THE MECHANISM OF PERI-IMPLANTATION SEXUAL INTERCOURSE AND OXIDATIVE STRESS ON PLACENTA HISTOPATHOLOGICAL CHANGES

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UNIVERSITI SAINS MALAYSIA

2025

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by

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Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

December 2025

ACKNOWLEDGEMENT

In the name of Allah, the Most Beneficent, the Most Merciful. Salutations and blessings be upon His Prophet Muhammad (peace be upon him). All praise and gratitude belong to Almighty Allah for His endless blessings, guidance, and mercy. Alhamdulillah, He granted me the strength to complete this thesis and my MSc studies. My deepest appreciation goes to my supervisor, Assoc Prof Dr. Nik Ahmad Zuky bin Nik Lah, for his unwavering support, mentorship, and encouragement. I am also grateful to my co-supervisors, Dr. Engku Husna Engku Ismail, Assoc Prof Dr. Anani Aila Mat Zain, Ass Prof Dr. Sarimah, and Prof Dr. Nik Hazlina Dr. Liza Nordin, and also our research assistance Ninie for their valuable guidance and feedback.

I sincerely appreciate Universiti Sains Malaysia (USM) for providing the platform for this research and the Federal Research Grant Scheme (FRGS) under the Ministry of Higher Education (FRGS/1/2020/SKK01/USM/02/3) for funding this work. Special thanks to the HUSM staff, particularly those at the Obstetrics and Gynecology Clinic, and to all study participants for their cooperation.

To my beloved family, my father, Alhaj Ibrahim Dangogo, and my mother, Rabiah Salisu, your sacrifices and prayers have been my greatest motivation. To my siblings, especially Auwal Ibrahim, Abdullahi Sulaiman (Dan Police), Aisha Ibrahim (Balaraba), and Aunty Nafisa, your support has been invaluable. To my dearest wife, Farida Sani Ibrahim, thank you for your patience, sacrifices, and unwavering belief in me. This work is dedicated to all who have contributed to my journey. May Allah reward you all abundantly.

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LIST OF ABBREVIATIONS

BSA	Bovine Serum Albumin
CAT	Catalase
ELISA	Enzyme-Linked Immunosorbent Assay
EP Tube	Eppendorf tube
FGR	Fetal Growth Restriction
FFPE	Formalin-Fixed Paraffin-Embedded
H&E	Hematoxylin and Eosin
IHC	Immunohistochemistry
IUGR	Intra Uterine Growth Restriction
LH	Leuteinizing Hormone
MDA	Malondealdehyde
MPFD	Massive Perivillous Fibrin Deposit
SOD	Superoxide Dismutase
T-AOC	Total Antioxidant Capacity
NICU	Neonatal Intensive Care Unit
PBS	Phosphate-Buffered Saline
OD	Optical Density
NBF	Neutral Buffered Formalin

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MEKANISME HUBUNGAN SEKSUAL SEMASA PERI-IMPLANTASI DAN TEKANAN OKSIDATIF KE ATAS PERUBAHAN HISTOPATOLOGI PLASENTA

ABSTRAK

Fasa peri-implantasi adalah tempoh penting dalam penubuhan kehamilan, yang dicirikan oleh penyelarasan rumit antara faktor ibu dan embrio yang memudahkan implantasi yang optimum dan perkembangan plasenta. Gangguan semasa fasa kritikal ini boleh menyebabkan akibat buruk kepada ibu dan janin, termasuk preeklampsia, sekatan pertumbuhan janin (FGR), dan placenta previa. Walaupun hubungan seksual peri-implantasi (PISI) secara tradisional dipercayai menyokong konsepsi, impaknya terhadap kesihatan plasenta dan hasil kehamilan masih belum diteroka dengan mendalam. Kajian ini menyiasat kesan penghindaran seksual peri-implantasi terhadap histomorfologi plasenta, penanda tekanan oksidatif, kedudukan plasenta, dan hasil ibu-janin. Satu kajian rawak terkawal telah dijalankan, melibatkan 33 peserta yang dibahagikan kepada kumpulan penghindaran (n=9) dan bukan penghindaran (n=24). Sampel plasenta dianalisis untuk perubahan histopatologi, termasuk keradangan, deposit fibrin, dan integriti korion/amnion, menggunakan pewarnaan H&E dan immunohistokimia CD31 untuk menilai angiogenesis. Penanda tekanan oksidatif—malondialdehid (MDA), dismutase superoksida (SOD), katalase (CAT), dan kapasiti antioksidan total (T-AOC), diukur melalui ELISA. Kedudukan plasenta dinilai melalui ultrasonografi, dan hasil ibu-janin, termasuk preeklampsia, placenta previa, FGR, kelahiran pramatang, skor Apgar, kemasukan NICU, dan keabnormalan neonatal, direkodkan. Analisis statistik dilakukan menggunakan GraphPad Prism versi 8.4.3.

Keputusan menunjukkan peningkatan histomorfologi yang signifikan dalam kumpulan penghindaran, termasuk kadar keradangan yang lebih rendah (33.3% vs. 70.8%, p=0.167), tiada MPFD (0% vs. 33.3%, p=0.0731), dan integriti korion dan amnion yang utuh (100% vs. 25%, p<0.0001). Pewarnaan CD31 menunjukkan ketumpatan vaskular yang lebih tinggi dalam kumpulan penghindaran (15.3 ± 3.1 vs. 8.9 ± 2.7 vesel/HPF, p=0.0136), menunjukkan peningkatan angiogenesis. Selain itu, penanda tekanan oksidatif mencerminkan pengurangan tekanan oksidatif dalam kumpulan penghindaran, dengan tahap MDA yang lebih rendah (6.54 ± 2.37 vs. 16.65 ± 4.98 μmol/L, p=0.0006) dan aktiviti antioksidan yang lebih tinggi (SOD: 52.16 ± 15.29 vs. 24.40 ± 6.30 U/mg protein, p=0.0038; CAT: 106.7 ± 23.24 vs. 57.33 ± 11.63 U/mg protein, p=0.0026; T-AOC: 137.5 ± 60.93 vs. 60.93 ± 22.35 μmol/L, p=0.0034). Analisis kedudukan plasenta menunjukkan tiada kes placenta rendah dalam kumpulan penghindaran berbanding 20.8% dalam kumpulan bukan penghindaran, walaupun perbezaan ini tidak signifikan secara statistik (p=0.137). Hasil ibu termasuk satu kes kelahiran pramatang dalam setiap kumpulan, dengan kemasukan NICU dilaporkan dalam kumpulan penghindaran, sementara FGR dilihat dalam satu kes dalam kumpulan bukan penghindaran. Tiada kes preeklampsia atau placenta previa dilaporkan. Hasil janin menunjukkan tiada perbezaan yang signifikan dalam skor Apgar atau keabnormalan neonatal antara kumpulan. Penghindaran seksual peri-implantasi dikaitkan dengan peningkatan yang signifikan dalam kesihatan plasenta, termasuk pengurangan tekanan oksidatif, peningkatan angiogenesis, dan pengurangan risiko kedudukan plasenta yang tidak normal. Penemuan ini menekankan potensi peranan pelindung penghindaran semasa tempoh peri-implantasi dalam mengoptimumkan hasil kehamilan. Kajian lanjut dengan kohort yang lebih besar

disyorkan untuk mengesahkan keputusan ini dan meneroka integrasinya dalam strategi penjagaan pra-konsepsi.

THE MECHANISM OF PERI- IMPLANTATION SEXUAL INTERCOURSE AND OXIDATIVE STRESS ON PLACENTA HISTOPATHOLOGICAL CHANGES

ABSTRACT

The peri-implantation phase is a pivotal period in pregnancy establishment, characterized by intricate synchronization between maternal and embryonic factors that facilitate optimal implantation and placental development. Disruptions during this critical phase can lead to adverse maternal and fetal outcomes, including preeclampsia, fetal growth restriction (FGR), and placenta previa. Although peri-implantation sexual intercourse (PISI) is traditionally believed to support conception, its potential impact on placental health and pregnancy outcomes remains underexplored. This study investigated the effects of peri-implantation sexual abstinence on placental histomorphology, oxidative stress markers, placental positioning, and maternal-fetal outcomes. A randomized controlled trial was conducted, involving 33 participants divided into abstinence (n=9) and non-abstinence (n=24) groups. Placental samples were analyzed for histopathological changes, including inflammation, fibrin deposits, and chorion/amnion integrity, using H&E staining and CD31 immunohistochemistry to assess angiogenesis. Oxidative stress markers, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (T-AOC), were quantified via ELISA. Placental positions were assessed through ultrasonography, and maternal-fetal outcomes, including preeclampsia, placenta previa, FGR, preterm birth, Apgar scores, NICU admissions, and neonatal anomalies, were documented. Statistical analyses were performed using GraphPad Prism version 8.4.3. The results demonstrated significant histomorphological improvements in the abstinence group, including lower inflammation rates (33.3% vs. 70.8%, p=0.167),

absence of MPFD (0% vs. 33.3%, p=0.0731), and intact chorion and amnion integrity (100% vs. 25%, p<0.0001). CD31 staining revealed significantly higher vascular density in the abstinence group (15.3 ± 3.1 vs. 8.9 ± 2.7 vessels/HPF, p=0.0136), indicating enhanced angiogenesis. Additionally, oxidative stress markers reflected reduced oxidative stress in the abstinence group, with lower MDA levels (6.54 ± 2.37 vs. 16.65 ± 4.98 μmol/L, p=0.0006) and higher antioxidant activity (SOD: 52.16 ± 15.29 vs. 24.40 ± 6.30 U/mg protein, p=0.0038; CAT: 106.7 ± 23.24 vs. 57.33 ± 11.63 U/mg protein, p=0.0026; T-AOC: 137.5 ± 60.93 vs. 60.93 ± 22.35 μmol/L, p=0.0034). Placental positioning analysis revealed no cases of low-lying placenta in the abstinence group compared to 20.8% in the non-abstinence group, though this difference was not statistically significant (p=0.137). Maternal outcomes included one case of preterm delivery in each group, with NICU admission reported in the abstinence group, while FGR was observed in one case in the non-abstinence group. No cases of preeclampsia or placenta previa were reported. Fetal outcomes showed no significant differences in Apgar scores or neonatal anomalies between groups. Peri-implantation sexual abstinence was associated with significant improvements in placental health, including reduced oxidative stress, enhanced angiogenesis, and a reduced risk of abnormal placental positioning. These findings highlight the potential protective role of abstinence during the peri-implantation period in optimizing pregnancy outcomes. Further studies with larger cohorts are recommended to validate these results and explore their integration into preconception care strategies.

CHAPTER 1
INTRODUCTION

1.1 Background:

Human reproduction presents a fundamental paradox: while essential for the continuation of the species, it is relatively inefficient. The maximum likelihood of conception within a single menstrual cycle is about 30% (Zinaman et al., 1996). Additionally, only 50–60% of all conceptions progress beyond 20 weeks of gestation(Norwitz et al., 2001). Among pregnancies that are lost, 75% result from implantation failure, which is not clinically identified as pregnancy (Wilcox et al., 1988). In most mammals, a zygote is naturally formed in vivo when a sperm fertilizes an egg. Following fertilization in the fallopian tube, the zygote travels toward the uterus while undergoing cell division and developing into a multicellular structure called a blastocyst (Su & Fazleabas, 2015). This blastocyst then attaches to the endometrium, marking the first step in the implantation process (Wilcox et al., 1999). Implantation and the successful establishment of pregnancy are vital components of human reproduction(Ahmadi et al., 2022)(S.-M. Kim & Kim, 2017). Implantation failure is a major contributor to pregnancy loss in humans (Junying Ma, 2023; J. Ma et al., 2023). Successful implantation requires a competent blastocyst and a receptive endometrium within a specific timeframe during the menstrual cycle, facilitating the bilateral communication necessary for a successful pregnancy(Su & Fazleabas, 2015).

Many factors must be carefully considered to ensure successful implantation, which is essential for proper placentation (S.-M. Kim & Kim, 2017). This, in turn, leads to the development of a healthy baby and a healthy mother (Herrick & Bordoni, 2025). A thorough understanding and preparation for the peri-implantation period are critical

for achieving optimal pregnancy outcomes. The peri-implantation period encompasses the pre-implantation, implantation, and post-implantation phases, each playing a vital role in the establishment of a healthy pregnancy (Zhai et al., 2022). Effective management of this period can significantly impact the success of implantation and placentation, ultimately contributing to favorable maternal and fetal health outcomes (Moreno et al., 2023; Zhai et al., 2022).

The peri-implantation phase is a vital stage in pregnancy establishment, characterized by complex interactions between the embryo and the maternal uterine environment (J. Zhou et al., 2021). For successful implantation, precise synchronization of maternal and embryonic factors is essential, enabling optimal placental development and favorable pregnancy outcomes(Su & Fazleabas, 2015). The placenta is crucial for supporting fetal growth and development, as it enables the exchange of nutrients and waste between the mother and the developing baby (Garnica & Chan, 1996). The placenta plays a pivotal role in fetal programming by influencing fetal responses to changes in the maternal environment, regulating nutrient transfer between mother and fetus, and adapting to environmental stimuli that impact maternal or fetal physiology (Jansson & Powell, 2007). Additionally, it serves as a pivotal regulator in shaping fetal development, actively adjusting to variations in the maternal environment and impacting nutrient transfer accordingly (Brett et al., 2014).

In human pregnancy, the placenta functions as the site for nutrient and oxygen exchange between the mother and the fetus (Herrick & Bordoni, 2025). A successful pregnancy relies on effective materno-fetal exchange, a function for which the placenta is uniquely adapted (Cindrova-Davies & Sferruzzi-Perri, 2022). It establishes an extensive and close interface between maternal and fetal bloodstreams (Jauniaux et al.,

2000). In humans, this is facilitated by the development of intricate fetal villous trees, which are directly immersed in maternal blood circulating within the intervillous space (Jauniaux et al., 2000). And this process is crucial for the natural growth and development of the fetus. When placental function is compromised fetal growth can be hampered, leading to fetal growth restriction (FGR) (Brodsky & Christou, 2004),. Successful implantation of the fertilized oocyte is vital for ensuring proper placental function (S.-M. Kim & Kim, 2017). After implantation, extravillous trophoblasts manage their invasion into the maternal decidua and spiral arteries, remodeling these arteries to establish low-resistance circulation within the intervillous space (Huang et al., 2021; Rizov et al., 2017). When the invasion of extravillous trophoblasts is impaired, placental function is disrupted (Illsley et al., 2020).

A key aspect of proper placental function is the effective development of its vascular network (Pollheimer et al., 2018). Inadequate vascularization of the placenta can result in fetal growth restriction, preeclampsia, and, in some cases, fetal death (Gagnon, 2003). Preeclampsia (PE) is a condition marked by maternal endothelial cell dysfunction, along with symptoms such as proteinuria and hypertension, often associated with fetal growth restriction (FGR) (Jsm et al., 2017). In cases of abnormal placentation, placental ischemia can arise from an imbalance between reactive oxygen species (ROS) and antioxidants (Schoots et al., 2018). This imbalance leads to oxidative stress, which can damage proteins, lipids, and DNA within the placental tissue (Vornic et al., 2024).. Within the placenta, evidence of morphological adaptation in reaction to hypoxia can be observed (Schoots et al., 2018). Various placental lesions, such as maternal or fetal vascular malperfusion or chronic villitis, can impair oxygen exchange between the mother and the fetus(Schoots et al., 2018). Clinically, several biomarkers reflecting oxidative stress, such as malondialdehyde and reduced levels of

free thiols, are often detected (Vornic et al., 2024). Oxidative stress (OS) arises when there is an imbalance between the generation of reactive oxygen and nitrogen species (ROS and RNS) and the body's capacity to neutralize their harmful effects through antioxidant defenses (Ibrahim et al., 2024). During pregnancy, the heightened metabolic demands and physiological changes can increase a woman's vulnerability to OS (Ibrahim et al., 2024).

Recent research has underscored the potential influence of peri-implantation sexual intercourse on implantation and subsequent pregnancy outcomes. While sexual activity is thought to promote conception through mechanisms like uterine contractions that facilitate sperm transport (Stanford et al., 2020), it may also trigger uterine contractions during the peri-implantation phase, potentially disrupting implantation (Steiner et al., 2014). Additionally, oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidants, plays a critical role in placental development and function. Elevated oxidative stress during pregnancy has been linked to conditions such as preeclampsia (PE), fetal growth restriction (FGR), and other adverse outcomes (C. Zhang et al., 2023). However, the interplay between peri-implantation sexual activity, oxidative stress, and pregnancy outcomes remains poorly understood.

1.2 Problem statement/Originality of research:

Successful human implantation and early placental development depend on highly coordinated mechanical, cellular and molecular interactions at the periconceptional maternal–embryonic interface (Lawless et al., 2023; Moreno et al., 2023; C. Zhang et al., 2023). Implantation typically occurs 6–10 days after ovulation (Wilcox et al., 1999), and the blastocyst remains mobile in the uterine cavity for several days before stable attachment, a period during which the uterine environment is especially vulnerable to perturbation (S.-M. Kim & Kim, 2017; S. Zhang et al., 2013). Sexual intercourse during the peri-implantation window (hereafter *peri-implantation sexual intercourse*, PISI) may be a double-edged phenomenon. On one hand, intercourse facilitates sperm transport and fertilization (Stanford et al., 2020); on the other hand, observational and prospective data suggest intercourse during the peri-implantation window is associated with lower fecundability**,** possibly because it increases the risk of failed implantation (i.e., loss after fertilization but before clinical recognition) (Steiner et al., 2014). Peri-implantation sexual intercourse (PISI) is biologically active beyond sperm delivery and therefore represents a plausible, modifiable influence on implantation (Steiner et al., 2014). Empirical evidence shows that intercourse increases intra-vaginal and intra-uterine pressures and stimulates myometrial contractions, effects that are augmented by female orgasm (Fox et al., 1970; Kafaei Atrian et al., 2014; Steiner et al., 2014; Wilcox, 2000). Such contractions at the time when a blastocyst is still mobile (the blastocyst remains free in the uterine cavity for ~3 days before firm attachment(M. Ma et al., 2024)) could mechanically displace a loosely apposed embryo (Steiner et al., 2014), cause shallow or incomplete attachment, or even expel the conceptus a plausible path to repeated implantation failure (RIF) (Steiner et al., 2014; J. Zhou et al., 2021). Furthermore, uterine relaxants such as atosiban improve

implantation and pregnancy rates to 43% in ART by suppressing contractions, supporting a causal role for myometrial activity in implantation outcomes (Lan et al., 2012).

Second, seminal plasma is not biologically inert. Seminal fluid contains cytokines, prostaglandins and other bioactive molecules that rapidly elicit an inflammation-like immune response in the female reproductive tract (local leukocyte recruitment, cytokine/chemokine upregulation) (Adefuye et al., 2016; Ahmadi et al., 2022; Sharkey, 2012). While controlled inflammation is essential for implantation (Dekel et al., 2014), The implantation site is already a controlled battlefield—an "open wound" where the embryo must break through the maternal tissue to embed itself (Dekel et al., 2010). This process requires a precise, finely-tuned inflammatory response to manage the invasion and subsequent repair (Dekel, 2014). The additional, potent inflammatory surge from seminal plasma is akin to pouring gasoline on a carefully controlled fire. It overwhelms the delicate balance, creating a state of excessive inflammation. This inflammatory chaos can confuse the crucial immune tolerance needed to accept the foreign embryo, disrupt the signalling for normal trophoblast invasion, and ultimately, corrupt the very architecture of the developing placenta.

Mechanical disturbance and seminal plasma–induced inflammation can impair trophoblast invasion, leaving spiral arteries poorly remodelled and causing placental ischemia (Roberts MD, 2014; Staff et al., 2022). The resulting ischemia–reperfusion generates excess reactive oxygen species (ROS), which overwhelm antioxidant defences and drive oxidative stress (Silvestro, 2020). Reactive oxygen species (ROS) are generated physiologically in the pre-implantation embryo and placenta and are required at controlled levels for signaling (Deluao et al., 2022; Yang et al., 2022);

however, excess ROS and impaired antioxidant responses produce oxidative damage that is strongly associated with defective placentation and downstream pregnancy disorders (Brooker et al., 2024; Ibrahim et al., 2024; Sultana et al., 2023). Oxidative injury at the maternal–fetal interface alters vascular development, endothelial function and trophoblast biology, contributing to placental insufficiency, preeclampsia (PE), fetal growth restriction (FGR), preterm birth and membrane weakening (Deluao et al., 2022; Myatt et al., 2000; Sultana et al., 2023; Wu et al., 2015; C. Zhang et al., 2023)

If peri-implantation events (mechanical displacement, inflammation, oxidative stress) increase the likelihood of shallow invasion, RIF or implantation in the lower uterine segment they may therefore seed a cascade culminating in placenta previa, placental insufficiency and FGR refer to figure 1.

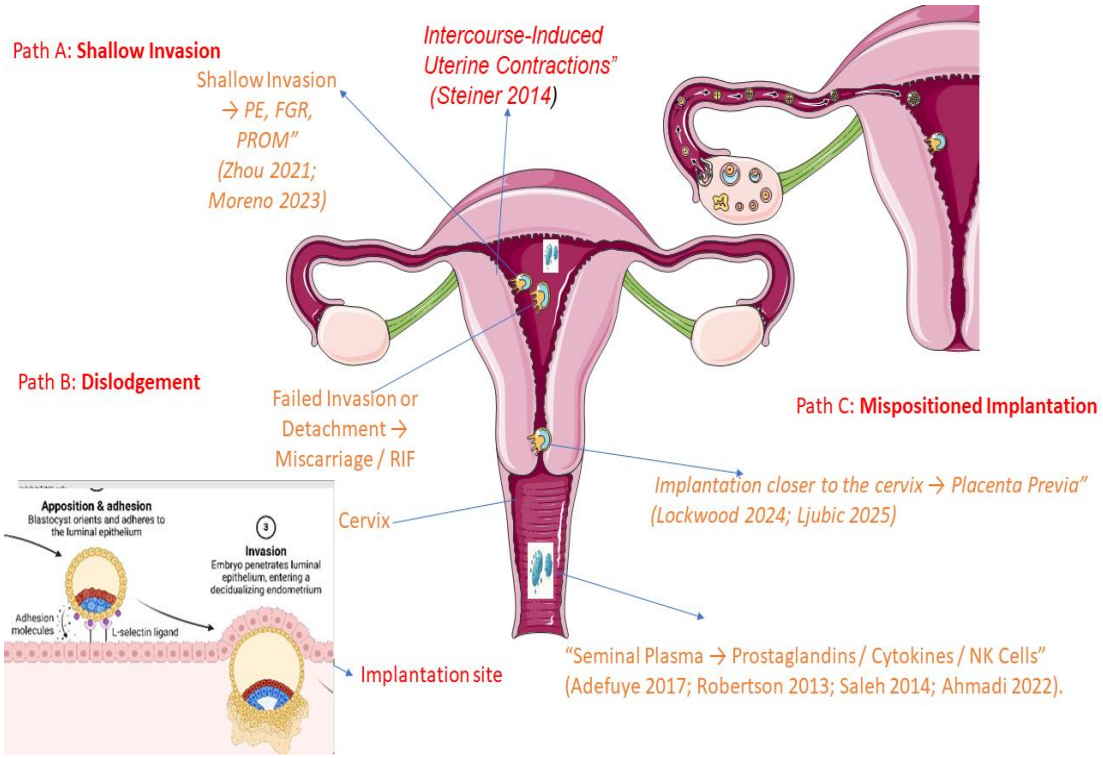

Figure 1.1 Hypothesis explained

Although the literature documents (a) increased myometrial activity during intercourse, (b) seminal-plasma-mediated inflammatory responses, and (c) the central role of oxidative stress in placental disease, there is no comprehensive, mechanistic clinical investigation that links peri-implantation sexual activity to oxidative stress, placental histomorphology (including implantation site and vascular remodelling), and subsequent pregnancy outcomes. Existing studies focus largely on implantation failure or time-to-pregnancy; they do not integrate histological placental assessment, oxidative-stress biomarkers and placental positioning to test the hypothesis that PISI contributes to abnormal implantation with downstream clinical sequelae. This study tests that pathway by randomizing peri-implantation behaviour (abstinence vs non-abstinence), measuring uterine/placental oxidative-stress biomarkers, and performing detailed placental histomorphology and positional analyses. By integrating these domains, the research aims to (i) evaluate whether peri-implantation sexual activity is a behaviourally modifiable risk factor for impaired implantation and abnormal placentation, and (ii) determine whether such effects explain a portion of clinically important outcomes (PE, FGR, placenta previa, preterm birth). Demonstrating these links would justify evidence-based guidance in preconception counselling and could provide a low-cost intervention to reduce implantation-related placental disease.

1.3 Research questions

1. How does peri-implantation sexual abstinence versus non-abstinence influence placental histomorphological findings?
2. Why are elevated oxidative stress markers (SOD, CAT, MDA, T-AOC) associated with changes in placental histomorphological findings?

3. How does peri-implantation sexual abstinence versus non-abstinence affect the normal implantation process, specifically the risk of low-lying placenta compared to normal placentation as assessed by early ultrasound?
4. How does abnormal implantation contribute to adverse pregnancy outcomes in the mother (preeclampsia, placenta previa, preterm birth) and the fetus (fetal growth restriction, low Apgar score, NICU admission, congenital anomalies)?

1.4 Research hypotheses

1. There is a significant association between peri-implantation sexual abstinence versus non-abstinence and placental histomorphological findings.
2. There is a significant association between peri-implantation sexual abstinence versus non-abstinence and oxidative stress markers (Superoxide Dismutase [SOD], Catalase [CAT], Malondialdehyde [MDA]) and total antioxidant capacity (T-AOC) in placental tissue.
3. There is a significant association between peri-implantation sexual abstinence versus non-abstinence and the likelihood of a low-lying versus normally implanted placenta as assessed by ultrasound at 5–6 weeks of pregnancy.
4. There is a significant association between peri-implantation sexual abstinence versus non-abstinence and adverse pregnancy outcomes, specifically maternal outcomes (preeclampsia, placenta previa, preterm birth) and fetal outcomes (fetal growth restriction, low Apgar score, NICU admission, congenital anomalies)

1.5 Research aims and objectives

Research Aim:

To investigate the effects of peri-implantation sexual abstinence on pregnancy outcomes, placental development, and oxidative stress markers in healthy women planning conception.

1.6 Specific Objectives:

1. To determine the effects of peri-implantation sexual abstinence and non-abstinence on placental histomorphological findings.

2. To assess the differences in oxidative stress markers (Superoxide Dismutase [SOD], Catalase [CAT], Malondialdehyde [MDA]) and Total antioxidant capacity (T-AOC) in placental tissue between the abstinence and non-abstinence groups.

3. To investigate whether peri-implantation sexual abstinence influences the placental implantation site (low-lying vs. normal) as assessed by ultrasound in early pregnancy (5^{th}-6^{th} weeks of gestation).

4. To compare maternal outcomes (preeclampsia, placenta previa, and preterm birth) and fetal outcomes (fetal growth restriction, low Apgar score, neonatal intensive care unit admission, and congenital anomalies) between women practicing peri-implantation sexual abstinence and those not practicing abstinence

CHAPTER 2

LITERATRE REVIEW

2.1 Implantatıon: A Precarious Foundation for Pregnancy

Implantation constitutes the pivotal process wherein the blastocyst adheres to and invades the receptive endometrium, establishing the foundational maternal-fetal interface essential for pregnancy (S.-M. Kim & Kim, 2017). This journey begins with the fertilized zygote, which undergoes cleavage to form a morula during its transit through the fallopian tube before maturing into a blastocyst upon entering the uterine cavity (Marieb, E. N., & Hoehn, K., 2019). Crucially, the human blastocyst then enters a period of intrauterine free suspension for approximately three days prior to initiating attachment (Yoshinaga, 2018). This pre-attachment window is a period of significant vulnerability, setting the stage for the meticulously orchestrated yet fragile events to follow.

The implantation process itself, typically occurring 6-10 days post-fertilization, can be delineated into three distinct stages: apposition, adhesion, and invasion (Norwitz et al., 2001). Apposition involves the initial, unstable contact between the blastocyst's trophectoderm and the uterine epithelium, facilitated by interactions with pinopodes transient, progesterone-induced protrusions on endometrial cells (Gauster et al., 2022). This is followed by stable adhesion, characterized by enhanced physical bonding, culminating in the invasion phase where syncytiotrophoblasts penetrate the endometrial lining, ultimately enabling the blastocyst to access the maternal blood supply (S. Zhang et al., 2013). The success of this invasion is paramount; extravillous trophoblasts must deeply infiltrate the maternal decidua and adequately remodel the spiral arteries to transform them into high-capacity, low-resistance vessels, thereby ensuring sufficient blood flow to the

developing fetoplacental unit (Burton & Jauniaux, 2018; Pereira et al., 2015a) Refer to Figure

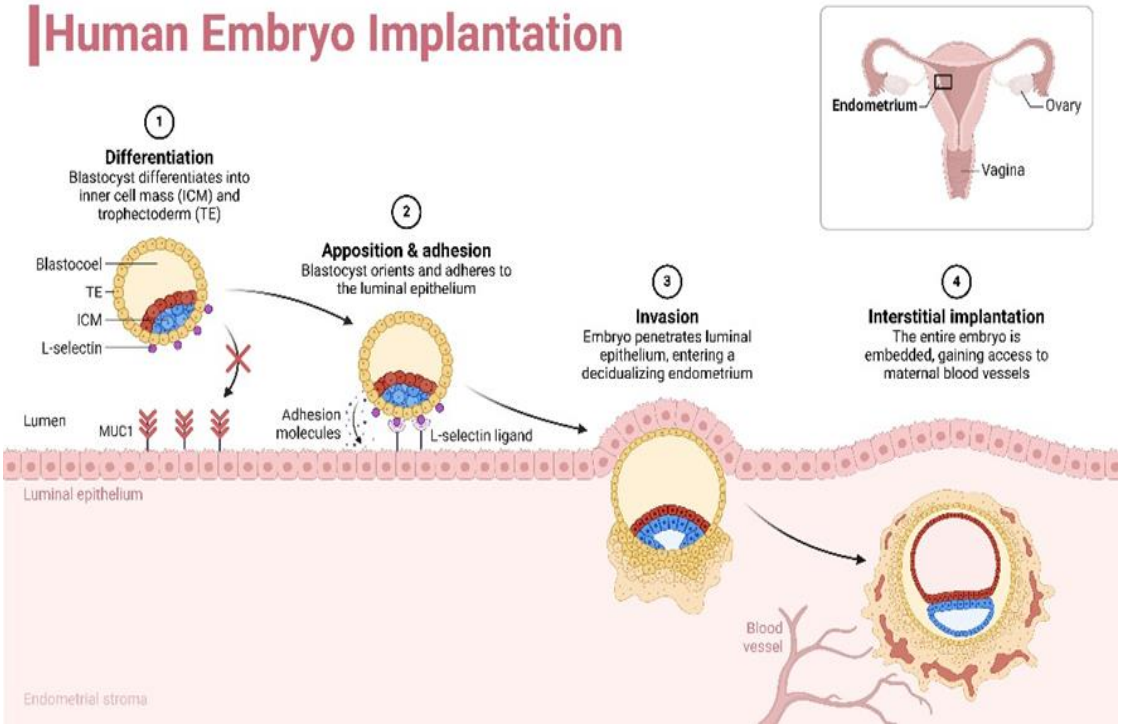

Figure 2.1 Key features of implantation

It is at this critical juncture that the literature reveals a profound paradox regarding sexual activity. While coitus is necessary for conception and may aid sperm transport via orgasm-induced uterine contractions (Puts et al., 2012; Stanford et al., 2020), evidence suggests its timing is paramount. A critical analysis of competing studies reveals a significant contradiction: the same mechanical forces that may facilitate conception could be detrimental during the implantation window (Steiner et al., 2014). The study by Steiner et al. (2014) provides compelling evidence for this, demonstrating that intercourse during the peri-implantation period (days 5-9 post-ovulation) was

associated with a 26% reduction in fecundability (Steiner et al., 2014). This finding forces a re-evaluation of traditional advice and suggests the mechanical forces of intercourse uterine contractions could disrupt the delicate apposition and adhesion phases, potentially displacing the unattached blastocyst, leading to implantation failure or predisposing to shallow, dysfunctional invasion.

The clinical significance of uterine quiescence is further underscored by interventional studies in assisted reproduction. The use of uterorelaxing agents like Atosiban during embryo transfer has been shown to significantly improve implantation and pregnancy rates in patients with a history of failure (Lan et al., 2012). This evidence directly supports the hypothesis that a tranquil uterine environment is not merely beneficial but essential for successful implantation.

Consequently, any disruption during this precise sequence, whether from aberrant biochemical signaling or physical disturbance, can precipitate a cascade of placental pathologies (Moreno et al., 2023). Inadequate trophoblast invasion and spiral artery remodeling are well-established precursors to major obstetric syndromes, including preeclampsia and fetal growth restriction (FGR) (Burton & Jauniaux, 2018). Furthermore, disordered implantation may result in the placenta developing in a low-lying position, a known risk factor for placenta previa (Amer, 2021; Babayan et al., 2024). Therefore, we posit that peri-implantation sexual intercourse (PISI) represents a plausible, modifiable risk factor that interferes with the implantation process. By potentially causing mechanical displacement (Steiner et al., 2014) or exacerbating inflammatory responses (Adefuye et al., 2016), PISI may initiate a trajectory of abnormal placentation, ultimately increasing the risk of adverse pregnancy outcomes through the downstream effects of placental hypoxia and oxidative stress.

2.2 Oxidative Stress Biomarkers:

Oxidative stress (OS) biomarkers are broadly classified into two categories: molecules Oxidative stress (OS) is defined as a pathophysiological state arising from a critical imbalance between the generation of reactive oxygen and nitrogen species (ROS/RNS) and the capacity of endogenous antioxidant defenses to mitigate their damaging effects (Jauniaux et al., 2000). In the context of pregnancy, the accurate assessment of OS is paramount, as it plays a dual role: it is a essential physiological mediator for key processes like trophoblast differentiation and invasion (Wang & Zhao, 2010; Wu et al., 2015), yet it is also a well-established pathological driver of placental dysfunction and associated complications (Ibrahim et al., 2024; Wu et al., 2015). Consequently, OS biomarkers, biological molecules that reflect this redox imbalance, serve as crucial indicators for evaluating maternal-fetal health and understanding the etiology of adverse outcomes (Myatt et al., 2000; Shigemitsu et al., 2016; Silvestro, 2020).

These biomarkers are broadly categorized into two classes: (1) molecules directly modified by ROS/RNS, which provide a measure of oxidative damage, and (2) components of the antioxidant system, whose concentration or activity changes in response to redox stress (Schoots et al., 2018). The most widely recognized biomarkers include malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS), which are reliable proxies for lipid peroxidation; 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage; and the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), which constitute the first line of enzymatic defense (Ibrahim et al., 2024). Furthermore, assays measuring total antioxidant capacity (TAC) offer a comprehensive assessment of the overall redox buffer capacity, integrating contributions from both enzymatic and non-enzymatic antioxidants (Basu et al., 2015).

A critical advancement in the field has been the exploration of placenta-specific biomarkers accessible via maternal circulation(Hong & Kumar, 2023). The placenta sheds trophoblast cells, mRNA, and cell-free DNA into the maternal blood, providing a unique window into its pathophysiological state (Taglauer et al., 2014). Research has demonstrated that altered expression levels of anti-angiogenic factors (e.g., sFlt-1, soluble endoglin) and pro-angiogenic factors (e.g., PlGF) in maternal blood are strongly associated with the subsequent development of preeclampsia (PE) and intrauterine growth restriction (IUGR), often weeks before clinical symptoms manifest (Phipps et al., 2019; Rana et al., 2022; Sekizawa et al., 2010). However, a critical appraisal of these findings is necessary. While these biomarkers are associative and attractive because they are non-invasive and reflect placental pathophysiology in real time, their standalone predictive accuracy has been questioned. The large WHO multicenter study by Widmer et al. (2015) concluded that measurements of sFlt-1, PlGF, and soluble endoglin before 20 weeks gestation lacked sufficient predictive power for PE on a population level (Widmer et al., 2015). This crucial finding highlights a significant limitation: the heterogeneous pathophysiology of PE. Not all cases are primarily driven by angiogenic imbalance; some may stem from metabolic, immunologic, or genetic factors that these biomarkers do not capture (N. Liu et al., 2021). Therefore, while promising, angiogenic markers likely need to be incorporated into multi-marker predictive algorithms that account for this heterogeneity.

This underscores a fundamental challenge in OS biomarker research: distinguishing cause from consequence. The consistent observation of elevated OS markers (e.g., MDA) in complications like PE, preterm birth, and miscarriage is robust (Ibrahim et al., 2024; Vornic et al., 2024). Yet, the temporal relationship is often unclear in human studies. Are elevated OS levels a primary instigator of placental pathology, or are they

a secondary byproduct of placental ischemia and inflammatory processes already underway? The failure of antioxidant supplementation trials (e.g., vitamins C and E) to prevent PE in large RCTs suggests that OS may often be a downstream effector or amplifier within a more complex pathological cascade, rather than a easily modifiable root cause (Rumbold et al., 2008).

In conclusion, oxidative stress biomarkers are indispensable tools for probing placental health. The field has evolved from measuring generic markers of damage (MDA) to investigating sophisticated placenta-derived signals (angiogenic factors). A critical synthesis of the literature reveals that while these biomarkers provide strong associative evidence, their application for prediction and their role in pathogenesis require careful, context-dependent interpretation. Future research must focus on longitudinal studies to establish causality and on integrating multiple biomarker classes to develop more accurate, personalized risk profiles for pregnancy complications.

2.3 Oxidative stress and placenta:

The placenta is central to fetal survival and development, functioning as a dynamic interface for nutrient exchange, oxygen supply, waste removal, and endocrine signalling (Cindrova-Davies & Sferruzzi-Perri, 2022). Its development is tightly regulated by oxygen concentration, making it highly susceptible to oxidative fluctuations (Basu et al., 2015). Reactive oxygen species (ROS), while often associated with tissue damage, also act as key regulators of trophoblast proliferation, invasion, angiogenesis, and gene transcription (Wu et al., 2015). Thus, ROS exert a paradoxical role: essential at controlled levels, yet pathogenic when excessive (Jauniaux & Burton, 2016). Placental oxidative stress integrates with autophagy and apoptosis, processes

that normally safeguard tissue homeostasis but, when dysregulated, contribute to miscarriage, preeclampsia, and intrauterine growth restriction (IUGR) (Ibrahim et al., 2024). Evidence from both experimental and clinical studies shows that redox imbalance is not simply a byproduct of placental dysfunction but an active driver of abnormal placentation and immune maladaptation (Sultana et al., 2023). However, the interpretation of oxidative stress as either a cause or consequence of placental pathology remains debated. For instance, increased oxidative stress markers are consistently observed in preeclampsia, yet it is unclear whether they originate from primary trophoblast dysfunction or secondary maternal vascular resistance (Jauniaux & Burton, 2016).

When comparing experimental models, a clearer picture emerges. *In vitro* studies using placental explants or cell cultures subjected to hypoxia-reoxygenation injury provide compelling and direct evidence for the causative role of OS in triggering apoptotic and inflammatory pathways (Hung et al., 2001). These models are strengthful for establishing mechanism but are limited by their simplification of the intricate *in vivo* maternal-fetal environment. Conversely, while animal models of PE or FGR (e.g., through reduced uterine perfusion pressure) allow for the study of OS within a whole organism, their relevance to the slow-onset, immune-mediated pathophysiology of human pregnancy is often questioned, representing a significant methodological challenge.

Furthermore, although placental oxidative stress is widely implicated in IUGR, gestational diabetes, and other adverse outcomes, current biomarker studies demonstrate substantial heterogeneity (Ibrahim et al., 2024). Some markers (e.g., MDA, 8-OHdG) show strong associations, while others fail to predict outcomes reliably (Scripcariu et al., 2019). This inconsistency reflects both methodological

differences across studies and the complex, stage-specific role of ROS in pregnancy physiology.

Taken together, oxidative stress is integral to placental development but remains a "double-edged sword." Its dual role, as both signalling mediator and pathological trigger, underscores the need for more nuanced investigation into the timing, sources, and thresholds of oxidative imbalance in pregnancy. Critically, the contribution of early peri-implantation disturbances (e.g., uterine contractions, seminal plasma exposure) to initiating placental oxidative stress is largely unexplored. Addressing this gap is central to justifying the present study.

2.4 Oxidative stress throughout pregnancy trimesters:

Embryo development takes place in a low-oxygen environment until the second trimester, when a substantial increase in oxygen delivery occurs to support the growing demands(Pereira et al., 2015a; Yang et al., 2022). This shift is crucial for meeting the metabolic requirements of the developing fetus. However, the increased oxygen availability also exposes the placenta to heightened metabolic activity and oxidative stress, which are typical during pregnancy (Huppertz et al., 2009). The first trimester of human pregnancy is a particularly vulnerable period, as it is highly susceptible to various adverse factors, including oxidative stress. This stress can interfere with embryonic and feto-placental development, potentially resulting in unfavorable outcomes (Gauster et al., 2017; Huppertz et al., 2009; Jauniaux et al., 2000).

Regarding oxidative stress in the first-trimester placenta, Watson et al. explored oxidative stress in the first-trimester placenta by evaluating the viability of human syncytiotrophoblasts obtained from chorionic villous tissue. Their study also involved

assessing mitochondrial activity and the ultrastructure of the villi (Watson et al., 1998a). They observed that high oxygen levels led to the deterioration of syncytiotrophoblasts, marked by vacuolization of the cells, a reduction in surface microvilli, and decreased mitochondria. However, this condition did not cause damage to cytotrophoblasts or stromal cells (Watson et al., 1998a). In another study, the same authors demonstrated that syncytiotrophoblasts in early pregnancy exhibit low levels of antioxidants (Watson et al., 1998b). Cytotrophoblasts and villous stromal cells exhibit higher levels of antioxidants compared to syncytiotrophoblasts (Schoots et al., 2018). Syncytiotrophoblasts can adapt to small increases in reactive oxygen species (ROS) by restoring the balance between oxidant and antioxidant activity, a process typically seen in normal pregnancy (Glasser et al., 1987). However, if the adaptation to oxidative stress is insufficient, there may be an inadequate increase in antioxidative capacity, leading to maladaptation of mitochondrial ultrastructure and intermediate damage to syncytiotrophoblasts. This could result in chronic oxidative stress. In severe cases, the damage to syncytiotrophoblasts may become so pronounced that it causes early pregnancy failure(Jauniaux et al., 2000).

The trajectory of OS across gestation further illuminates its dual role. Longitudinal studies indicate that after the first-trimester peak (Żelaźniewicz et al., 2015), OS levels typically decline during the second trimester as antioxidant capacity matures, representing a period of relative redox stability in uncomplicated pregnancies (Basu et al., 2015; Rejc et al., 2017). However, a major controversy arises in the interpretation of third-trimester findings. While OS increases again in late gestation, it is methodologically difficult to distinguish between a *physiological* rise associated with high metabolic demand and the *pathological* oxidative stress that characterizes conditions like preeclampsia (Basu et al., 2015). This ambiguity underscores a

significant limitation in the literature: the reliance on cross-sectional studies that provide a snapshot rather than a continuous narrative of redox balance, making it challenging to establish causal timelines Studies have shown that oxidative stress levels in the placenta change throughout pregnancy, with a significant decline occurring during the second and third trimesters.

The focus has largely been on the pathological endpoints (e.g., preeclampsia), not on the initial insults that occur during placental development. Therefore, this study is justified in investigating peri-implantation sexual intercourse as a novel, modifiable external factor that could act as an early pro-oxidant insult, potentially overwhelming the limited adaptive capacity of the early placenta and setting it on a trajectory toward dysfunction.

2.5 Implantation, oxidative stress and Preeclamapsia (PE):

Pre-eclampsia (PE), a pregnancy-specific condition that has been recognized for over 200 years, is closely linked to its more severe form, eclampsia, which has been documented for over 2,400 years (Lindheimer, 2013). Preeclampsia, affecting 3% to 10% of pregnancies, is a condition characterized by high blood pressure and either proteinuria or dysfunction of vital organs after the 20th week of pregnancy (Garrido-Gómez et al., 2022). It significantly impacts multiple bodily systems and poses serious risks to the health and well-being of pregnant individuals worldwide(Duley, 2009). Globally, preeclampsia and eclampsia are responsible for approximately 10% to 15% of maternal deaths (Duley, 2009). This condition is associated with complications such as an elevated risk of premature birth, low birth weight, and, in severe cases, life-threatening outcomes for both the mother and the developing fetus (Dimitriadis, 2023; McNestry et al., 2023; Sundheimer & Pisarska, 2017).

Preeclampsia is linked to abnormal implantation, a process that involves tightly regulated spatial and temporal invasion (Goldman-Wohl & Yagel, 2002). An imbalance between the factors that promote and those that restrict this invasion can lead to pregnancy-related complications, with preeclampsia being a prominent example (Merviel et al., 2004). Two main theories aim to explain the origins of pre-eclampsia: the *'pure vascularist'* theory, which attributes the condition primarily to vascular dysfunction caused by oxidative stress, often considering it a disorder predominantly affecting first pregnancies. In contrast, the immunological perspective regards pre-eclampsia as an immune-mediated condition, whether localized or systemic (Chaouat et al., 2005).

A unique aspect of human pregnancy is the occurrence of a second wave of trophoblastic invasion later in gestation (Robillard et al., 2022). To date, this phenomenon has not been observed in other primate species (Chaouat et al., 2005). The importance of this secondary invasion lies in its abnormality in pre-eclampsia, often described as "shallow invasion," which has traditionally led many clinicians to attribute pre-eclampsia to deficiencies in the second wave of trophoblastic invasion (Geldenhuys et al., 2018). However, comparative studies in non-human primates challenge this assumption. Research by Palmer et al. on Patas monkeys, along with studies by Sophie L, Baird, Thornton, and Onwude on lowland gorillas, reveal that pre-eclampsia can occur even in the absence of the second-wave invasion seen in humans (Baird, 1981; Palmer et al., 1979; Thornton & Onwude, 1992; Walter et al., 2018). These findings suggest that pre-eclampsia may not be solely driven by defects in the second wave of invasion but could originate from impaired implantation during the blastocyst stage. Such early implantation abnormalities may initiate a cascade of

events, ultimately resulting in shallow trophoblastic invasion, a hallmark of pre-eclampsia.

Recent advancements in the study of implantation sites have highlighted a significant role for the immune system in transforming uterine arteries (Dunn et al., 2003). A notable discovery is that the peri-implantation uterine stroma resembles an immune organ, akin to a lymph node, rather than traditional stroma, with events occurring in distinct waves(Dunn et al., 2003). Theoretically, an implanting embryo could become the target of various forms of immunological aggression, including standard cell-mediated lysis, lysis by cytotoxic antibodies with complement activation, and NK cell-mediated lysis (Dekel, 2014; Yao-Kai Ho, 2019).

The connection between impaired implantation and immune system activation has been further clarified by recent studies. King et al. investigated the role of inflammatory markers, particularly CD40 Ligand (CD40L), in impaired implantation. Their findings revealed a cascade of immune responses triggered by abnormal implantation, leading to elevated CD40L expression in uterine tissues (A. E. King et al., 2001). Matsubara et al. built upon this by demonstrating that immune activation of CD40L during implantation induced pre-eclampsia-like symptoms in murine models (Matsubara et al., 2015, 2016). Peri-implantation sexual intercourse may disrupt implantation by triggering inflammation and oxidative stress, which impair proper trophoblast invasion and increase the risk of developing preeclampsia.

The link between preeclampsia and oxidative stress is well-documented (Aouache, 2018). In preeclampsia, oxidative stress imbalance can cause damage to the vascular endothelium, leading to endothelial dysfunction, elevated blood pressure, and reduced blood flow, all of which are key characteristics of preeclampsia (Aouache, 2018; Hansson, 2015; Smith et al., 2021). Oxidative stress also impacts trophoblast function

and implantation by hindering the invasion of trophoblast cells into the uterine lining. This disruption leads to shallow implantation and inadequate placental development, which are believed to play a role in the onset of preeclampsia(Hung et al., 2001; Jauniaux & Burton, 2016; Sultana et al., 2023). While OS has been extensively studied in the context of placental dysfunction later in pregnancy, mounting evidence suggests that oxidative damage begins much earlier, during implantation and early placentation (Ahmad et al., 2019; Fujii et al., 2005; Governini et al., 2021; Mrozikiewicz et al., 2021). During the implantation window, physiological oxidative stress is required for trophoblast differentiation, angiogenesis, and decidual remodeling (Grzeszczak et al., 2023; Pereira et al., 2015b). However, when ROS levels exceed physiological limits, due to maternal conditions like obesity, infections, or environmental exposures, these same processes are disrupted. Excessive ROS during early pregnancy impairs blastocyst-endometrium cross-talk, inhibits trophoblast proliferation, and reduces vascular endothelial growth factor (VEGF) expression, resulting in poor vascularization and defective spiral artery remodeling (Gusella et al., 2024; Schoots et al., 2018; Vornic et al., 2024)

Oxidative stress also triggers apoptosis of cytotrophoblasts and syncytiotrophoblasts, further weakening placental development and nutrient exchange (Mukherjee et al., 2021; Xie et al., 2024; Zheng et al., 2024). As a result, the placenta becomes ischemic, setting off a feedback loop of inflammation, hypoxia, and systemic endothelial dysfunction. key mechanisms in PE pathogenesis (Gusella et al., 2024; D. Liu et al., 2022; McElwain et al., 2020; Wu et al., 2016). Furthermore, ROS can modify gene expression in endometrial cells, alter immune tolerance, and interfere with decidualization, creating a hostile uterine environment even before clinical signs of PE emerge (Soczewski et al., 2025; C. Zhang et al., 2023). Elevated OS biomarkers during

the first trimester are increasingly being explored as predictive tools for PE, offering a promising avenue for early risk stratification (Chiarello et al., 2020; Ibrahim et al., 2024; X. Liu et al., 2025). Thus, oxidative stress at the implantation, placentation interface serves not merely as a downstream effect of placental dysfunction, but as a primary initiator of the pathological cascade leading to PE. Addressing oxidative imbalance in early pregnancy could be key to preventing the onset of preeclampsia.

2.6 Implantation, oxidative stress and Fetal Growth Restriction (FGR):

Intrauterine growth restriction (IUGR) occurs when the fetus does not reach its expected genetic growth potential (Chew et al., 2025). It is typically defined by an estimated fetal weight below the 10th percentile for its gestational age (Chew et al., 2025). IUGR is a major factor contributing to mortality and health complications in fetuses, newborns, and throughout the perinatal period (Brodsky & Christou, 2004). Epidemiologically, the primary measurable indicator of IUGR is a birth weight below the third percentile (adjusted for gestational age and gender) or below the tenth percentile with optional confirmation of abnormal cord blood flow observed in Doppler studies (Krishna & Bhalerao, 2011). Various factors can lead to fetal growth restriction (FGR), including chronic maternal conditions, fetal chromosomal anomalies, and infections. Maternal smoking, infections, obesity, malnutrition, and chromosomal abnormalities are particularly associated with late-onset FGR (Tsikouras et al., 2024). However, a significant number of cases remain unexplained. The primary cause of late-onset FGR is often uteroplacental dysfunction, which results in inadequate delivery of oxygen and nutrients essential for normal fetal aerobic development (Brodsky & Christou, 2004; Krishna & Bhalerao, 2011).