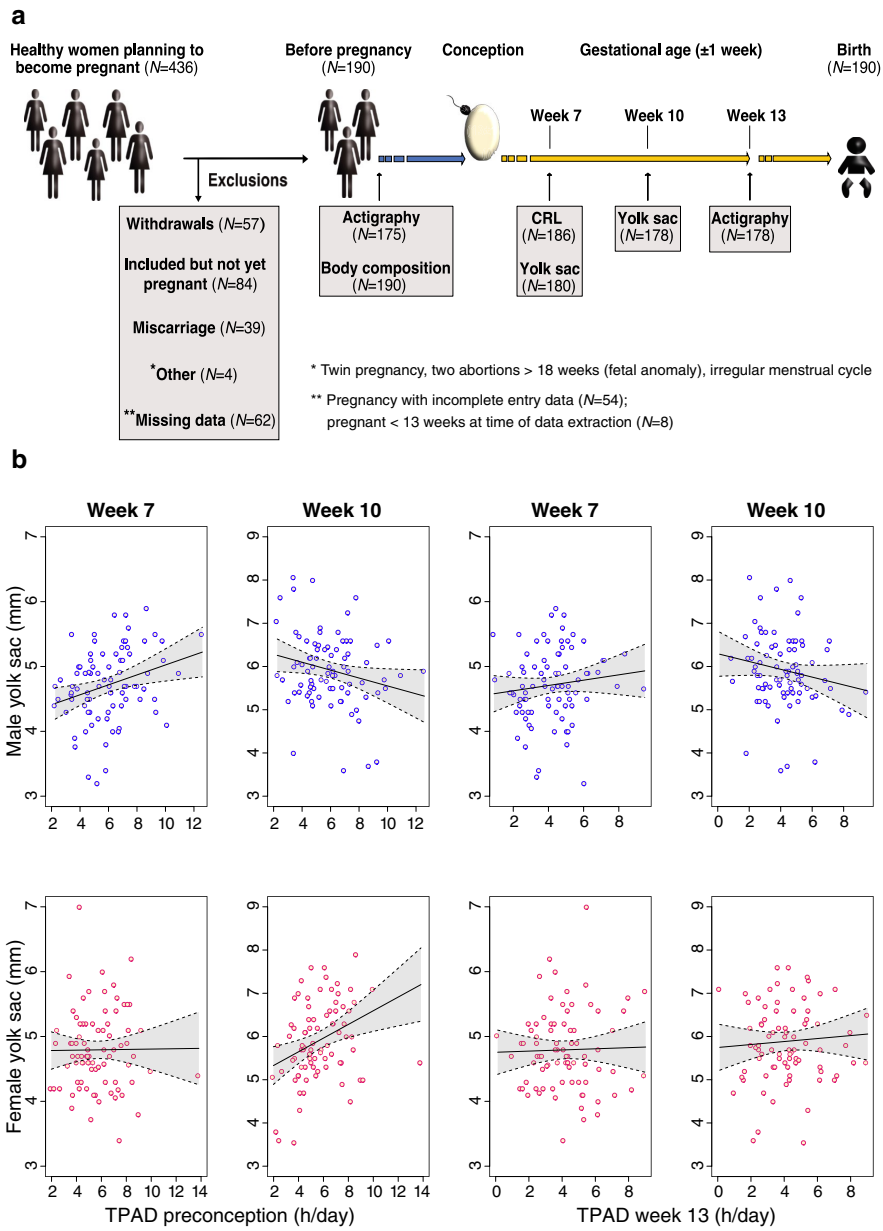

Figure 3. (**a**) Study protocol including the study population, participant exclusions, and study visits with the type and number of successful measurements. (**b**) Effect of the total daily activity duration (TPAD) before pregnancy (left half) and at gestational week 13 (right half) on the yolk sac size at 7 weeks and 10 weeks. A regression line and its 95% confidence interval are presented and grouped according to embryonic sex (males (blue) and females (red)).

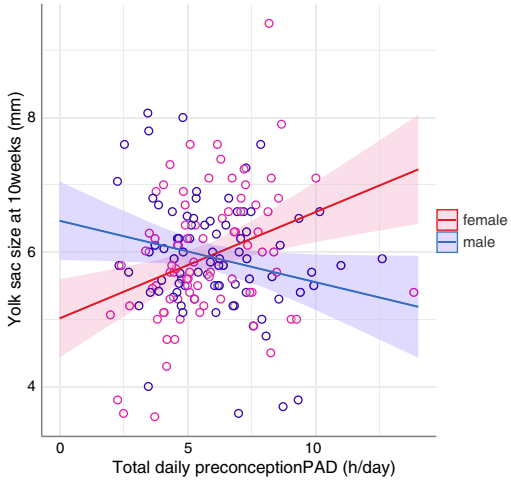

Figure 4. Yolk sac size of male and female embryos at 10 weeks of gestation according to total daily maternal physical activity (PAD) before pregnancy. The presented regression lines and their 95% confidence intervals are grouped according to embryonic sex.

capture such effects. Animal studies have also provided some evidence supporting the effect of environmental factors, such as temperature, nutrition, and noise, on the yolk sac[28–31], but yolk sac development and implantation mechanisms vary among species[5,10,11].

Nevertheless, the findings of the present study support the concept of the compensatory enlargement of the yolk sac surface to ensure adequate nutritional support for embryonic growth[15]. In nonpregnant individuals, exercise is known to reduce visceral blood flow to meet the metabolic demand of the working muscles[32]. This reduction in visceral blood flow is accompanied by increased vasodilatation through the release of nitric oxide (NO)[33] and endothelium-dependent hyperpolarization[34]. It has also been suggested that physical activity induces shear stress and intermittent fluctuations in substrate and oxygen delivery, resulting in hypoxic strain, which generates a repetitive stimulus triggering a feto-maternal response with increased placental vascularization[26,35].

However, compared with the increase in yolk sac size, the association of physical activity and fetal body composition[36–39], fetal growth, placental size[36,40–43], and placental circulation[23] are relatively late pregnancy responses to multiple factors and events. A significant feto-maternal connection via placental circulation is not established before twelve weeks of gestation[44] and therefore is unlikely to explain variations in yolk sac size. Nevertheless, the underlying mechanisms may be similar because they both occur within the same organ, the uterus, with the same supplying vasculature, myometrium, and endometrium that includes glands surrounded by vessels. Therefore, it is plausible that fluctuations capable of influencing placental development may also influence histotrophic nutrition at earlier stages of pregnancy via the uterine glands and vasculature[10,11]. Furthermore, sex steroid levels, which are associated with physical activity in women[45], are widely recognized to influence both the menstrual cycle and the timing of ovulation, as well as the composition of the endometrium and its glands.

The sex-specific response in size and growth dynamics shown in the present study is in line with other reports on sexual dimorphism in response to environmental factors during pregnancy in animal and in vitro studies[2]. We envisage that physical activity might act as a natural stress factor leading to the physiological adaptive response of the yolk sac (i.e., increasing size and thereby surface area that facilitates gas exchange and nutrient uptake during the period before the placenta is sufficiently developed).

Study strengths

The strengths of this study lie in its prospective longitudinal design, which includes maternal data from the preconception period, and the inclusion of many healthy women who conceived naturally, without the confounding influence of hormonal treatments commonly used in assisted reproduction. Furthermore, the observed opposite effects on male and female embryos at 10 gestational weeks provide robust evidence for a sex-dependent effect of maternal physical activity on yolk sac size.

Additionally, the effect on yolk sac size at gestational weeks 7 and 10 is corroborated by the sex-specific effect on the yolk sac growth rate and strengthens the internal validity of the study.

Another strength is the utilization of alternative statistical models and quantile regression models, which consistently yielded the same results. This demonstrates that the results were not dependent on skewed data, systematic distribution differences, or extreme values.

To ensure the accuracy of yolk sac measurements, intra- and interobserver variability were calculated, confirming sufficient measurement precision, which was unbiased by any observer. Confounding observer effects are unlikely since maternal physical activity is not inherently related to the ultrasound procedure itself.

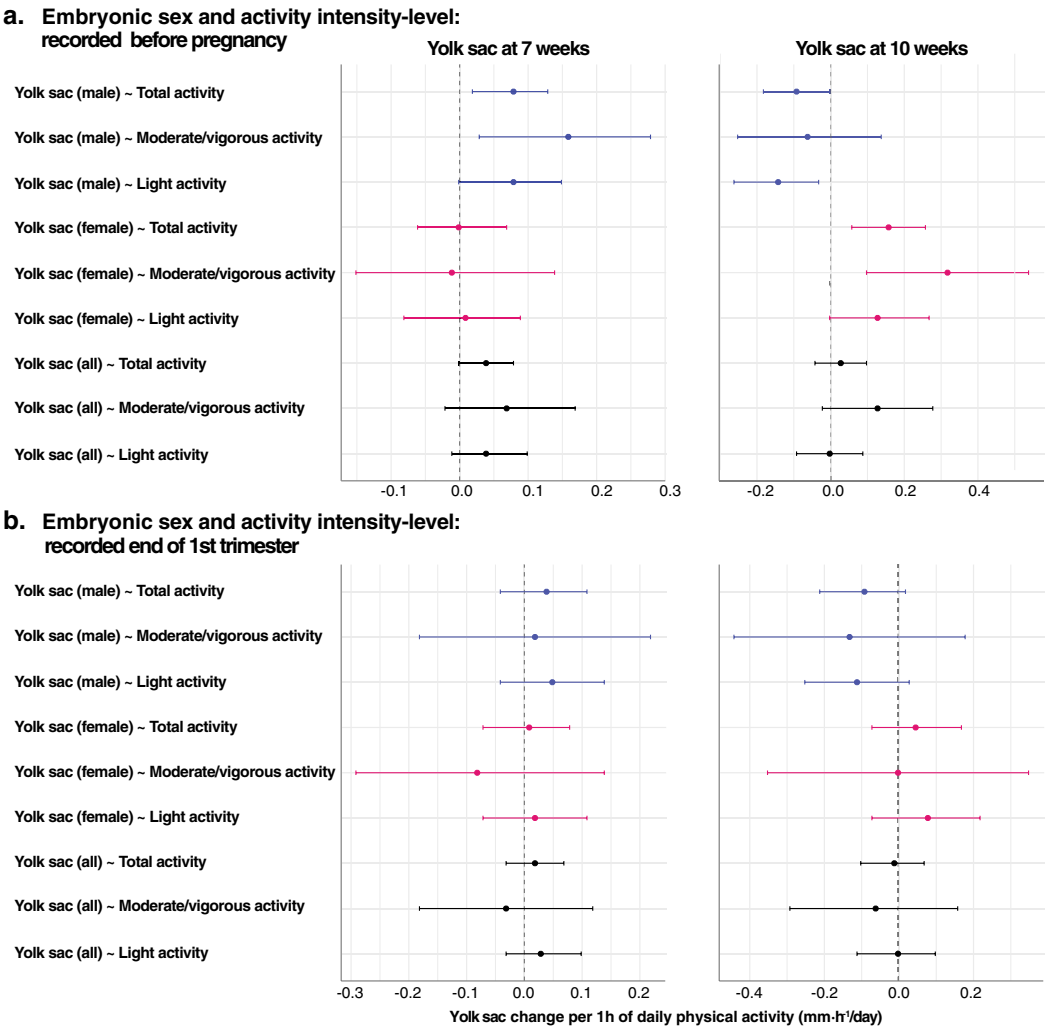

Figure 5. Forest plot showing the effect of physical activity intensity on yolk sac size according to embryonic sex and the time of the actigraphy recording: (**a**) before pregnancy and (**b**) at the end of the 1st trimester. Coefficients are presented with 95% confidence intervals.

The ultrasound operators were blinded to embryonic sex, as embryonic sex was unknown at the time of observation.

Multiple regression analyses examining the association between maternal physical activity and yolk sac size did not reveal any significant observer effects (ultrasound operator) or effects of maternal age, parity, weight, height, BMI, lean body mass, body fat percentage, GA, time of inclusion, or the inclusion of the few participants with pregnancy complications.

Study limitations

Due to the study design, we cannot be certain that the activity patterns recorded before pregnancy, although close to conception (Table 2), continued into early pregnancy. In addition, the study included only two yolk sac measurements (gestational weeks 7 and 10). Both factors imply that we cannot infer whether the variation in yolk sac observations stems from pre- or periconception variations in the intrauterine environment (i.e., the vasculature, myometrium, and endometrium with endometrial glands) or whether we observed an ongoing sex-specific impact of maternal physical activity during gestation within the specific time windows of gestational weeks 7 and 10.

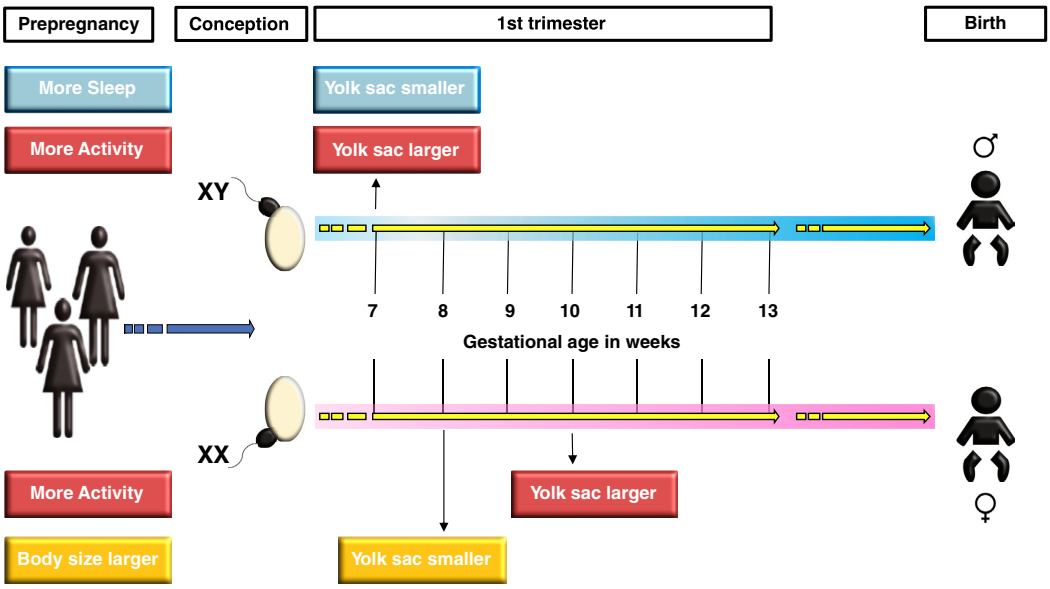

Figure 6. Effect of preconception maternal factors on yolk sac size: i.e., the effect of maternal sleep duration[16], total maternal physical activity duration, and maternal body size (weight and height)[15]. The figure illustrates the sex and time-dependent effects during the 1st trimester. The time windows, where these effects can be observed, are short.

An additional use of activity diaries or continuous measurement as well as more frequent yolk sac measurements would have strengthened the conclusions and provided deeper insight. However, the effect of different intensities of physical activity on the yolk sac could also be traced later in pregnancy (at 13 weeks' gestation), suggesting that the physical activity pattern was similarly distributed in the population throughout the entire period.

Other challenges to control for are sex steroid levels, mental stress, and maternal nutrition. Stress causes a hormonal response similar to that of exercise, and nutrition is closely related to energy metabolism; thus, both might be confounders. The study population, however, consisted of healthy women with no history of chronic diseases or risk factors, and the chance of chronic psychological stress in this population should therefore be low. Confounding by differences in maternal nutrition also seems unlikely, as maternal body composition, which is closely related to energy metabolism and nutrition, did not significantly affect our results (Supplementary Tables S4–S7).

Conclusion

Normal human embryonic development is sensitive to maternal cues. Here, we showed that maternal physical activity influences human yolk sac development in a graded fashion. Second, embryonic sex determines the timing, degree, growth dynamics, and direction of the effect. Third, the time frames for these effects seem to be rapidly changing, short phases at this stage of pregnancy.

Methods

We studied the effects of maternal physical activity on the yolk sac in a prospective, longitudinal study of healthy nonsmoking women who planned to conceive naturally. The study is embedded in the ongoing CONIMPREG research program[16,46].

Data collection

During the period 2014–2020, women aged 20–35 years with a BMI of 18–30 kg/m^2 were recruited through social media (targeted Facebook® advertisements) and posters, provided that they had an uncomplicated obstetric history, a regular menstrual cycle, did not use contraceptives during the month before study entry and had no chronic diseases or fertility problems. If the women did not conceive within six sampling cycles, they were excluded from the study.

The participants were assessed at four consecutive study visits (Fig. 3a). At the first visit—before conception—maternal height and body composition were measured, immediately followed by the first actigraphy recording. The second visit was scheduled—based on the first day of the LMP—at 7 ± 1 weeks' gestation. At this time, we confirmed the viability of the embryo and the length of gestation[47] and assessed the yolk sac. At the third visit

(10 ± 1 weeks' gestation), the yolk sac measurements were repeated, and finally, at the fourth visit (13 ± 1 weeks' gestation), maternal body composition and activity duration were reassessed.

Height, weight, and maternal body composition

Before conception, height was measured with a wall-mounted stadiometer[48], and weight was measured digitally using bioelectrical impedance analysis (model BC-418, Tanita, Tokyo, Japan). The percentage of body fat was estimated using the instrument's computer software, and lean body mass was calculated by subtracting body fat mass from total body weight. Measurements were carried out as recommended by the manufacturer[49].

Physical activity

Maternal physical activity was recorded before conception and at gestational week 13 using the SenseWear Mini Armband Actigraph (model MF-SW, BodyMedia, Pittsburgh, PA, USA). This wireless, noninvasive activity monitor incorporates triaxial accelerometry, heat flux, galvanic skin response, skin temperature, and near-body temperature measurements with a sampling frequency of 32 Hz. All information, plus information on sex, age, height, and weight, is considered in proprietary algorithms to predict physical activity at the level of 1.4 metabolic equivalents (METs)[50]. In accordance with the Sedentary Behavior Research Network (SBRN) consensus and American College of Sports Medicine (ASM) guidelines, the recordings were classified as light activity at ≥ 1.5 METs < 3.0, moderate at ≥ 3.0 METs < 6.0, and vigorous at ≥ 6.0 METs[51,52]. The monitor was worn on the upper posterior part of the nondominant arm for 4 days[53], and the recording started at midnight. Raw data were processed and summarized using SenseWear Pro analysis software (SenseWear Professional, version 8.0.0.2903, Body Media) and exported into Excel workbooks (Microsoft Office, Excel version 2016, Redmond, WA, USA). Sampling days were excluded from the statistical analyses when data loss in a single day exceeded 6%. This or earlier versions of this actigraph have been validated for physical activity measurements[50,54,55], including measurements during pregnancy[56,57]. The results of the included pregnant women were in good agreement with results from earlier versions of this monitor and other actigraphs or methods[46].

Embryonic measurements

At gestational weeks 7 and 10, ultrasound measurements were carried out by a group that consisted of seven obstetricians using a 6–12 MHz transvaginal transducer (Voluson Expert E8; GE Medical Systems, Kretz Ultrasound, Zipf, Austria). The transducer output power was set to be low, with a thermal index (TI) always below 1.0[58]. Viability of the embryo was ensured by employing clinical guideline safety criteria[59], and the length of gestation was confirmed by the CRL[47] determined as the mean of three measurements. The yolk sac size was determined as the average of two perpendicular outer diameters measured thrice[15] (Fig. 1c).

Inter- and intraobserver variability of the yolk sac measurements

To calculate the inter- and intraobserver variability of yolk sac size measurements, we expanded our study in 2023 by utilizing prospectively collected data (Supplementary Tables with keys in S10 and S11) from the same study cohort (CONIMPREG). Embryonic yolk sacs ($n = 19$) were assessed either at gestational week 7 or week 10, and video sequences (ultrasound loops) were generated and stored in the machine's local archive.

All seven ultrasound operators were instructed to select the best yolk sac image from the sequence and measure the yolk sac using the previously described method. This involved measuring the perpendicular diameters three times and calculating the mean size. After a minimum of one day, the procedure was repeated to assess intraobserver variability (repeatability).

Statistics

Statistical analysis was performed using R (Foundation for Statistical Computing, version 4.1, Vienna, Austria) and R-studio (Integrated development for R, Boston, MA, USA) software.

The mean and standard deviation (SD) with minimum and maximum values were calculated for each continuous variable, and frequencies and proportions were calculated for categorical variables. When the distribution was asymmetric, the median and IQR are reported. In addition, the 95% CIs of the mean were calculated for the recorded physical activity intensities, the number of days with recorded data, the frequency of physical activity on weekend days, and the CRL and yolk sac size with GA at the time of the measurements.

Ordinary least square linear (OLS) regression models were used to analyze the association of yolk sac size with maternal physical activity duration before pregnancy and at the end of the first trimester (week 13). Linearity assumptions and normal distribution of the residuals were ascertained. The regression models were fitted with and without embryonic sex stratification. In addition, we tested the effect of embryonic sex on the maternal physical activity-yolk sac relation by adding the interaction term (embryonic sex*maternal physical activity) to the OLS model. OLS regression results were compared with results from quantile regression, including iterated reweighted least squares regressions (Huber weights and bisquare weighting), and heteroskedastic methods (sandwich variance estimators). In the subanalysis of our main findings, we replaced the yolk sac diameter with the yolk sac Z score that was calculated employing multilevel growth models, accounting for repeated measurements and GA (Supplementary Equation EQ1 and code C1). In addition, we controlled for physical activity effects in the original OLS model for GA. Likewise, maternal age, parity, and body composition parameters (i.e., height, weight, body mass index, lean body mass, and body fat percent) were added one by one to the primary model and were included if they notably altered the effect size of the association. We also stratified by time of inclusion and assessed the effect of three equally sized time categories between 2014 and 2020 by adjusting the regression model for these strata. Finally, we performed regression analyses with and without participants who experienced complications or unfavorable obstetric outcomes (i.e., hypertensive complications, gestational

diabetes, preterm birth, and a 5-min Apgar score less than seven). As measures of fit, the adjusted *R*-squared and Akaike information criterion were calculated. Differences between the regression models were tested using analysis of variance (ANOVA) methods. Differences in variables from the summary statistics were tested with unpaired and paired parametric or nonparametric tests.

The assessment of intra- and interobserver variability, along with the associated SEM (SEM-intraobserver and SEM-interobserver), as well as the minimum detectable difference, was conducted using a two-way ANOVA method, as outlined by Popović and Thomas[60]. The necessary variances were derived either directly or indirectly by utilizing the variances expressed as multiple squares for the various factors of the model (i.e., observer, subject, the interaction between the observer and subject) and the residual variation (Code is provided in Supplementary Code C2).

Ethics declaration and consent

The study was approved by the Regional Committee for Medical Research Ethics Southeast Norway (REK Southeast, ref. 2013/856a). E-mail: rek-sorost@medisin.uio.no. Written informed consent was obtained from all participants, and all research was performed in accordance with relevant guidelines and regulations.

Data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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References

1. Hanson, M. A. & Gluckman, P. D. Early developmental conditioning of later health and disease: physiology or pathophysiology. *Physiol. Rev.* **94**, 1027–1076 (2014).
2. Rosenfeld, C. S. Sex-specific placental responses in fetal development. *Endocrinology* **156**, 3422–3434 (2015).
3. Workalemahu, T. *et al.* Genetic and environmental influences on fetal growth vary during sensitive periods in pregnancy. *Sci. Rep.* **8**, 7274 (2018).
4. Ljubic, A., Abazovic, D., Ljubic, D., Pirkovic, A. & Perovic, A. *Induced Abortion and Spontaneous Early Pregnancy Loss—Focus on Management* (IntechOpen, 2020).
5. Carter, A. M. Unique aspects of human placentation. *Int. J. Mol. Sci.* **22**, 8099 (2021).
6. Bermejo-Alvarez, P., Rizos, D., Lonergan, P. & Gutierrez-Adan, A. Transcriptional sexual dimorphism during preimplantation embryo development and its consequences for developmental competence and adult health and disease. *Reproduction* **141**, 563–570 (2011).
7. Deegan, D. F. & Engel, N. Sexual dimorphism in the age of genomics: How, when, where. *Front. Cell Dev. Biol.* **7**, 186 (2019).
8. Eriksson, J. G., Kajantie, E., Osmond, C., Thornburg, K. & Barker, D. J. Boys live dangerously in the womb. *Am. J. Hum. Biol.* **22**, 330–335 (2010).
9. Burton, G. J., Hempstock, J. & Jauniaux, E. Nutrition of the human fetus during the first trimester—A review. *Placenta* **22**(Suppl A), S70–S77 (2001).
10. Burton, G. J., Cindrova-Davies, T. & Turco, M. Y. Review: Histotrophic nutrition and the placental-endometrial dialogue during human early pregnancy. *Placenta* **102**, 21–26 (2020).
11. Ross, C. & Boroviak, T. E. Origin and function of the yolk sac in primate embryogenesis. *Nat. Commun.* **11**, 3760 (2020).
12. Mäkikallio, K., Tekay, A. & Jouppila, P. Yolk sac and umbilicoplacental hemodynamics during early human embryonic development. *Ultrasound Obstet. Gynecol.* **14**, 175–179 (1999).
13. Chen, S., Yang, J., Wei, Y. & Wei, X. Epigenetic regulation of macrophages: From homeostasis maintenance to host defense. *Cell Mol. Immunol.* **17**, 36–49 (2020).
14. Goh, I. *et al.* Yolk sac cell atlas reveals multiorgan functions during human early development. *Science* **381**, eadd7564 (2023).
15. Odland Karlsen, H. *et al.* The human yolk sac size reflects involvement in embryonic and fetal growth regulation. *Acta Obstet. Gynecol. Scand.* **98**, 176–182 (2018).
16. Vietheer, A., Kiserud, T., Lie, R. T., Haaland, Ø. A. & Kessler, J. Effect of maternal sleep on embryonic development. *Sci. Rep.* **12**, 17099 (2022).
17. Ming, W. K. *et al.* The effect of exercise during pregnancy on gestational diabetes mellitus in normal-weight women: A systematic review and meta-analysis. *BMC Pregnancy Childbirth* **18**, 440 (2018).
18. Reyes, L. M. & Davenport, M. H. Exercise as a therapeutic intervention to optimize fetal weight. *Pharmacol. Res.* **132**, 160–167 (2018).
19. Mudd, L. M., Owe, K. M., Mottola, M. F. & Pivarnik, J. M. Health benefits of physical activity during pregnancy: An international perspective. *Med. Sci. Sports Exerc.* **45**, 268–277 (2013).
20. Barakat, R., Franco, E., Perales, M., López, C. & Mottola, M. F. Exercise during pregnancy is associated with a shorter duration of labor. A randomized clinical trial. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **224**, 33–40 (2018).
21. Witvrouwen, I., Mannaerts, D., Van Berendoncks, A. M., Jacquemyn, Y. & Van Craenenbroeck, E. M. The effect of exercise training during pregnancy to improve maternal vascular health: Focus on gestational hypertensive disorders. *Front. Physiol.* **11**, 450 (2020).
22. Russo, L. M., Harvey, M. W., Pekow, P. & Chasan-Taber, L. Physical activity and risk of cesarean delivery in hispanic women. *J. Phys. Act. Health* **16**, 116–124 (2019).
23. Jackson, M. R., Gott, P., Lye, S. J., Ritchie, J. W. & Clapp, J. F. The effects of maternal aerobic exercise on human placental development: Placental volumetric composition and surface areas. *Placenta* **16**, 179–191 (1995).
24. Bhattacharjee, J., Mohammad, S., Goudreau, A. D. & Adamo, K. B. Physical activity differentially regulates VEGF, PlGF, and their receptors in the human placenta. *Physiol. Rep.* **9**, e14710 (2021).
25. Hardy, D. B., Mu, X., Marchiori, K. S. & Mottola, M. F. Exercise in pregnancy increases placental angiogenin without changes in oxidative or endoplasmic reticulum stress. *Med. Sci. Sports Exerc.* **53**, 1846–1854 (2021).
26. Ramírez-Vélez, R., Bustamante, J., Czerniczyniec, A., Aguilar-de-Plata, A. C. & Lores-Arnaiz, S. Effect of exercise training on eNOS expression, NO production and oxygen metabolism in human placenta. *PLoS ONE* **8**, e80225 (2013).
27. Robinson, H. P. & Fleming, J. E. A critical evaluation of sonar "crown-rump length" measurements. *Br. J. Obstet. Gynaecol.* **82**, 702–710 (1975).
28. Fiksen, Ø. & Folkvord, A. Maternal effects and the benefit of yolk supply in cod larvae in different environments—A simulation model. ICES Council Meeting, 1–6 (1999).

29. Lara, R. A. & Vasconcelos, R. O. Impact of noise on development, physiological stress and behavioural patterns in larval zebrafish. *Sci. Rep.* **11**, 6615 (2021).
30. Mikec, M. *et al.* Influence of environmental and nutritional stressors on yolk sac utilization, development of chicken gastrointestinal system and its immune status. *World's Poultry Sci. J.* **62**, 31–40 (2006).
31. Watkins, A. J. *et al.* Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. *Biol. Reprod.* **78**, 299–306 (2008).
32. Delp, M. D. Differential effects of training on the control of skeletal muscle perfusion. *Med. Sci. Sports Exerc.* **30**, 361–374 (1998).
33. Schuler, G., Adams, V. & Goto, Y. Role of exercise in the prevention of cardiovascular disease: Results, mechanisms, and new perspectives. *Eur. Heart J.* **34**, 1790–1799 (2013).
34. Gündüz, F. *et al.* Exercise training enhances flow-mediated dilation in spontaneously hypertensive rats. *Physiol. Res.* **60**, 589–597 (2011).
35. Bergmann, A., Zygmunt, M. & Clapp, J. F. Running throughout pregnancy: Effect on placental villous vascular volume and cell proliferation. *Placenta* **25**, 694–698 (2004).
36. Clapp, J. F. *et al.* Continuing regular exercise during pregnancy: effect of exercise volume on fetoplacental growth. *Am. J. Obstet. Gynecol.* **186**, 142–147 (2002).
37. Hopkins, S. A., Baldi, J. C., Cutfield, W. S., McCowan, L. & Hofman, P. L. Exercise training in pregnancy reduces offspring size without changes in maternal insulin sensitivity. *J. Clin. Endocrinol. Metab.* **95**, 2080–2088 (2010).
38. Harrod, C. S. *et al.* Physical activity in pregnancy and neonatal body composition: the Healthy Start study. *Obstet. Gynecol.* **124**, 257–264 (2014).
39. Bisson, M. *et al.* Influence of maternal physical activity on infant's body composition. *Pediatr. Obes.* **12**(Suppl 1), 38–46 (2017).
40. Clapp, J. F., Kim, H., Burciu, B. & Lopez, B. Beginning regular exercise in early pregnancy: Effect on fetoplacental growth. *Am. J. Obstet. Gynecol.* **183**, 1484–1488 (2000).
41. Clapp, J. F. Influence of endurance exercise and diet on human placental development and fetal growth. *Placenta* **27**, 527–534 (2006).
42. Rodríguez, I. & González, M. Physiological mechanisms of vascular response induced by shear stress and effect of exercise in systemic and placental circulation. *Front. Pharmacol.* **5**, 209 (2014).
43. Hilde, G., Eskild, A., Owe, K. M., Bø, K. & Bjelland, E. K. Exercise in pregnancy: an association with placental weight. *Am. J. Obstet. Gynecol.* **216**(168), e1-168.e9 (2017).
44. Burton, G. J. & Jauniaux, E. Development of the human placenta and fetal heart: Synergic or independent. *Front. Physiol.* **9**, 373 (2018).
45. Ennour-Idrissi, K., Maunsell, E. & Diorio, C. Effect of physical activity on sex hormones in women: A systematic review and meta-analysis of randomized controlled trials. *Breast Cancer Res.* **17**, 139 (2015).
46. Vietheer, A., Kiserud, T., Lie, R. T., Haaland, Ø. A. & Kessler, J. Sleep and physical activity from before conception to the end of pregnancy in healthy women: A longitudinal actigraphy study. *Sleep Med.* **83**, 89–98 (2021).
47. Robinson, H. P. Sonar measurement of fetal crown-rump length as means of assessing maturity in first trimester of pregnancy. *Br. Med. J.* **4**, 28–31 (1973).
48. Lohman, T. *Anthropometric Standardization Reference Manual* (Human Kinetics Book, 1988).
49. *Body Composition Analyser BC-418-MA. Instruction Manual* (Tanita Corporation of America. https://www.tanita.com/es/.downloads/download/?file=855638086&fl=en_US,
50. Liden, C. B. *et al.* Benefits of the SenseWear armband over other physical activity and energy expenditure measurement techniques. *White Papers Body Media* **1**, 1–14 (2001).
51. Tremblay, M. S. *et al.* Sedentary behavior research network (SBRN)—Terminology consensus project process and outcome. *Int. J. Behav. Nutr. Phys. Act.* **14**, 75 (2017).
52. Thompson, P. D. In *ACSM' Guidelines for Exercise Testing and Prescription* (eds Pescatello, L. S., Arena, R., Riebe, D. & Thompson, P. D.) 2–14 (Lippincott Williams & Wilkins, 2014).
53. Matthews, C. E., Ainsworth, B. E., Thompson, R. W. & Bassett, D. R. Sources of variance in daily physical activity levels as measured by an accelerometer. *Med. Sci. Sports Exerc.* **34**, 1376–1381 (2002).
54. Andre, D. et al. The development of the SenseWear® armband, a revolutionary energy assessment device to assess physical activity and lifestyle. *BodyMedia Inc* (2006).
55. Bhammar, D. M., Sawyer, B. J., Tucker, W. J., Lee, J.-M. & Gaesser, G. A. Validity of SenseWear® Armband v5.2 and v2.2 for estimating energy expenditure. *J. Sports Sci.* **34**, 1830–1838 (2016).
56. Berntsen, S., Stafne, S. N. & Mørkved, S. Physical activity monitor for recording energy expenditure in pregnancy. *Acta Obstet. Gynecol. Scand.* **90**, 903–907 (2011).
57. Smith, K. M., Lanningham-Foster, L. M., Welk, G. J. & Campbell, C. G. Validity of the SenseWear® Armband to predict energy expenditure in pregnant women. *Med. Sci. Sports Exerc.* **44**, 2001–2008 (2012).
58. Bhide, A. *et al.* ISUOG practice guidelines: use of Doppler ultrasonography in obstetrics. *Ultrasound Obstetr. Gynecol.* **41**, 233–239 (2013).
59. Preisler, J. *et al.* Defining safe criteria to diagnose miscarriage: prospective observational multicentre study. *BMJ* **351**, h4579 (2015).
60. Popović, Z. B. & Thomas, J. D. Assessing observer variability: a user's guide. *Cardiovasc. Diagn. Ther.* **7**, 317–324 (2017).
61. Spencer, T. E. Biological roles of uterine glands in pregnancy. *Semin. Reprod. Med.* **32**, 346–357 (2014).

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Author contributions

A.V.: Conceptualization, methodology, investigation, software, data collection and curation, visualization, validation, formal analysis, interpretation, writing—original draft preparation, review and editing; T.K.: Project administration, conceptualization, methodology, investigation, data collection and curation, formal analysis, interpretation, visualization, writing—review & editing; C.E.: Data collection, interpretation, visualization, writing—review & editing; H.R.: Data collection, writing—review & editing; R.T.L.: Methodology, formal analysis, validation, visualization, writing—review & editing; Ø.A.H.: Methodology, software, formal analysis, validation, visualization, writing, review and editing; R.R.: Interpretation, visualization, writing—review & editing; J.K.:

Project administration, methodology, investigation, formal analysis, validation, visualization, writing—review & editing.

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Competing interests
The authors declare no competing interests.

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Supplementary

Figure S1 Longitudinal changes of the daily physical activity duration from before pregnancy to the end of the 1st trimester (week 13). The mean difference of the activity duration was 1 h 36 min (95% CI [1 h 55 min–1 h 19 min], *p<0.001)*.

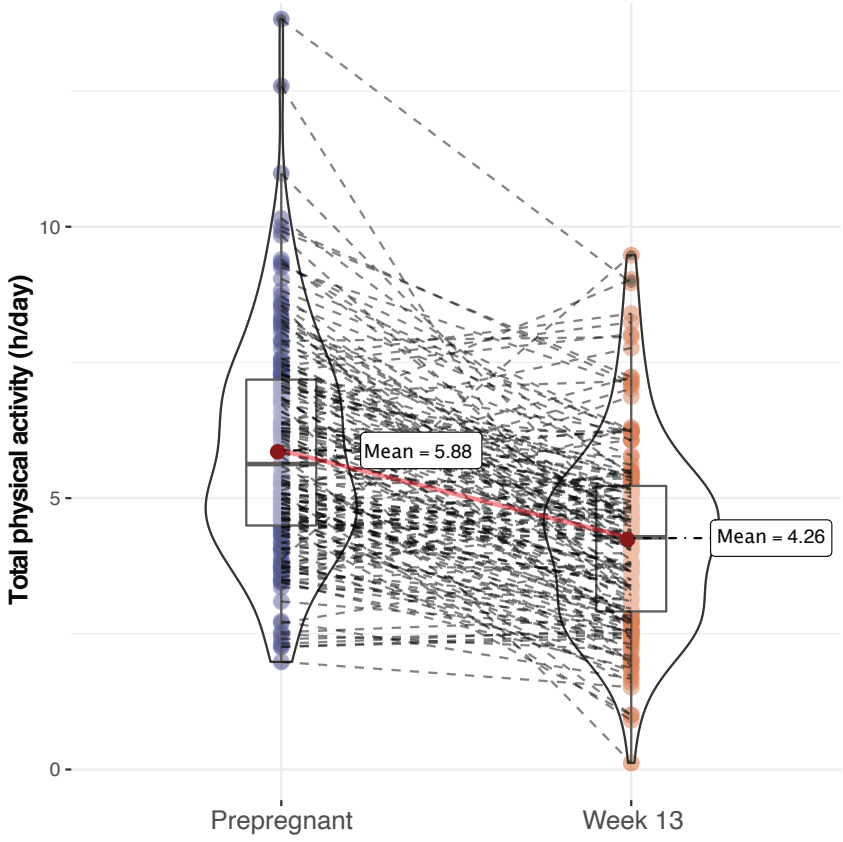

Table S1 (Dataset 1)

Please find the table in "*Supplementary Table S1_dataset-1.csv*".

Table S2 (Dataset-1 key)

Variable name	Description
id	Participant ID
inc.date_cat	Inclusion during the first, second, or third tertial of the study period
age	Maternal age before conception (at study entry)
para	Number of previous births before conception (at study entry)
ht	Maternal body height before conception (at study entry)
wt.1	Maternal body weight before conception (at study entry)
bmi.1	Maternal body mass index before conception (at study entry)
lbm.1	Maternal lean body mass before conception (at study entry)
bfp.1	Maternal body fat percent
sw.ndays.1	Number of recorded days at the first actigraphy recording (at study entry)
sw.ndays.2	Number of recorded days at the second actigraphy recording (at week 13)
pad.1.h	Total daily physical activity duration in hours at the first actigraphy recording before conception (at study entry)
pad.2.h	Total daily physical activity duration in hours at the second actigraphy recording (at week 13)
modvig_pad.1.h	Moderate and vigorous daily physical activity duration in hours at the first actigraphy recording before conception (at study entry)
modvig_pad.2.h	Moderate and vigorous daily physical activity duration in hours at the second actigraphy recording (at week 13)
lt_pad.1.h	Light daily physical activity duration in hours at the first actigraphy recording before conception (at study entry)
lt_pad.2.h	Light daily physical activity duration in hours at the second actigraphy recording (at week 13)
g_date.1	Date of the first yolk sac measurement
lmp_ga.1	Gestational age in weeks at the first yolk sac measurement at week 7 (by LMP*)
g_date.2	Date of the second yolk sac measurement
lmp_ga.2	Gestational age in weeks at the second yolk sac measurement at week 10 (by LMP)
day.diff.1	Number of days between the two yolk sac measurements
ys.1	Yolk sac size in mm at the first yolk sac measurement at week 7
ys.2	Yolk sac size in mm at the second yolk sac measurement at week 10
ys.growth	Average yolk sac growth in mm per week between the first and second measurement (week 7–10)
childSex	Sex of the child determined at birth

* LMP, the first day of the last menstrual period.

Table S3 Estimated *yolk sac diameter* at gestational week 7 (upper half) and week 10 (lower half) by total daily physical activity duration (TPAD) before pregnancy and at the end of the first trimester (week 13): Ungrouped (all); grouped according to fetal sex (male, female), or by the interaction term male sex and TPAD (Male:TPAD). Modeled using ordinary least square regression—degrees of freedom (DF); unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion).

Group	DF	Effect	95% CI	Adj.*r*2	AIC	*p*
Yolk sac at week 7 by TPAD before pregnancy						
All	165	0.04 mm·h^{-1}	(-0.00–0.08)	0.014	295.2	0.07
Male	82	0.08 mm·h^{-1}	(0.02–0.13)	0.077	137.2	**<0.01**
Female	81	0.00 mm·h^{-1}	(-0.06–0.07)	-0.012	158.7	0.93
Male:TPAD	163	0.07 mm·h^{-1}	(-0.01–0.16)	0.024	295.4	0.09
Yolk sac at week 7 by TPAD at the end of 1st trimester						
All	167	0.02 mm·h^{-1}	(-0.03–0.07)	-0.002	301.9	0.47
Male	83	0.04 mm·h^{-1}	(-0.04–0.11)	-0.000	146.0	0.34
Female	82	0.01 mm·h^{-1}	(-0.07–0.08)	-0.011	159.8	0.88
Yolk sac at week 10 by TPAD before pregnancy						
All	164	0.03 mm·h^{-1}	(-0.04–0.09)	-0.002	446.1	0.42
Male	81	-0.09 mm·h^{-1}	(-0.18 to -0.00)	0.038	209.8	**0.04**
Female	81	0.16 mm·h^{-1}	(0.06–0.26)	0.094	227.9	**<0.01**
Male:TPAD	162	-0.24 mm·h^{-1}	(-0.38 to -0.12)	0.064	436.7	**<0.001**
Yolk sac at week 10 by TPAD at the end of 1st trimester						
All	166	-0.01 mm·h^{-1}	(-0.10–0.07)	-0.005	451.1	0.72
Male	82	-0.09 mm·h^{-1}	(-0.21–0.02)	0.020	214.8	0.10
Female	82	0.05 mm·h^{-1}	(-0.07–0.17)	-0.003	238.1	0.41

Table S4 Estimated *male yolk sac diameter* at gestational *week 7* by total daily activity duration (TPAD) *before pregnancy*. Calculated by ordinary least square regression models—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion). The crude model (grey background) with its estimates and significance level can be compared with the adjusted models listened below, controlling for maternal health parameters: i.e., age, parity, height, weight, BMI, lean body mass (LBM), body fat percent (BFP), gestational age (GA), and stratified date of inclusion (inc. date; 3 time-categories). The last row represents the crude model but based on women without pregnancy complications (compl. excl.). The activity-yolk sac relation remained significant after adjustments that caused minute changes in the estimated effect.

Model	DF	Effect	95% CI	Adj.*r2*	AIC	*p*
Crude	82	0.07 mm·h^{-1}	(0.02–0.13)	0.077	137.2	**<0.01**
Adjusted by age	81	0.08 mm·h^{-1}	(0.03–0.14)	0.085	137.0	**<0.01**
Adjusted by parity	79	0.07 mm·h^{-1}	(0.01–0.13)	0.062	141.0	**0.02**
Adjusted by height	81	0.07 mm·h^{-1}	(0.01–0.13)	0.085	136.9	**0.02**
Adjusted by weight	81	0.09 mm·h^{-1}	(0.03–0.14)	0.099	135.6	**<0.01**
Adjusted by BMI	81	0.09 mm·h^{-1}	(0.03–0.15)	0.071	138.3	**<0.01**
Adjusted by LBM	81	0.08 mm·h^{-1}	(0.02–0.13)	0.114	134.2	**<0.01**
Adjusted by BFP	81	0.08 mm·h^{-1}	(0.02–0.14)	0.065	138.7	**<0.01**
Adjusted by GA	81	0.07 mm·h^{-1}	(0.02–0.13)	0.118	133.9	**0.01**
Adjusted by incl. date	80	0.08 mm·h^{-1}	(0.02–0.14)	0.059	140.2	**<0.01**
Crude compl. excl.	70	0.06 mm·h^{-1}	(0.00–0.12)	0.045	111.2	**0.04**

Table S5 Estimated *female yolk sac diameter* at gestational *week 7* by total daily activity duration *(TPAD) before pregnancy*. Calculated by ordinary least square regression models—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion). The crude model (grey background) with its estimates and significance level can be compared with the adjusted models listened below, controlling for maternal health parameters: i.e., age, parity, height, weight, BMI, lean body mass (LBM), body fat percent (BFP), gestational age (GA), and stratified date of inclusion (inc. date; 3 time-categories). The last row represents the crude model but based on women without pregnancy complications (compl. excl.). The activity-yolk sac relation remained constant after adjustments that caused minute changes in the estimated effect.

Model	DF	Effect	95% CI	Adj.*r*2	AIC	*p*
Crude	81	0.00 mm·h^{-1}	(-0.06–0.07)	-0.012	158.7	0.93
Adjusted by age	80	-0.00 mm·h^{-1}	(-0.07–0.07)	0.020	160.3	>0.99
Adjusted by parity	79	-0.00 mm·h^{-1}	(-0.07–0.07)	-0.035	162.4	>0.99
Adjusted by height	80	-0.00 mm·h^{-1}	(-0.07–0.07)	-0.020	160.3	0.96
Adjusted by weight	80	-0.01 mm·h^{-1}	(-0.08–0.06)	-0.012	159.6	0.81
Adjusted by BMI	80	-0.01 mm·h^{-1}	(-0.08–0.07)	-0.018	160.1	0.87
Adjusted by LBM	80	-0.00 mm·h^{-1}	(-0.07–0.07)	-0.022	160.4	>0.99
Adjusted by BFP	80	-0.01 mm·h^{-1}	(-0.08–0.06)	-0.010	159.5	0.77
Adjusted by GA	80	0.01 mm·h^{-1}	(-0.06–0.07)	0.060	153.4	0.86
Adjusted by incl. date	79	0.00 mm·h^{-1}	(-0.06–0.07)	-0.002	159.7	0.90
Crude compl. excl.	75	0.00 mm·h^{-1}	(-0.08–0.07)	-0.013	147.3	0.94

Table S6 Estimated *male yolk sac diameter* at gestational *week 10* by total daily activity duration *(TPAD) before pregnancy*. Calculated by ordinary least square regression models—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion). The crude model (grey background) with its estimates and significance level can be compared with the adjusted models listened below, controlling for maternal health parameters: i.e., age, parity, height, weight, BMI, lean body mass (LBM), body fat percent (BFP), and stratified date of inclusion (inc. date; 3 time-categories). The last row represents the crude model but based on women without pregnancy complications (compl. excl.). The activity-yolk sac relation remained significant after adjustments that caused minute changes in the estimated effect.

Model	DF	Effect	95% CI	Adj.*r*2	AIC	*p*
Crude	81	-0.09 mm·h^{-1}	(-0.18 to -0.00)	0.038	209.8	**0.04**
Adjusted by age	80	-0.09 mm·h^{-1}	(-0.18–0.00)	0.023	211.6	0.06
Adjusted by parity	78	-0.09 mm·h^{-1}	(-0.18–0.00)	0.027	213.7	0.06
Adjusted by height	80	-0.09 mm·h^{-1}	(-0.18 to -0.00)	0.026	211.8	**0.04**
Adjusted by weight	80	-0.08 mm·h^{-1}	(-0.17–0.01)	0.036	211.0	0.07
Adjusted by BMI	80	-0.08 mm·h^{-1}	(-0.04–0.02)	0.037	210.9	0.10
Adjusted by LBM	80	-0.09 mm·h^{-1}	(-0.18 to -0.00)	0.032	211.4	**0.04**
Adjusted by BFP	80	-0.08 mm·h^{-1}	(-0.17–0.01)	0.034	211.2	0.09
Adjusted by GA	80	-0.09 mm·h^{-1}	(-0.18 to -0.00)	0.026	211.8	**0.04**
Adjusted by incl. date	79	-0.09 mm·h^{-1}	(-0.18–0.00)	0.021	213.2	**0.04**
Crude compl. excl.	69	-0.08 mm·h^{-1}	(-0.18–0.01)	0.027	181.2	0.09

Table S7 Estimated the *female yolk sac diameter* at gestational *week 10* by total daily activity duration *(TPAD) before pregnancy*. Calculated by ordinary least square regression models—unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion). The crude model (grey background) with its estimates and significance level can be compared with the adjusted models listened below, controlling for maternal health parameters: i.e., age, parity, height, weight, BMI, lean body mass (LBM), body fat percent (BFP), and stratified date of inclusion (inc. date; 3 time-categories). The last row represents the crude model but based on women without pregnancy complications (compl. excl.). The activity-yolk sac relation remained significant after adjustments that caused minute changes in the estimated effect.

Model	DF	Effect	95% CI	Adj.*r*2	AIC	*p*
Crude	81	0.16 mm·h^{-1}	(0.06–0.26)	0.094	227.9	**<0.01**
Adjusted by age	80	0.15 mm·h^{-1}	(0.05–0.25)	0.093	229.0	**<0.01**
Adjusted by parity	79	0.14 mm·h^{-1}	(0.04–0.24)	0.108	228.5	**<0.01**
Adjusted by height	80	0.16 mm·h^{-1}	(0.06–0.26)	0.083	229.9	**<0.01**
Adjusted by weight	80	0.15 mm·h^{-1}	(0.04–0.26)	0.087	229.5	**<0.01**
Adjusted by BMI	80	0.14 mm·h^{-1}	(0.03–0.25)	0.090	229.3	**0.01**
Adjusted by LBM	80	0.16 mm·h^{-1}	(0.05–0.26)	0.083	229.9	**<0.01**
Adjusted by BFP	80	0.13 mm·h^{-1}	(0.02–0.24)	0.099	228.4	**0.01**
Adjusted by GA	80	0.17 mm·h^{-1}	(0.07–0.27)	0.204	212.4	**<0.01**
Adjusted by incl. date	79	0.16 mm·h^{-1}	(0.06–0.27)	0.074	231.6	**<0.01**
Crude compl. excl.	75	0.17 mm·h^{-1}	(0.06–0.28)	0.099	211.2	**<0.01**

Table S8 Estimated yolk sac Z-score at gestational week 7 (upper half) and week 10 (lower half) by total daily physical activity duration (TPAD) before pregnancy and at the end of the first trimester (week 13): Grouped according to fetal sex (male, female). Modeled using ordinary least square regression—degrees of freedom (DF); unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion).

Group	DF	Effect	95% CI	Adj.*r*2	AIC	*p*
Yolk sac Z-score at week 7 by daily PAD before pregnancy						
Male	80	$0.20 \cdot h^{-1}$	(0.07–0.33)	0.09	270.4	**<0.01**
Female	78	$-0.03 \cdot h^{-1}$	(-0.19–0.12)	-0.01	278.7	0.65
Yolk sac Z-score at week 7 by daily PAD at the end of 1st trimester						
Male	80	$0.06 \cdot h^{-1}$	(-0.11–0.23)	-0.006	278.8	0.49
Female	78	$0.02 \cdot h^{-1}$	(-0.14–0.19)	-0.012	278.8	0.80
Yolk sac Z-score at week 10 by daily PAD before pregnancy						
Male	79	$-0.14 \cdot h^{-1}$	(-0.26 to -0.01)	0.044	263.8	**0.03**
Female	78	$0.21 \cdot h^{-1}$	(0.06–0.35)	0.085	270.7	**<0.01**
Yolk sac Z-score at week 10 by daily PAD at the end of 1st trimester						
Male	79	$-0.12 \cdot h^{-1}$	(-0.28–0.05)	0.012	266.4	0.16
Female	78	$0.03 \cdot h^{-1}$	(-0.13–0.20)	-0.011	278.7	0.69

Equations Eq.1 Z-score calculation:

In this section, we will explain how Z-scores were obtained, and how means and standard deviations (SDs) were estimated for each of the measurements. First, we modelled the mean yolk sac size for individual *j* at time *i* using a 2nd order polynomial mixed model with random intercept,

$$\mu_{ij} = \beta 0_j + \beta 1_j \times GA_{ij} + \beta 2_j \times GA_{ij}^2 + \epsilon_{ij} \ , \qquad (S1)$$

where GA is gestational age and ϵ_{ij} is a normally distributed error term. From *(S1)* we now get

$$\hat{\mu}_{ij} = b0_j + b1_j \times GA_{ij} + b2_j \times GA_{ij}^2 \ , \qquad (S2)$$

where the *b*'s are the estimated β's from *(S1)*. Because the SD will change with GA we also calculate an SD for each GA. Letting $c.res = \sqrt{\left(obs_{ij} - \hat{\mu}_{ij}\right)^2}$, where obs_{ij} are the observed yolk sac sizes. For individual *j* at time *i*, we get

$$c.res_{ij} = \alpha_0 + \alpha_1 \times GA_{ij} + \tau_{ij} \ , \qquad (S3)$$

where τ_{ij} is a normally distributed error term. Now, our modelled SD for individual *j* at time *i* becomes

$$SD_{ij} = a_0 + a_1 \times GA_{ij} \ , \qquad (S4)$$

where the a's are the estimated α's from *(S3)*. Finally, from *(S2)* and *(S4)*, we now get that the Z-score for individual *j* at time *i* is

$$Z_{ij} = \frac{obs_{ij} - \hat{\mu}_{ij}}{SD_{ij}} \ .$$

Code C1 Syntax (r-code) Z-score calculation:*)*

```
# Load necessary packages and libraries
if(T){
  rm(list=ls(all=T))
  pkgs <- c("here", "lme4")
  pkgs2 <- which(!(pkgs %in% installed.packages()))
  if (length(pkgs2)>0) install.packages(pkgs[pkgs2])
library(here)
library(lme4)
}
# Import Supplementary Table S1 (Dataset-A)
df.w1.short.ys <- read.csv(here("Supplementary Table S1_dataset-1.csv"), header =
TRUE, sep = ",", row.names = 1)
# longformat the dataset
df.l1.short.ys <- reshape(df.w1.ys.short, direction ="long",
                          varying= c("ys.1","ys.2","lmp_ga.1","lmp_ga.2"),
                          idvar="id",
                          sep=".")
# reorder by id
df.l1.short.ys <- df.l1.short.ys[order(df.l1.short.ys$id,df.l1.short.ys$time),]
df.l1.short.ys <- df.l1.short.ys %>% rename("Time of measurement"=time)
df.l1.short.ys$"Time of measurement"<- recode_factor (df.l1.short.ys$"Time of
measurement", "1"="Week 7", "2"="Week 10")

## statistical model (yolk sac by gestational age; quadratic linear random intercept
model: LME4)
Model.1 <- lmer(ys ~ lmp_ga + I(lmp_ga^2) + (1 |id), na.action = na.exclude, data
= df.l1.short.ys , REML = FALSE)

## column of predicted values
df.l1.short.ys$ys_predicted <- NA
tmp <- predict(Model.1)
## adding predicted model values to the correct individuals
df.l1.short.ys[names(tmp),"ys_predicted"] <- tmp

### Create new yolk sac standard deviation "sd-variable" with only NA
df.l1.short.ys$c.res <- NA
```

Create temporary vector based and the calculated standard deviation from the residuals (converted residuals "c.res" from Model.1

```
c.res <- sqrt (residuals(Model.1)^2)
```

Adding converted residuals of the model.1 (c.res) to the correct individuals

```
df.l1.short.ys[names(c.res),"c.res"] <- c.res
```

Model the converted residuals (c.res) values

```
Model.2 <- lm(c.res ~ lmp_ga, data = df.l1.short.ys, na.action = na.exclude)
```

get the predicted standard deviations (sd_pred) and create new variable with only NA

df.l1.short.ys$sd_pred <- NA

Create temporary vector from predicted standard deviations

```
tmp.sd.pred <- predict(Model.2)
```

Adding predicted yolk sac standard deviations to the correct individuals

```
df.l1.short.ys[names(tmp.sd.pred),"sd_pred"] <- tmp.sd.pred
```

Calculate the final yolk sac Z-scores based gestational age adjusted predicted mean and gestational age adjusted predicted standard deviation

Create new variable with only NA

```
df.l1.short.ys$ys_Zscore <- NA
```

Create temporary vector with the calculated Z-scores

```
tmp.Zscore <- (df.l1.short.ys$ys-
df.l1.short.ys$ys_predicted)/df.l1.short.ys$sd_pred
```

####### Adding yolk sac Z-scores to the correct individuals

```
df.l1.short.ys$ys_Zscore <- tmp.Zscore
```

Figure S2 Male and female yolk sac diameter at week 7 (left, *N*=180) and week 10 (right, *N*=178) by the total daily physical activity duration (TPAD) before pregnancy. Prediction lines are calculated by sex-stratified quantile regression models—median (thick black); 5th, 20th, 30th, 40th, 60th, 70th, 80th, and 95th percentile (grey); ordinary least square regression-line (stippled).

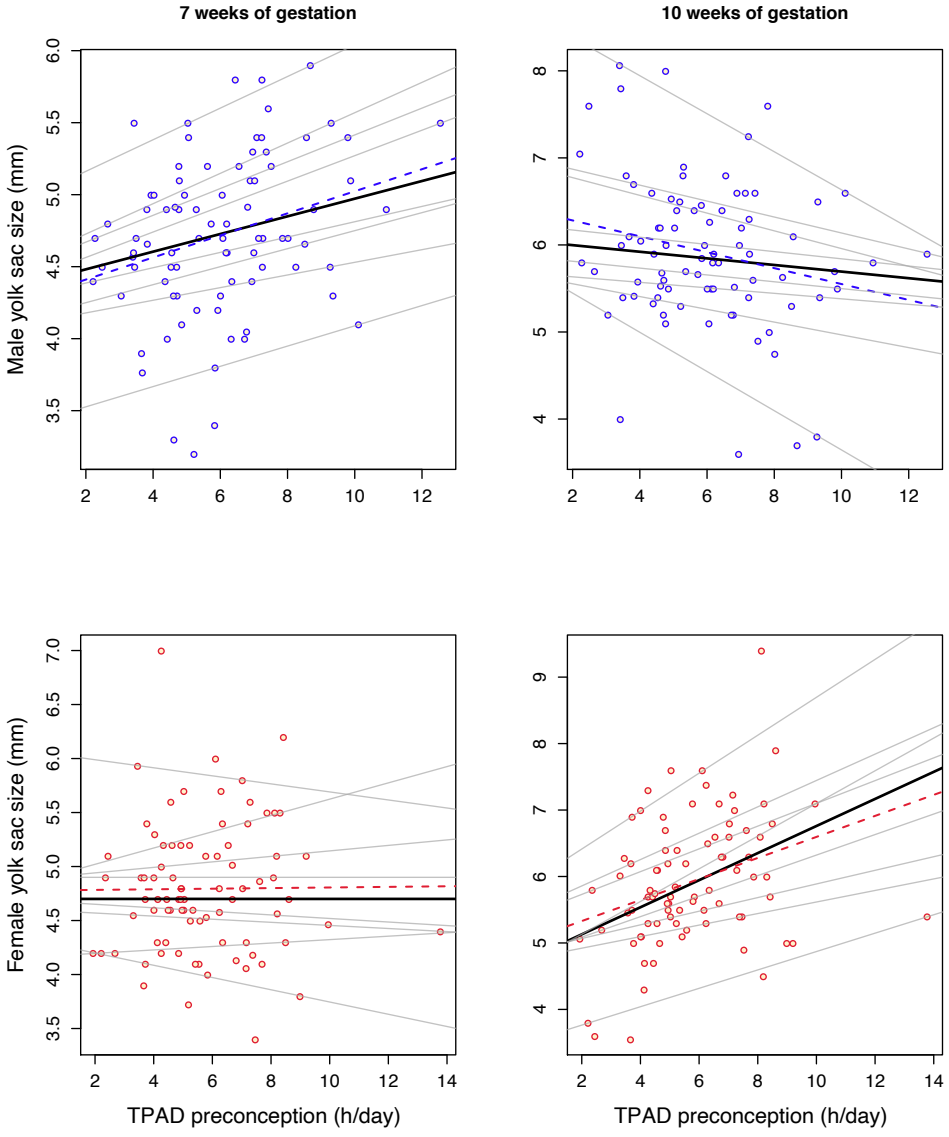

Figure S3 Overview of the sex- and time-stratified results of the quantile regression models: The **preconception** activity estimates on the y-axis with 95% confidence intervals (change of yolk sac size in mm per h of daily physical activity duration (TPAD) before conception) were calculated for yolk sac deciles on the x-axis; zero-effect (red line)

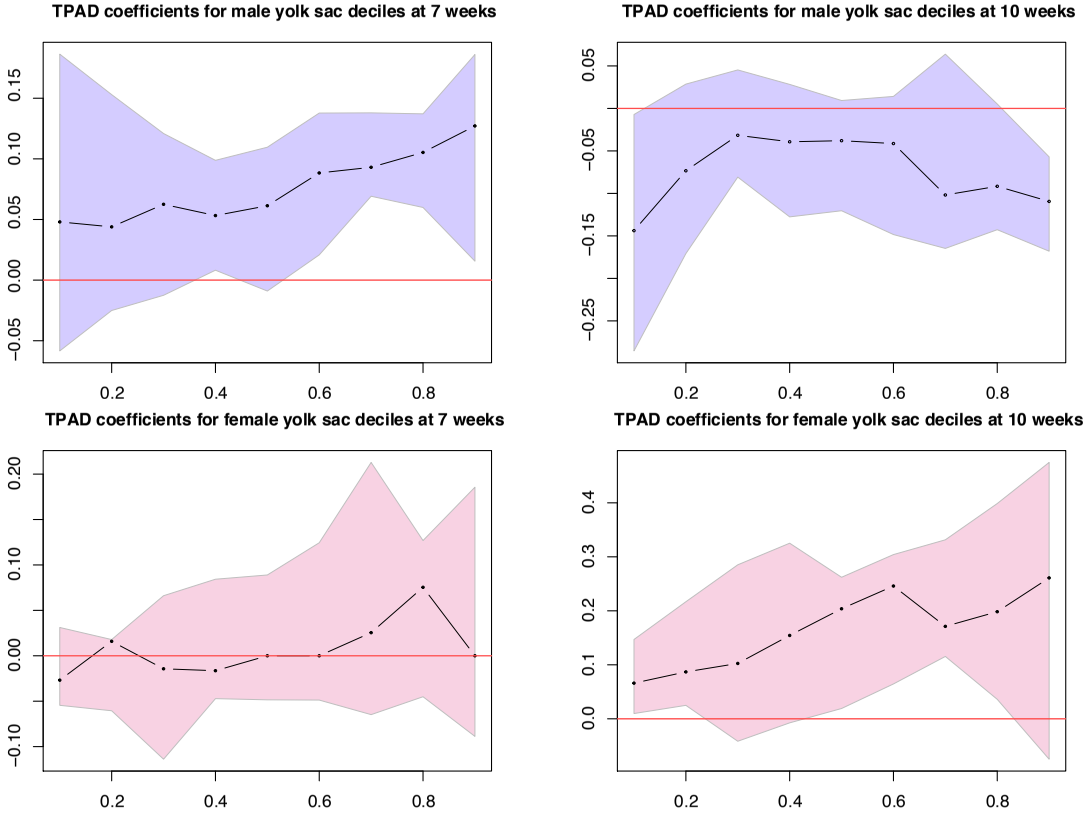

Figure S4 Unadjusted quantile regression-lines for the sex specific yolk sac measurements from the first (week 7, *N*= 180) and second measurement (week 10, *N* = 178) by daily physical activity duration at the end of the 1st trimester (week 13); median (thick black line) and 5th, 20th, 30th, 40th, 60th, 70th, 80th, and 95th percentile (grey); Individual observations (open circles); ordinary least square regression-line (stippled).

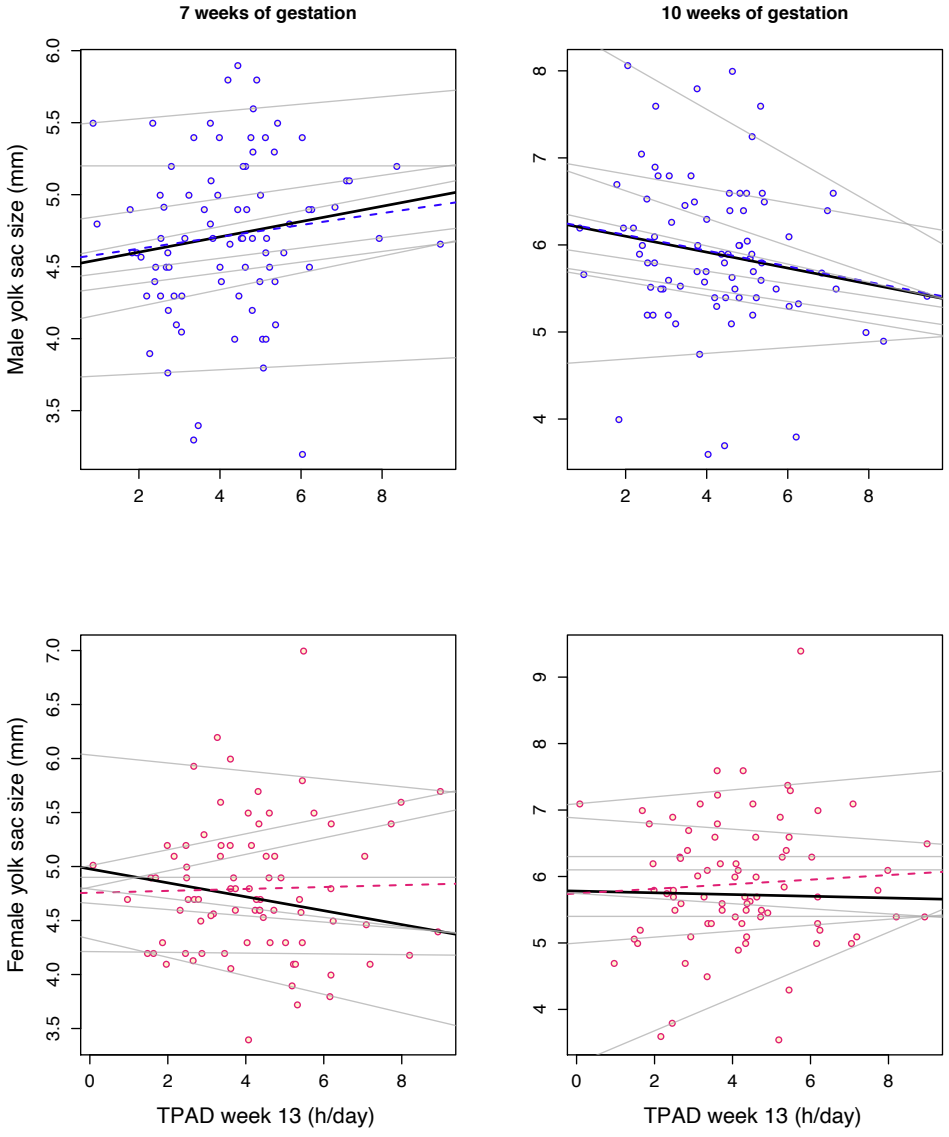

Figure S5 Overview of the sex- and time-stratified results of the quantile regression models: The **<u>end of 1st trimester</u> (week 13)** activity estimates on the y-axis with 95% confidence intervals (change of yolk sac size in mm per h of daily physical activity duration (TPAD) before conception) were calculated for yolk sac deciles on the x-axis; zero-effect (red line)

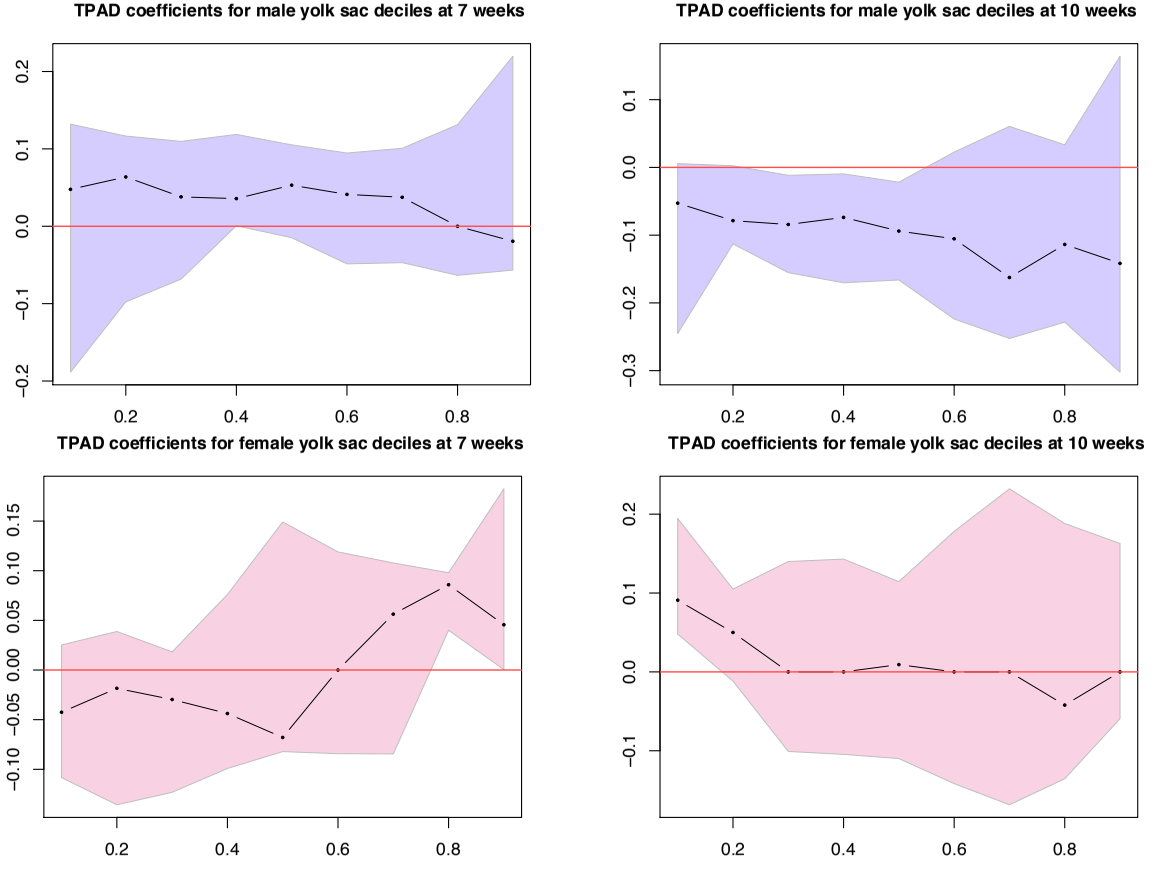

Table S9 Estimated *Yolk sac growth rate (mm·week^{-1}·h^{-1})* by total daily physical activity duration (TPAD) before pregnancy and at the end of the first trimester (week 13): Ungrouped (all); grouped according to fetal sex (male, female), or by the interaction term male sex and TPAD (Male:TPAD). Modeled using ordinary least square regression—degrees of freedom (DF); unstandardized regression coefficient (Effect); adjusted R squared (Adj.R2); 95% confidence interval (95%CI); AIC (Akaike information criterion).

Model	DF	Effect	95% CI	Adj.*r*2	AIC	*p*
Yolk sac growth rate by daily PAD before pregnancy						
All	156	-0.00 mm·week^{-1}·h^{-1}	(-0.03–0.02)	-0.006	103.3	0.77
Male	75	-0.05 mm·week^{-1}·h^{-1}	(-0.08 to -0.02)	0.126	26.4	**<0.01**
Female	79	0.05 mm·week^{-1}·h^{-1}	(0.01–0.09)	0.064	62.4	**0.01**
Male:PAD	154	-0.1 mm·week^{-1}·h^{-1}	(-0.15 to -0.05)	0.084	90.5	**<0.01**
Yolk sac growth rate by daily PAD at the end of first trimester						
All	158	-0.01 mm·week^{-1}·h^{-1}	(-0.04–0.02)	-0.004	96.2	0.53
Male	76	-0.03 mm·week^{-1}·h^{-1}	(-0.07–0.01)	0.021	34.6	0.11
Female	80	0.01 mm·week^{-1}·h^{-1}	(-0.03–0.05)	-0.010	63.3	0.66
Male:PAD	156	-0.04 mm·week^{-1}·h^{-1}	(-0.10–0.02)	-0.003	98.1	0.15

Please find the code for corresponding statistics as supplementary code in:
"*R-Code-growth-rate-stats_2023-08-30.pdf*"

Table S10 Dataset 2

Please find the table in the file *“Supplementary Dataset 2*”.

Table S11 Key dataset-2

Variable name	Description
id	Subject number; 1–19 .
measurement_nr	Measurement groups; 1–2 .
Observer	Observer (Ultrasound operator; 1–7)
mean_all	Value of the yolk sac measurement in mm.

Code C2 Syntax (r-code) for dataset-2:
(Analysis of the intra- and inter-observer variation, and determination of the standard error of measurements (SEM's).

```
# Load necessary packages and libraries

if(T){rm(list=ls(all=T))

pkgs <- c("here")

pkgs2 <- which(!(pkgs %in% installed.packages()))

if (length(pkgs2)>0) install.packages(pkgs[pkgs2])

}

library(here)

# Import Dataset-2

AOV_data <- read.csv(here("Supplementary Table S10_dataset-2.csv "), header = TRUE,
sep = ",", row.names = 1))

# id (index for the study subjects), Observer (index for the observers), measurement_nr (index
for the measurement at week 7 or 10, and mean_all is the measured value

attach(AOV_data)

# Fitting the two-way ANOVA

(fit<-aov(mean_all~1+factor(Observer)+factor(id)+factor(Observer)*factor(id)))

# Getting the fitted output of the model

(fita<-anova(fit))

# Output of the residual variation corresponds to intra-observer variation (repeatability)

(intra.obsvar<-fita$`Mean Sq`[4])

# Calculation observer variation (reproducibility)

(observervar<- (fita$`Mean Sq`[1]-fita$`Mean Sq`[3])/(length(unique(id))*length
               (unique(exam_nr))))

# Calculation interaction variation

(interactionvar<-(fita$`Mean Sq`[3]-fita$`Mean Sq`[4])/length(unique(exam_nr)))

# Inter-observer variation

(inter.obsvar <- intra.obsvar+observervar+interactionvar)

# Calculation SEM of the intra-observer variation

(SEMintra<-sqrt(intra.obsvar))

# SEM of the inter-observer variation fixed effect

(SEMinter.fixed<-sqrt(observervar))

# SEM's of the inter observer variation random effect

(SEMinter.random<-sqrt(inter.obsvar))

detach(AOV_data)
```

#****************Yolk Sac Growth-Rate by Maternal Physical Activity**********
#************************Alexander Vietheer 2023-08-30*********************

 # Clear environment in Rstudio

```
if(T){ rm(list=ls(all=T))
  # Install and load necessary packages in Rstudio
  pkgs <- c("here","haven", "tidyverse")
  pkgs2 <- which(!(pkgs %in% installed.packages()))
  if (length(pkgs2)>0) install.packages(pkgs[pkgs2])
  library (here)
  library (haven)
  library (tidyverse) }
```

 # Read in Supplementary Dataset-1

```
if(T){
  df.w1.short <- read.csv(here("Supplementary Table S1_dataset-1.csv"), header = TRUE, sep = ",",
row.names = 1)}
```

 # Stratifying dataset by fetal sex

```
if(T){ df.w1.short.girls <- df.w1.short %>% filter (childSex == "female")
df.w1.short.boys <- df.w1.short %>% filter (childSex == "male") }
```

 # Yolk sac growth-rate per week (ys.growth) by per 1 hour daily physical activity before conception (pad.1)

Not stratified by embryonic sex

```
  g.mod.p.1w <- lm (ys.growth ~ pad.1.h, data = df.w1.short, na.action = na.exclude)
  summary ( g.mod.p.1w)
  confint( g.mod.p.1w)
  AIC( g.mod.p.1w)
```

male embryos

```
  g.mod.p.2w <- lm (ys.growth ~ pad.1.h, data = df.w1.short.boys, na.action = na.exclude)
  summary ( g.mod.p.2w)
  confint( g.mod.p.2w)
  AIC( g.mod.p.2w)
```

female embryos

```
  g.mod.p.3w <- lm (ys.growth ~ pad.1.h, data = df.w1.short.girls, na.action = na.exclude)
  summary ( g.mod.p.3w)
  confint( g.mod.p.3w)
  AIC( g.mod.p.3w)
```

interaction term Physical activity:embryonic sex

```
  g.mod.p.int.1.w<- lm (ys.growth ~ pad.1.h*childSex, data = df.w1.short, na.action = na.exclude)
  summary ( g.mod.p.int.1.w)
  confint( g.mod.p.int.1.w)
  AIC( g.mod.p.int.1.w)
```

 # Yolk sac growth-rate per week (ys.growth) by per 1 hour daily physical activity at 13 weeks (pad.2)

Not stratified by embryonic sex

```
  g.mod.p.4 <- lm (ys.growth ~ pad.2.h, data = df.w1.short, na.action = na.exclude)
  summary ( g.mod.p.4)
  confint( g.mod.p.4)
  AIC( g.mod.p.4)
```

male embryos

```
  g.mod.p.5 <- lm (ys.growth ~ pad.2.h, data = df.w1.short.boys, na.action = na.exclude)
  summary ( g.mod.p.5)
  confint( g.mod.p.5)
  AIC( g.mod.p.5)
```

female embryos

```
  g.mod.p.6 <- lm (ys.growth ~ pad.2.h, data = df.w1.short.girls, na.action = na.exclude)
  summary ( g.mod.p.6)
  confint( g.mod.p.6)
  AIC( g.mod.p.6)
```

interaction term Physical activity:embryonic sex

```
  g.mod.p.int.2 <- lm (ys.growth ~ pad.2.h*childSex, data = df.w1.short, na.action = na.exclude)
  summary ( g.mod.p.int.2)
  confint( g.mod.p.int.2)
  AIC( g.mod.p.int.2)
```

Errata for
Human Embryonic Development:

Effects of Physical Activity and Sleep in Physiological Pregnancies

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Thesis for the degree philosophiae doctor (PhD)
at the University of Bergen

19.03.2024,
(date and sign. of candidate)

20.03.24
(date and sign. of faculty)

Errata

Page 5, Paragraph 4: "Associate professor Cathrine Ebbing" amended to "Professor Cathrine Ebbing" as the title of dr. Cathrine Ebbing changed after submission of the thesis.

Page 8, Table of abbreviations: Replaced a comma by a period after "i.e." in row 7.

Page 8, Table of abbreviations: Corrected the spelling of the word "nitrogen" in row 9.

Page 8, Table of abbreviations: Corrected the spelling of the word "oxygen" in row 10.

Page 10, Glossary: Deleted a space in the word "extraembryonic" in row 4.

Page 11, Glossary: Added a comma after the word "form" in row 4.

Page 12, Glossary: Corrected the spelling of the word "monoamine" in row 1.

Page 12, Glossary: Corrected for subject-verb agreement; the verb "breaks" in row 1.

Page 12, Glossary: Added an extra space after the word "neurotransmitter" in row 1.

Page 13, Glossary: Corrected spelling of "satisfaction".

Page 16, English abstract: Added an extra space in "2 SD" in paragraph 6.

Page 19, Norwegian abstract: Added extra spaces in "2 SD" in paragraph 2.

Page 20, Column 2, last bullet point: Amended the word "effects" to the—in this context—more correct word "affect "and corrected for subject-verb agreement.

Page 21, third paragraph: Corrected spelling of the name Ebbing.

Page 24: Added a hyphen in "intra- and inter- observer variability" for consistency.

Page 28, List of figures: Changed the word "sleeping" to the, in this context, more correct word "sleep".

Page 31, Capture Fig. 1: Corrected the spelling of the word "reprinted".

Page 32, Capture Fig. 2: Corrected the spelling of the word "syncytiotrophoblast".

Page 33: Added a comma after the word "and".

Page 33, Capture Fig. 3: Amended the license agreement abbreviation to the more common form "Creative Commons Attribution-NonCommercial-NoDerivs".

Page 34, Fig. 4: Added a space in the annotation "Maternal blood in lacunae".

Page 38, Capture Fig. 7: Deleted a period before and replaced a comma with a period after "with permission" for consistency.

Page 39, Capture Fig. 8: Subject-verb agreement correction of the verb "adhere", as it refers to the plural "vessels". Deleted a period before and replaced a comma with a period after "with permission" for consistency.

Page 40: Corrected the spelling of the word "not".

Page 40: Corrected the noun "veins" to its singular form.

Page 40, Capture Fig. 9: Amended the license agreement to "Creative Commons Attribution-NonCommercial-NoDerivs" for consistency.

Page 40: Moved the comma behind the brackets.

Page 41, Capture Fig. 10: Added a period at the end.

Page 42: Added a comma after "tail fold".

Page 42, Capture Fig. 11: Replaced "secondary yolk" sac with "vitelline duct" as it specifies the anatomical location more precisely.

Page 43, Capture Fig. 12: Added a period after the word "permission" and added a necessary space after the period before "Original annotations".

Page 45: Amended the sentence from "of a human embryonic liver that remained..." to "that the human embryonic liver remained" for better clarity.

Page 45, Last paragraph: Removed the unnecessary "and" after reference (43) and corrected the punctuation accordingly. Subject-verb agreement correction, changing the plural form of the word "indicate" to "indicates", as it refers to "recent evidence".

Page 46: Merged the words "can not" to the generally preferred expression "cannot".

Page 46, Capture Fig. 13: Changed the license agreement to "Creative Commons Attribution-NonCommercial-NoDerivs" for consistency.

Page 45, Paragraph 2: Amended the expression "methods of secretion" to the generally preferred expression "secretory mechanisms".

Page 47: Removed the abbreviation (ECC) for consistency in the text body.

Page 48, Capture Fig. 14: Deleted a period before and replaced a comma with a period after "with permission" for consistency.

Page 49: Amended the spelling to "cathepsins", which is more commonly used.

Page 50, Capture Fig. 16: Deleted a period before and replaced a comma with a period after "with permission" for consistency.

Page 52: Added a comma for better clarity.

Page 52: Added a space in the word "γ-glutamyl cysteine".

Page 52: Corrected the spelling of "defense".

Page 54: Added "For example" after "immunohistochemistry studies" for better clarity. Replaced a comma by "and" after reference (135) for better readability. Removed a redundant sentence describing the difference between humans and rodents concerning the orientation of the yolk sac endoderm.

Page 54: Added an "s" to human according to the context. Replaced the comma after "outer layer" by a period to break the text for better readability.

Page 56, Chapter 1.4: Added a space after the abbreviation "LDL" in the legend of Fig. 17.

Page 56, Capture Fig. 17: Removed a comma before "or".

Page 58, Capture Fig. 18: Added "according to" before "embryonic CRL". Replaced a comma with a period after "with permission" for consistency.

Page 59, Capture Fig. 19: Deleted a period before and replaced a comma with a period after "with permission" for consistency.

Page 60, Second paragraph: The spelling of "edema" has been corrected.

Page 62, Table 1, last column: Deleted "with" after "secondary YS"; deleted a comma before "and", amended "entoderm" to "endoderm" for consistency in the text.

Page 63, Table 1: The spelling of "vitelline duct" has been corrected in the last row.

Page 63, Table 1: The word "Hematopoietic" and mid-layer have been corrected in the fifth columns of the second row.

Page 63, Table 1: The spelling of "notochord" has been corrected in the fifth columns of the third row.

Page 63, Table 1: Subject-verb agreement correction of the verb "begins" corrected in the fifth columns of the fourth row.

Page 63, Table 1: The spelling of "thickened" has been corrected in the fifth columns of the fourth row.

Page 63, Table 1: The spelling of "Macrophages" has been corrected in the fifth columns of the fifth row.

Page 66, Table 2: Added a comma before "and" in the third row of the second column.

Page 67, Table 2: Added and extra line and corrected the spelling of "equation" in the third row of the fourth column.

Page 68: Removed a closing bracket.

Page 73: The last sentence has been amended to "—including human embryos" for more clarity and a stronger emphasis on the fact that the mechanism also was based on findings in human embryos.

Page 74: Repeated the word "being" before "expressed in the offspring" for better clarity and flow.

Page 75: "the" before pathology has been omitted for better flow and a more concise sentence.

Page 77: Added a space in "± 24h".

Page 78, Capture Fig. 21: Separated the words "biosampling" and "biosamples" into "bio sampling" and "bio samples".

Page 78, Last paragraph: Added a comma after "weight".

Page 79, Capture Fig. 22: Deleted a period before and replaced a comma with a period after "with permission" for consistency.

Page 80: Added 2 extra spaces in 7 ± 1 for consistency.

Page 80, Capture Fig. 23: Replaced the "p" in "Paper II" with the capital letter "P" for consistency.

Page 81: Deleted unnecessary "a".

Page 81: Replaced the word "provided" with "presupposed" for better clarity.

Page 82, Second paragraph: Deleted the word "of" for better flow.

Page 83, Second paragraph: Replaced single quotation marks (') with ordinary quotation marks (") for consistency. Deleted unnecessary "the". Corrected the spelling of the official name for "Sedentary Behaviour Research Network".

Page 85: Replaced a comma with "and" for better readability.

Page 85, Second paragraph: Added a hyphen in "intra- and inter-observer variability" for consistency.

Page 85, Last sentence: Last sentence slightly rephrased for better consistency and readability.

Page 86: Deleted "an" before "analysis" in the penultimate sentence to be more concise.

Page 87, Second paragraph: Replaced "considering" with "accounting for" in the first sentence for clarity.

Page 87, Third paragraph: Moved the abbreviation "OLS" after the complete term "ordinary least square linear regression" in the first sentence.

Page 87, Third paragraph: Corrected the word "regression" for plurality by adding "s".

Page 88: added a hyphen in "SEM intra-observer and SEM-inter-observer variability" for consistency.

Page 89: Added additional space in ± 2 SD for consistency.

Page 90, Capture Fig. 27: Deleted a period before and replaced a comma with a period after "with permission" for consistency.

Page 90: Correction: Deleted "and" before "as well as".

Page 91, Table 3: Added additional space before and after " ± " for consistency.

Page 92, Capture Fig. 28: Deleted a period before and replaced a comma with a period after "with permission" for consistency.

Page 93, Table 4: Replaced "*N*" with "*n*" for consistency in the first column of the first row.

Page 95, Second paragraph: Added a space after "p. "for consistency.

Page 95, Third paragraph: Added brackets for better readability.

Page 95, Capture Fig. 29: Deleted a period before and replaced a comma with a period after "with permission" for consistency.

Page 96, Capture Table 5: Added a space in "95% CI" for consistency.

Page 96, Table 5: ($mm \cdot week^{-1}$) amended to ($mm \cdot week^{-1} \cdot h^{-1}$) for consistency.

Page 97: Added space in (Fig. 30 A) for consistency.

Page 97: Added a comma before "and" for better readability.

Page 99, Capture Table 6: Added space in "95% CI) for consistency.

Page 100, Table 7: Added a hyphen in the second row of the first column. Replaced a comma with a period to separate the decimals in the second row of the fourth column.

Page 101: Added space in "95% CI" for consistency.

Page 104, Title: Units amended to (mm·week^{-1}·h^{-1}) for consistency.

Page 104: Units amended to (mm·week^{-1}·h^{-1}) for consistency.

Page 105, Capture Table 9: Amended abbreviation to (adj.*R*2) and added space in (95% CI) for consistency.

Page 105, Capture Fig. 33: Added "with 95% confident bands" to be more exact.

Page 108: Added "longitudinal" and "from" for clarity.

Page 106: Added a comma after the introductory phrase "Here".

Page 106: Replaced indefinite article by definite article as more appropriate in this context.

Page 106: Deleted a misplaced comma after "and".

Page 106, Second paragraph: Deleted redundant "also" to be more concise and added m-dash before "—also in pregnancy" for clarity in the last sentence.

Page 114, Capture Fig. 36: Amended the license agreement to "Creative Commons Attribution-NonCommercial-NoDerivs" for consistency.

Page 115, Second paragraph: Added space after reference (407).

Page 115: Deleted "also" in the last sentence as it was redundant in this context and added indefinite article before "contributing factor" for better flow.

Page 116, Second Paragraph: Amended the spelling of "remodeling" to American English to be in line with the rest of the thesis.

Page 116, Second Paragraph: Amended the sentence slightly from "However, the lack of placental circulation before weeks 10 to 12 would mean that YS variation would rather be seen at the second measurement and not as early as six–eight weeks of gestation because the remaining embryo probably needs continuous nourishment" to " However, the lack of placental circulation before weeks 10 to 12 could also mean that YS variation would rather be seen at the second measurement and not as early as six–eight weeks of gestation because the larger embryo needs more nourishment" for more clarity.

Page 117: Replaced "mean" with "mode" as this is in line with the reference (39) and consistent with the text, where this fact has been mentioned before.

Page 117: Replaced "typically begins with" with "corresponds to" for clarity.

Page 118, Capture Fig. 37: Corrected language and replaced "than" with "compared to that".

Page 119: Amended the sentence for clarity from "Moreover, these responses differ from those associated with maternal sleep patterns, as discussed in (Paper II)" to " Moreover, the present yolk sac responses differed from those associated with maternal sleep patterns reported in (Paper II)".

Page 122: Added the word "probably" to clarify uncertainty.

Page 123: Removed space before reference (59).

Page 125, Third paragraph, last sentence. Amended the sentence slightly for better coherence with the previous sentence and clarity. From " However, focusing a study exclusively on the effects of preconception body composition offers benefits, as it reduces the risk of inaccurately distinguishing between maternal and fetal compartments." *to* " In the current study, the focus was exclusively on the effects of pre-conception body composition, as it offers benefits such as reducing the risk of inaccurately distinguishing between maternal and fetal compartments."

Page 125, Fifth paragraph: Replaced "the control of" with "controlling for" as this expression is generally preferred in the present context.

Page 126, Second paragraph. Omitting "as opposed to the 7-week measurement".

Page 126, Second paragraph: Added "similar", "as for example at week 7", and "sleep and activity" for clarity.

Page 127, Second paragraph: Specified the specific papers II and III to be more precise.

Page 127, Last paragraph: Replaced "until" with "before" for clarity.

Page 129: Omitted "a" to be more concise.

Page 129: Omitted a word that was accidentally written as "weekdays" and, as such, makes no sense in the given context.

Page 129, Last paragraph: Added a comma before "nor" for readability.

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