ENDOMETRIAL GLYCOGEN METABOLISM DURING EARLY PREGNANCY IN MICE

BY

ZITING CHEN

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois Urbana-Champaign, 2022

Urbana, Illinois

Master's Committee:

Assistant Professor Matthew Dean, Advisor
Professor Romana Nowak
Professor David Miller

ABSTRACT

In humans, at least 30-40% of pregnancies fail, most during the preimplantation period. Before implantation, too much or too little glucose impairs embryo development. Pregnancy also requires successful decidualization of the stroma, a glucose intensive process. Therefore, optimal levels of endometrial glucose are required to achieve a successful pregnancy. Glycogen, the storage form of glucose, is found in the uterus and may contribute to maintaining glucose concentrations. Our objectives were to 1) determine how glycogen levels change in the luminal epithelium, glandular epithelium, and stroma during early pregnancy; 2) understand the distribution of glycogen metabolizing enzymes (hexokinase I, HK1; glucose-6-phosphatase, G6PC; glycogen synthase, GYS; and glycogen phosphorylase, PYG); and 3) determine if the decidua stores glycogen independently of pregnancy.

To characterize glycogen during pregnancy, uteri from CD-1 mice were collected at proestrus, days post-coitum 1.5 (DPC 1.5), DPC 3.5, and DPC 5.5. To artificially induce decidualization, CD-1 mice were ovariectomized, primed with steroids, and one uterine horn was stimulated by an infusion of corn oil. Periodic acid-Schiff staining, with and without diastase pre-treatment, was used to measure glycogen content in endometrial tissues. Western blot was carried out to quantify glycogen metabolizing enzymes. Immunohistochemistry (IHC) was performed to localize the glycogen metabolizing enzymes.

Our results showed that in the glandular epithelium, glycogen content was highest at proestrus and decreased 71.4% at DPC 1.5 and 62.13% at DPC 3.5. Similarly, in the luminal epithelium glycogen was highest at proestrus, was 46.2% lower at DPC 1.5 and 63.2% lower at DPC 3.5. In contrast, the stroma stored little glycogen during the preimplantation period, and the

amount did not change. However, at DPC 5.5, glycogen content increased 7-fold in the implantation site compared to the stroma of proestrus. IHC showed that HK1 was present in the glandular and luminal epithelium, but was undetectable in the stroma. GYS in the luminal and glandular epithelium was highest in the preimplantation period compared to proestrus, but was not detected in stromal cells. At the implantation site, there was a dramatic increase in GYS in the decidua. G6PC was highly expressed in the luminal and glandular epithelium from DPC 1.5 to DPC 5.5. PYG increased from proestrus to DPC 3.5 in the glandular and luminal epithelium. The artificially decidualized stroma showed a 5-fold increase in glycogen content. The levels of GYS, G6PC, and PYG were increased in the decidualized uterine horn compared to the control horn.

In conclusion, glycogen in the luminal and glandular epithelium of murine uterus decreased during early pregnancy, which correlated with the increased expression of G6PC and PYG. This suggests that glycogen in the uterine epithelium supports the preimplantation embryos. In contrast, glycogen in the stroma was constantly low until after implantation. After which, glycogen and GYS expression increased dramatically in the decidua. Thus, the decidua stores glycogen, which later may be released to support remodelling of the decidua or to provide energy for embryo development.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Matthew Dean, for taking me in as one of his lab members and providing me with an unforgettable opportunity to conduct research during the special time of COVID-19. He is an amazing, knowledgeable, and caring mentor that I could not ask for more. His enthusiasm for science and passion for life is contagious. He is always the first person I came to for the answers and advice about any science or career-related questions. Never once he was impatient on any of those questions. He was also instrumental in helping me prepare presentations and cranking out this thesis. Without his support and guidance, none of these works and writings would be possible.

I would also like to thank my committee members, Dr. Romana Nowak and Dr. David Miller, for their feedback and guidance throughout the process. The supports they provided are invaluable and I am sincerely grateful for it.

I am also grateful to have wonderful lab members, especially Malia Berg and Alexis Gonzalez. I am very lucky to get to know these lovely people and build a great friendship with them. It is impossible to count how many times they helped me overcome any kinds of problems and supported me in everything. Their warmth and kindness made my time at the University of Illinois forever unforgettable.

Last but not least, I would like to thank my parents and friends for respecting my decisions and unconditionally encouraging and supporting me to pursue a degree in animal science.

TABLE OF CONTENTS

List of Abbreviations

1, 3-BPG: 1, 3-bisphosphoglycerate

20α-HSD: 20α-hydroxysteroid dehydrogenase

2PG: 2-phosphoglycerate

3PG: 3-phosphoglycerate

6PG: 6-phosphogluconate

6PGDH: 6-phosphogluconate dehydrogenase

6PGL: 6-phosphogluconolactone

ABC: avidin-biotin complex

ADP: adenosine diphosphate

ATP: adenosine 5'-triphosphate

cAMP: cyclic adenosine monophosphate

CL: corpus luteum

DHAP: dihydroxyacetone phosphate

DPC: days post-coitum

E2: estradiol

ER: estrogen receptor

F-1,6-BP: fructose 1, 6-bisphosphate

F6P: fructose-6-phosphate

FOXO: forkhead box protein

FSH: follicle-stimulating hormone

G1P: glucose-1-phosphate

G3P: glyceraldehyde 3-phosphate

G6P: glucose-6-phosphate

G6PC: glucose-6-phosphatase

G6PDH: glucose-6-phosphate dehydrogenase

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

GBE: glycogen branching enzyme

GDE: glycogen debranching enzyme

GlcN6P: glucosamine-6-phosphate

GlcNAc-1P: N-acetylglucosamine-1-phosphate

GlcNAc-6P: N-acetylglucosamine-6-phosphate

GLUT: facilitative glucose transporter

GnRH: gonadotrophin-releasing hormone

GPI: glucose phosphate isomerase

GYG: glycogenin

GYS: glycogen synthase

hCG: human chorionic gonadotropin

HESC: human endometrial stromal cell

HK: hexokinase

Hox: homeobox

IGFBP-1: insulin-growth factor binding protein-1

IHC: immunohistochemistry

IIS: inter-implantation site

IL: interleukin

IS: implantation site

LH: luteinizing hormone

LIF: leukemia inhibitory factor

MPA: medroxyprogesterone

NAD+: nicotinamide adenine dinucleotide

NADP: nicotinamide adenine dinucleotide phosphate

NK cell: natural killer cell

P4: progesterone

PAS: periodic acid Schiff

PDZ: primary decidual zone

PEP: phosphoenolpyruvate

PFK: phosphofructokinase

PGK: phosphoglycerate kinase

PGLS: 6-phosphogluconolactonase

PGM: phosphoglucomutase

PKM2: pyruvate kinase M2

PP1: protein phosphatase-1

PPP: pentose-phosphate pathway

PR: progesterone receptor

PRL: prolactin

PRPP: phosphoribosyl pyrophosphate

PYG: glycogen phosphorylase

R5P: ribose-5-phosphate

RIPA: radioimmunoprecipitation assay buffer

RPI: ribulose-5-phosphate isomerase

Ru5P: ribulose-5-phosphate

SGLT: sodium-coupled glucose transporters

TCA: tricarboxylic acid

UDP-GlcNAc: uridine diphosphate N-acetylglucosamine

UDP: uridine diphosphate

UGKO: uterine gland knockout

UGP: UDP-glucose pyrophosphorylase

UTP: uridine 5’-triphosphate

Xu5P: xylulose-5-phosphate

List of Figures

CHAPTER 2:

CHAPTER 3:

CHAPTER 1: INTRODUCTION

Pregnancy loss is common in humans, and at least 30-40% of pregnancies fail. Among those pregnancy failures, most occur during the pre-or peri-implantation period. Unfortunately, pregnancy failure may be higher than expected as early embryo loss can take place before human chorionic gonadotropin (hCG) is produced by the trophoblast (Wilcox et al., 1988; Zinaman et al., 1996). In addition, some women experiencing infertility hesitate to visit their doctors (Iino et al., 2022). Therefore, given the high rate of pregnancy failure before and around the implantation period, understanding the uterine environment during early embryonic development and the interactions between the uterus and the embryo may provide insights to minimize early pregnancy failure.

Before implantation, the free-floating embryo relies on the nutrients secreted into the uterine lumen. Glucose is one of the most important nutrients required for early embryonic development, and optimal glucose concentration is required to prevent pregnancy loss. Glucose uptake by the embryo is low until the morula stage and too much glucose before compaction is toxic to the embryo (Gardner et al., 1996). From the fertilization stage until the 8-cell stage, the embryo primarily utilizes lactate and pyruvate as main energy sources (Brinster, 1969; Leese & Barton, 1984; Martin & Leese, 1995). A switch of energy sources from pyruvate and lactate to glucose occurs around the blastocyst stage. The uptake of glucose by the blastocyst increases significantly compared to the cleavage stage embryo (Gardner et al., 2011). Not surprisingly, the increase in glucose uptake by the blastocyst correlates with higher glucose concentrations found in the uterine lumen than in the oviduct (Gardner et al., 1996).

At the implantation site, the stromal cells undergo morphological and physiological changes to form decidua. Expression of glucose transporter-1 (GLUT1) and uptake of glucose

increased in both humans and mice uterine stromal cells as they decidualize (Frolova et al., 2009). An increase in glucose metabolism through the pentose-phosphate pathway (PPP) was observed in the decidua and inhibiting this pathway was detrimental to the decidual response (Frolova et al., 2011; Tsai et al., 2013). After decidualization, a shift to Warburg-like metabolism resulted in higher glucose uptake (Zuo et al., 2015). Therefore, the glucose needs for the embryo and uterus change in a spatiotemporal manner during early pregnancy.

Glycogen is a storage form of glucose, and it acts as a glucoses reservoir to regulate glucose concentrations. The uterus can temporarily store glucose as the macromolecule glycogen. In humans, female infertility is associated with low glycogen content in the endometrium, suggesting that proper glycogen storage is important for successful pregnancies (Gupta et al., 2013; Hughes et al., 1969; Maeyama et al., 1977). However, glycogen metabolism and glucose regulation are not fully understood in the uterus. Uterine glycogen metabolism has never been characterized during the pre-and peri-implantation period of mice. In addition, which tissues in the endometrium contribute to the storage of glycogen remains unclear. The locations of glycogen metabolizing enzymes during early pregnancy have not been illustrated. Therefore, our objectives were to 1) characterize endometrial glycogen storage in the murine uterus from proestrus until the implantation period in the luminal epithelium, glandular epithelium and stroma; 2) localize key glycogen metabolizing enzymes in the uterus during the same period; and 3) determine whether decidualization is sufficient to drive glycogen accumulation independently of pregnancy.

CHAPTER 2: LITERATURE REVIEW

2.1 Pregnancy Failure

Infertility is a common problem in reproductive health, and 10-15% of couples have difficulties trying to conceive. It is estimated that by 2050, 15% of couples will be childless because of infertility (Wang & Dey, 2006). Even with advanced assisted reproductive technologies, such as *in vitro* fertilization and utilizing high-quality embryos for embryo transfer, failures in implantation and loss of embryos are common. To successfully implant, complex interactions between a viable embryo and an appropriately primed uterus are necessary (Ng et al., 2020). Even though preimplantation embryonic death is common in many mammals and is considered to be a selection process leading to higher survival of superior embryos, the dysregulation of embryonic development and uterine receptivity may be the causes for poor pregnancy rates (Dey et al., 2004).

Pregnancy failure can occur at any time from fertilization, implantation, up until term. Even with optimal conditions, the maximal clinically detected pregnancy rate is 30-40% per cycle in humans (Macklon et al., 2002). However, optimal conditions normally do not reflect real-life. As a result, the pregnancy rate generated from a population-based study will be more reliable and practical. A study analyzing daily urinary hCG levels in 221 women for 6 months was able to provide pregnancy rates. The study implemented a highly sensitive and immuno-radiometric assay for hCG to detect embryo. The result showed that the clinically detectable pregnancy rate was 25% per cycle during the first three cycles (Wilcox et al., 1988). Similarly, in another study of 200 couples, the maximal fertility rate was about 30% per cycle in the first two cycles (Zinaman et al., 1996).

Pregnancy loss is also significant in a proportion of women. In support of that, both of the studies also reported pregnancy loss after hCG was detectable. In the study of Wilcox et al. (1988), they found that 22% of early pregnancy losses detected utilizing a highly sensitive hCG assay were clinically unrecognized. Combining these pre-clinically recognized early losses with clinically detectable pregnancy losses, the overall pregnancy loss rate was about 31%. This rate of overall pregnancy loss was consistent with the study of Zinaman et al. (2019).

However, the actual rate of preclinical pregnancy loss may be higher. Losses occurring from fertilized zygote to the morula stage were undetectable in these studies. This is because hCG is secreted by the trophoblasts of the embryo and the embryo at early developing stages has not yet differentiated into trophoblastic cells. Therefore, these losses were missed. In addition, the hCG produced by the blastocyst may not reach urine by the time of detection. Moreover, early pregnancies that failed to produce enough hCG to meet the criterion were also overlooked in these studies.

2.2 Carbohydrate Metabolism

2.2.1 Types of Glucose Transporters

Glucose transport across the plasma membrane and into cells is mediated by sodium-coupled glucose transporters (SGLT) or the facilitative glucose transporter (GLUT). SGLTs are also known as the *SLC5A* gene family while GLUTs are known as the *SLC2A* gene family (Frolova & Moley, 2011). Different tissues express different glucose transporters. The brain takes up glucose mainly through GLUT1 and GLUT3. GLUT3 is highly expressed by neurons (Simpson et al., 2008). Skeletal muscle coordinates GLUT1 and GLUT4 to take in glucose.

GLUT1 lies within the plasma membrane to facilitate glucose transport whereas GLUT4 is found in the intracellular vesicles of cells and is then transported to the membrane when stimulation occurs. Stimulatory signals include binding of insulin or muscle contraction. In the liver, GLUT2 is most utilized to transport glucose (Navale & Paranjape, 2016).

The expression of glucose transporters was also reported in the uterine endometrium. Immunostaining showed that SGLT1 was found in the luminal epithelium and glandular epithelium during day 1 and day 2 of pregnancy in the murine uterus. The expression of SGLT1 shifted to the stroma on day 3 and day 4 (Zhang et al., 2021). In addition, GLUT1, 3, 4, and 8 are also presented in both the human and murine uterus as discussed below.

GLUT1 was the first GLUT identified in the endometrium. It was expressed in the uterus of both cycling and pregnant rodents. In non-pregnant mice, GLUT1 expression was higher in the uterine epithelium than in the stroma, but further research is needed to characterize GLUT1 expression in the epithelial cells (Frolova & Moley, 2011). In pregnant rats, GLUT1 protein was found localized to the luminal epithelium, the stroma and the decidual cells (Korgun et al., 2001). On the other hand, although low expression of GLUT1 was found in the undifferentiated stromal cells, its expression was increased dramatically in the decidualized stromal cells. Yamaguchi et al. (1996) initially detected expression of GLUT1 mRNA in the decidua and the mRNA expression increased during gestation in mice. GLUT1 mRNA and protein levels both increased in the murine stromal cells throughout the decidualization process both *in vivo* and *in vitro* (Frolova et al., 2009). Furthermore, the mRNA expression of GLUT1 was most abundant in the endometrial stroma compared with other GLUTs, suggesting that GLUT1 may play an important role in maintaining pregnancy and regulating glucose availability to the developing embryo (Frolova & Moley, 2011).

GLUT3 has a higher glucose affinity than GLUT1, 2 and 4. It is well characterized in tissues that require higher metabolic demands, such as the brain, the sperm, and the preimplantation embryo (Simpson et al., 2008). GLUT3 is less studied when compared with GLUT1, but has been shown to be presented in the endometrium. Immunohistochemistry (IHC) showed that GLUT3 was expressed in the decidual cells in the rat uterus (Korgun et al., 2001). In addition, mRNA expression of GLUT3 was reported in the decidua of the murine uterus. The expression decreased slightly as pregnancy proceeded (Yamaguchi et al., 1996). In humans, GLUT3 mRNA expression was low during the proliferative phase and increased in the late secretory phase and in the decidua (Von Wolff et al., 2003).

GLUT4 is the most studied among all the GLUTs as it regulates whole-body glucose homeostasis and is important for understanding type II diabetes (Wang et al., 2020). It is less studied in the uterus when compared with other tissues. Frolova & Moley (2011) reported that the mRNA expression of GLUT4 was 100- to 1000-fold lower than other GLUTs in the endometrial stroma, possibly explaining the fewer studies focusing on GLUT4 in the uterus. Even though GLUT4 is low in the uterus, it is ubiquitously expressed in the epithelium, the stroma, and the decidua throughout early pregnancy in rats (Korgun et al., 2001).

GLUT8 is expressed at high levels in the testis, and is also found in other tissues such as the uterus, the ovary, the brain and the liver. GLUT8 was localized entirely intracellularly except in the blastocyst. In the blastocyst, GLUT8 was translocated to the plasma membrane in response to insulin stimulation similar to GLUT4 (Carayannopoulos et al., 2000; Diril et al., 2009). In the uterus, the expression of GLUT8 has only been identified recently. GLUT8 was found to be the highest at estrus in the murine uterus and increased during decidualization (Kim & Moley, 2009). Significant upregulation of GLUT8 was also reported in human decidualized cells (Frolova &

Moley, 2011). GLUT8 knockout mice exhibited small litter size and impaired decidualization, suggesting GLUT8 was required for completing the decidualization process (Adastra et al., 2012)

2.2.2 Glycogen Structure and Storage

After glucose enters the cell, it can be stored as glycogen. Glycogen is a branched polymer of glucose and acts as reservoir of glucose storage. The backbone linear chain of glycogen is composed of glucose resides linked via α-1,4-glycosidic bonds, and the branched points of glycogen are created by α-1,6-glycosidic linkages (Figure 1). The branches are evenly distributed, resulting in the spherical structure of glycogen. In general, glycogen can store up to 55,000 glucose residues, with a size of 10-44 mm in diameter in skeletal muscle and 110-290 nm in liver. The skeletal muscle and the liver are the main tissue types for glycogen storage, and the glycogen in the liver and muscle plays an important role in maintaining whole-body blood glucose homeostasis. Glycogen-containing cells are also found in the brain, the heart, the skin, the adipose tissue, the leukocytes, the thymus, the retina, and the skin (Ryu et al., 2009). Moreover, several studies have shown that glycogen can be stored in the uterus and glycogen content changes throughout the reproductive cycle and pregnancy (Dean et al., 2014; Demers & Jacobs, 1998; Demers et al., 1972). However, glycogen storage in specific uterine tissues has never been fully understood.

2.2.3 Glucose Metabolism

Once the glucose is transported into the cell, hexokinase (HK) phosphorylates glucose, yielding glucose-6-phosphate (G6P) while adenosine 5'-triphosphate (ATP) is hydrolyzed to adenosine diphosphate (ADP). Phosphorylation of glucose traps the glucose moiety inside the cell and this reaction maintains the gradient necessary for glucose diffusion into the cell (Printz et al., 1993). G6P can enter four major pathways, the glycogenic pathway, the glycolytic pathway, the hexosamine biosynthetic pathway, and the pentose-phosphate pathway (Figure 2).

2.2.3.1 Glycogen Metabolism

Glycogenesis is the pathway that stores glucose as glycogen, which is an important energy reserve for animals. After G6P is generated from glucose, phosphoglucomutase (PGM) isomerizes G6P to glucose-1-phosphate (G1P) by transferring a phosphate group from C6 to C1. Catalyzed by uridine diphosphate (UDP)-glucose pyrophosphorylase (UGP), the phosphoric-anhydride bond on uridine 5'-triphosphate (UTP) is replaced by the phosphate group on G1P, yielding UDP-glucose and pyrophosphate. Glycogen synthase (GYS) is a glycosyltransferase that elongates the linear chain of glycogen. It forms α-1,4-glycosidic linkages between the glycosyl residues from the UDP-glucose and the growing glycogen molecule, leading to the release of UDP. As the linear chain elongates through the α-1,4-glycosidic bond, the glycogen branching enzyme (GBE) transfers 6-8 glycosyls to glycogen via α-1,6-glycosidic linkages, creating the multi-branched macromolecule glycogen (Figure 3).

To break down glycogen, several enzymes are also involved in the process. Glycogen catabolism occurs in the cytoplasm as well as inside the lysosome, but different enzymes are required. In the cytoplasm, glycogen phosphorylase (PYG) breaks the α-1,4-glycosidic bond in

the linear chain of glycogen, releasing G1P. After PYG releases G1P, four glycosyl residues remain on the branch, and glycogen debranching enzyme (GDE) is required to break the branch and reattach the residues to form a linear chain. To achieve this, the transferase activity of the enzyme relocates three glycosyl residues from the branch to a linear strand. Then, the glucosidase activity of the enzyme breaks the α-1,6-glycosidic bond, which forms the branching point, releasing a single glucose molecule (Figure 4). On the other hand, glycogen breakdown in the lysosomes is accomplished by the lysosomal enzyme acid α-glucosidase, yielding G1P. The G1P generated from glycogen degradation in both cytoplasm and lysosome is isomerized by PGM, producing G6P. Lastly, glucose-6-phosphatase (G6PC) dephosphorylates G6P in the endoplasmic reticulum to liberate free glucose (Adeva-Andany et al., 2016)

The protein that was thought to form new glycogen molecules is called glycogenin (GYG). It is a glycosyltransferase that catalyzes the transfer of glucose residues from UDP-glucose to itself and forms a linear chain of the first dozen glucose residues through α-1,4-glycosidic linkage (Adeva-Andany et al., 2016; Skurat et al., 2006). The linear chain of glucose residues generated by GYG acts as the precursor for glycogen (Gibbons et al., 2002). However, new evidence indicates that GYG may not be required for glycogen formation (see section 2.4 Glycogen Metabolizing Enzymes below).

2.2.3.2 Glycolysis

Glycolysis is a universal pathway in all living cells and is a major pathway to generate the energy-carrying molecule ATP (Akram, 2013). G6P is first converted to fructose-6-phosphate (F6P) by glucose phosphate isomerase (GPI). F6P can either proceed with further glycolysis or enter the hexosamine biosynthetic pathway (see section 2.3.3 The Hexosamine

Biosynthetic Pathway below). In glycolysis, the phosphoryl group from ATP is transferred to F6P under the catalytic effect of phosphofructokinase (PFK), yielding fructose 1, 6-bisphosphate (F-1,6-BP) and ADP. F-1,6-BP is then cleaved by aldolase, generating dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P) (Harris, 2013). The DHAP and G3P can inter-convert in the process. From there, G3P is oxidized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH), forming 1,3-bisphosphoglycerate (1,3-BPG). Phosphoglycerate kinase (PGK) transfers the phosphate group on 1,3-BPG to ADP, producing ATP and 3-phosphoglycerate (3PG), which is then isomerized to 2-phosphoglycerate (2PG) by PGM. Enolase dehydrolyzes 2PG, forming phosphoenolpyruvate (PEP). PEP is converted to pyruvate by pyruvate kinase, which also generates ATP. When oxygen is present, pyruvate can enter the tricarboxylic acid (TCA) cycle, also known as Kreb cycle, to generate significant amounts of ATP. In the absence of oxygen, pyruvate can be converted to lactate, which produces more nicotinamide adenine dinucleotide (NAD+) to continue the process of glycolysis (Zimmermann, 2001; Figure 2).

2.2.3.3 The Hexosamine Biosynthetic Pathway

The hexosamine biosynthetic pathway is responsible for the production of key substrates for glycosylation. In the hexosamine biosynthetic pathway, 2-5% of F6P together with glucosamine is converted to glucosamine-6-phosphate (GlcN6P). GlcN6P is further converted to N-acetylglucosamine-6-phosphate (GlcNAc-6P), followed by PGM conversion to N-acetylglucosamine-1-phosphate (GlcNAc-1P). GlcNAc-1P is converted to uridine diphosphate N-acetylglucosamine (UDP-GlcNAc). From there, UDP-GlcNAc can be used to form N-linked and O-linked glycosylation in the endoplasmic reticulum or the Golgi apparatus (Akella et al., 2019; Figure 2).

2.2.3.4 The Pentose-Phosphate Pathway

The pentose-phosphate pathway serves two purposes that are important for the proliferation of cells: 1) to synthesize nucleotides and nucleic acids; and 2) to generate NADPH (Horecker, 2002). NADPH is required for fatty acid synthesis and cell survival, suggesting the indispensable role of PPP for cells. There are two branches of PPP, the oxidative branch generating NADPH and ribose-5-phosphate (R5P), and the non-oxidative branch producing glycolytic intermediates such as F6P or G3P. In the oxidative phase, G6P is oxidized by G6P dehydrogenase (G6PDH) to generate 6-phosphogluconolactone (6PGL), while nicotinamide adenine dinucleotide phosphate (NADP+) is reduced to NADPH. 6PGL is then hydrolyzed by 6-phosphogluconolactonase (PGLS) producing 6-phosphogluconate (6PG). From there, 6PG converts to ribulose-5-phosphate (Ru5P) by 6-phosphogluconate dehydrogenase (6PGDH), generating another NADPH in this step. In contrast, in the non-oxidative branch of PPP, Ru5P undergoes isomerization by ribulose-5-phosphate isomerase (RPI) to produce R5P, which can be converted to phosphoribosyl pyrophosphate (PRPP) and used for ribonucleotide synthesis. In addition, xylulose-5-phosphate (Xu5P) can also be produced from R5P and can be used to generate F6P and G3P, the glycolytic intermediates (Patra & Hay, 2014; Figure 2).

2.2.4 Glycogen Metabolizing Enzymes

There are four isomers of HKs, I, II, III, and IV (HK IV is also known as glucokinase). HK II is expressed in the skeletal muscle. An increased concentration of G6P has an inhibitory effect on HK I, II, and III (Grossbard & Schimke, 1966). HK IV is different from the other three HKs in the family. It has a higher molecular weight and a lower glucose affinity (Printz et al.,

1993). G6P does not have an inhibitory effect on glucokinase, and neither do ATP nor ADP (Weinhouse, 1976).

GYG has two isoforms in humans: GYG1, which is ubiquitously expressed, and GYG2, which is mainly found in the liver, the cardiac muscle, and the pancreas. In contrast to humans, rats and mice only express one isoform of GYG (Zhai et al., 2001). GYG was considered as an initiator protein in glycogenesis, but whether GYG is indispensable for glycogen synthesis is debatable. GYG knockout mice showed an accumulation of glycogen in the heart and the muscle (Testoni et al., 2017). In addition, patients with depletion or absence of GYG1 were observed with various amounts of glycogen stored in the skeletal muscle (Malfatti et al., 2014). As a result, these observations suggested that GYG may not be required for glycogenesis.

GYS is regulated by the allosteric effect of G6P, and reversible phosphorylation of GYS leads to the inactivation of this enzyme. The presence of G6P overcomes phosphorylation, leading to restoring the activity of the GYS (Roach et al., 2012). In addition, an increased concentration of glycogen inhibited GYS activity (Adeva-Andany et al., 2016). In humans and mice, there are two forms of GYS, GYS1 and GYS2. GYS1 (encoded by *GYS1*) is most abundant in the heart and the skeletal muscle, whereas GYS2 (encoded by *GYS2*) is a liver isoform of GYS (Browner et al., 1989; Nuttall et al., 1994).

Three isoforms of PYG are found in the skeletal muscle (encoded by *PYGM*), the brain (encoded by *PYGB*), and the liver (encoded by *PYGL;* Burwinkel et al., 1998). The activity of PYG, similar to GYS, is regulated through allosteric effects. Reversible phosphorylation by protein phosphatase-1 (PP1) regulates the activation and deactivation of PYG. PP1 regulates glycogen concentration through the activation of GYS and the inhibition of PYG. An increase of

G6P and UDP-glucose inhibits the activity of PYG (Ercan-Fang et al., 2002). In the muscle, a higher concentration of glycogen also activates PYG (Munger et al., 1993).

In humans and mice, the *G6PC* gene includes three members, G6PC1, G6PC2, and G6PC3 (Marcolongo et al., 2013). They are expressed in different tissues. G6PC1 is found in the endoplasmic reticulum of the liver, the kidney, the pancreas as well as the intestinal mucosa (Schaftingen & Gerin, 2002). G6PC2 is only expressed in pancreatic β-cells, whereas G6PC3 is ubiquitously expressed. G6PC is activated when G6P is at a high concentration. The activated G6PC results in release of free glucose and inorganic phosphate (Bhagavan, 2002). In addition, glucagon, glucocorticoid, and fatty acids also up-regulate G6PC. Down-regulation of G6PC by insulin has also been reported (Hutton & O'Brien, 2009).

2.3 Characteristics of Uterus

2.3.1 Uterine Structure and Secretions

The uterus body is composed of three layers, the endometrium, the myometrium, and the perimetrium. The endometrium is a mucosa layer consisting of (1) a layer of columnar secretory cells made up of the luminal epithelial lining, (2) uterine glands lined with epithelial cells, and (3) endometrial stroma containing numerous fibroblasts, fine collagenous, and elastic fibers. The myometrium is a thick layer of smooth muscle and the perimetrium is made of connective tissues (Verma, 1983).

Histotroph is the accumulation of components and nutrients that are secreted into the uterine gland and the uterine lumen. It contains many substances such as glucose, enzymes, hormones, growth factors, cytokines, and amino acids (Bazer & Words, 1975). These secretions

play important roles in maintaining pregnancies. During the secretory phase, glandular epithelial cells are transformed into polarized secretory cells that secrete substances into the lumen of glands. Optimal levels of growth factors in histotroph are crucial for successful implantation. During the preimplantation period, the embryo is free-floating in the oviductal and uterine lumen and is dependent on the nutrients secreted into histotroph to sustain growth (Spencer et al., 2007). As a result, histotroph is considered an indispensable nutrient source during early pregnancy. This is supported by studies in uterine gland knockout (UGKO) ewes. UGKO ewes could not maintain a pregnancy to day 25, even when embryos from control ewes were transferred into the UKO ewes. This result suggests that uterine glands and their secretions are required for the survival and development of the peri-implantation embryo (Gray et al., 2001). In addition, the major phenotype of uterine-specific forkhead box protein (FOXO) 2 knockout mice was the absence of uterine glands. Implantation and decidualization were impaired in these mice due to loss of leukemia inhibitory factor (LIF) produced by the glandular epithelium, and injection of LIF partially rescued the decidual response in these mice (Jeong et al., 2010). Similarly, implantation was also rescued with the replacement of LIF in these mice (Kelleher et al., 2018). These results suggested that uterine gland secretions, such as LIF, are critical to embryo implantation and proper stromal cells decidualization. Furthermore, in humans as the embryo implants into the uterine wall, histotroph can be phagocytosed by the trophectoderm of the blastocyst and by trophoblast cells of the placenta later during early pregnancy (Burton et al., 2002).

2.3.2 Menstrual and Estrous Cycles

Menstruation refers to the periodic shedding of the outer layer of the endometrium when the embryo is absent. It is restricted to a limited number of mammals such as higher-order primates, including humans and some old-world monkeys. Interestingly, menstruation has also been observed in non-primate mammals such as the spiny mouse, some bat species, and the elephant shrew (Bellofiore et al., 2017; Hamlett, 1934; Horst, 1954; Rasweiler, 1991). The menstrual cycle can be divided into two phases, proliferative and secretory phases. In humans, the proliferative phase, which coincides with the ovarian follicular phase, starts after menstruation (day 5 to day 14). The endometrium increases its thickness from 1 mm to 5 mm and estradiol (E2) is the most influential steroid hormone during this phase. The secretory phase, coinciding with the luteal phase (day 15 to day 28), starts following ovulation at the end of the proliferative phase in the menstrual cycle. The endometrium increases about 3 mm to 8 mm at this stage and progesterone (P4) concentrations start to increase dramatically.

The progression of menstrual cycles depends on the hypothalamus-pituitary-ovarian axis. During the follicular phase, P4 is low due to the regression of the corpus luteum (CL), which leads to the activation of gonadotrophin-releasing hormone (GnRH). GnRH neurons release GnRH which stimulates gonadotroph cells in the anterior pituitary to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The action of LH and FSH on theca cells and granulosa cells of follicles respectively induces follicular growth and ovulation (Caligioni, 2009). LH stimulates the production of androgen in the theca cell, which subsequently diffuses through the membrane into the granulosa cells. As FSH binds to its receptor on the granulosa cells, aromatase converts androgen to E2, resulting in higher production of E2 from the developing follicles (Pohler et al., 2019). Under the effect of FSH, the pre-ovulatory follicles

develop and produce more E2. The high concentration of E2 results in the GnRH surge from the surge center of the hypothalamus. The GnRH surge induces the LH surge, which leads to ovulation followed by the secretory phase of the menstrual cycle. This stage is characterized by the collapse of the corpus hemorragicum and the formation of CL. The CL continue to produce P4, which has a negative feedback on the hypothalamus and anterior pituitary (Crowe, 2022). As the CL regresses, the inhibitory effect of P4 decreases due to lower P4 concentration, leading to the increase of GnRH secretion and the beginning of the next cycle.

The estrous cycle represents the cyclic manner of the ovarian and uterine activities. As reproductive receptivity proceeded in female animals, they either enter another cycle or establish pregnancy after mating (Crowe, 2022). Unlike the menstrual cycle where the endometrial wall is shed during each cycle, in the estrous cycle the endometrium does not undergo menstruation (Catalini & Fedder, 2020). The length of the estrous cycle varies between species. In mice, the estrous cycle repeats every 4-5 days, while in the cattle the cycle is 17-24 days (Byers et al., 2012; Pohler et al., 2019). The estrous cycle can be divided into four stages in rodents, proestrus, estrus, metestrus, and diestrus, which are controlled by a variety of hormones through both negative and positive feedback.

2.3.3 Endometrial Structural Changes and Glycogen Deposition during Reproductive Cycle

Some ultrastructural studies on human endometrial biopsies characterized structural changes and glycogen deposits in the glandular epithelium**.** During the early proliferative phase, clusters of glycogen granules were found at sub-nuclear locations on day 8 in glandular epithelial cells (Verma, 1983). From the proliferative phase to the secretory phase, one of the major changes in the glandular epithelial cells during the transition was the abundance in glycogen

deposition (Dockery et al., 1988, 1993; Verma, 1983). At the beginning of the secretory phase, glycogen was limited to the basal side and moved more towards the apical side as the phase progressed. Glycogen and glycoproteins started to accumulate on the second day after the LH surge and reached a peak on the third day. The volume of cytoplasm decreased due to the presence of sub-nuclear glycogen. In addition, glycogen was also seen enclosed in lysosomes (Verma, 1983). Once active secretion of glycogen occurred, large masses of glycogen were no longer found in the epithelial cells (Dockery et al., 1988).

During the early proliferative phase, the stromal cells had a narrow and elongated appearance. As the phase progresses toward the middle and end of the proliferative phase, the size of the cells increases and they have a more round appearance. During the secretory phase, the accumulation of extracellular fluid leads to dispersion of stromal cells. Glycogen deposition starts to increase at the beginning of the secretory phase. As the progression continues toward the end of the secretory phase, cells become hyperhydrated (Verma, 1983).

2.3.4 Endometrial Structural Changes and Glycogen Deposition during Early Pregnancy

In humans, the structures of luminal epithelial cells and glandular epithelial cells change during early pregnancy. In the luminal epithelial cells, the apical membrane, basal membrane, as well as lateral membrane became looser. Glycogen has occasionally been observed in the luminal epithelium. Accumulation of glycogen and large quantities of glycogen budding off from the apical into the lumen of the uterus were also detected during early pregnancy. The number of microvilli on the apical and lateral plasma membrane decreased compared to the early secretory phase of the menstrual cycle in humans (Demir et al., 2002). Similar observations have also been found in the murine and rat uterus. In the first two days of pregnancy, the long, thin and regular

microvilli observed during the estrous phase were replaced by shorter and irregular microvilli or protrusions (Murphy, 1993). The apical microvilli disappeared around day 5 to day 6 of pregnancy, when implantation is occurring (Murphy, 1993; Tachi et al., 1970).

In the glandular epithelial cells, the number of free or attached ribosomes increased. The increased ribosomes can be distributed into polysomes, which play an important role in synthesizing proteins. Glycogen-rich cells and glycogen-poor cells have been found in the glandular epithelium. In the glycogen-rich cells, glycogen formation is advanced during early pregnancy and the apical side of the cells was filled with glycogen. Glycogen secretion was seen from the apical region to the lumen of glands, where large lipid droplets and glandular mucus were also present (Demir et al., 2002). These observations were similar to the findings that the uterine glands during the first six weeks of pregnancy resembled the luteal phase of the menstrual cycle and glycogen particles were observed in the glandular secretion under electron micrograph (Burton et al., 2002). Moreover, microvilli were abundant on the surface adjacent to the lateral side near the lumen of glands, but the number of microvilli decreased as days of pregnancy proceeded (Demir et al., 2002).

Not only does the epithelium have a different appearance during early pregnancy, but the stroma also undergo significant changes. In early implantation, the stromal cells are loosely structured with a high proportion of extracellular spaces between cells (Potts, 1968). Around day 4 just before implantation in mice, the stromal cells beneath the luminal epithelium enlarge and establish a larger contact area with the neighbouring cells. In rodents, as the blastocyst initiates the first contact with the uterine epithelium, the fibroblasts become transformed into decidual cells. The decidual cells contained abundant cytoplasm and some tight junctions are found between these cells (Finn & Lawn, 1967). The decidual cells are more cuboidal in shape and rich

in glycogen, polyribosomes and lipids (Enders & Schlafke, 1967). The formation of the decidua is a significant and important change in the stroma in the human and rodents uterus during early pregnancy.

2.4 Preimplantation and Implantation Development

2.4.1 Embryonic Development during Early Pregnancy

Preimplantation embryonic development involves the differentiation of the totipotent fertilized egg to the blastocyst containing the multipotent trophectoderm and the pluripotent inner cell mass (Wang & Dey, 2006). Embryonic development commences with a transition of oocytes to embryos, whereby the molecular program of oocytes is degraded while those of embryos are activated. Following fertilization in the oviduct, the embryo undergoes a series of mitotic cell divisions into 2-cell, 4-cell, and 8-cell stages (Gardner et al., 2000; Figure 5). Around day 4, human embryos are compact around the morula stage, where the inner blastomeres are surrounded by the zona pellucida composed of glycoproteins. Then the blastocyst, which is a fluid-filled structure containing the inner cell mass and trophectoderm, forms on day 5. The inner cell mass gives rise to the fetus, whereas the trophoblasts are differentiated into the extraembryonic structures such as the placenta (Norwitz et al., 2001). Next, the blastocyst hatches from the zona pellucida and implants into the endometrial uterine wall around day 7 (Niakan et al., 2012). By day 10, the embryo is completely buried within the endometrial lining. Since the placenta has not yet developed a vasculature, the nutrition sources for the embryo is through the histotroph (Ng et al., 2020). The secretion of histototroph is active until at least 10

weeks of pregnancy in human when the maternal circulation to the placenta is fully established (Burton et al., 2002).

In mice, the timeline of preimplantation embryonic development is somewhat different from that in humans. The cleavage to the 2-cell stage of the murine embryo happens on 1.5 days post-coitum (DPC), 4-cell stage on DPC 2.5 and 8-cell stage late on DPC 2.5 (Moore et al., 2019). On DPC 3.5, the embryo is developing towards the blastocyst stage (Wang & Dey, 2016). Early on day 4 of pregnancy, the embryo enters the uterine horn, and the shedding of the zona pellucida occurs late on that same day. Implantation of the blastocyst is initiated on the night of day 4 (Yoshinaga, 2013). On DPC 5.5, the blastocyst is implanted in the decidua (Figure 5).

Protein synthesis, linked to transcriptional activation, was observed around the 4-cell to the 8-cell stage in human embryos, suggesting that human zygotic genome activation starts around those stages (Braude et al., 1988). In mice, 90% of the maternal transcripts are completely degraded by the 2-cell stage (Hamatani et al., 2004). The first major wave of murine zygotic genome activation occurs around the 2-cell stage to the 4-cell stage and the second major wave of genetic activation peaks around the 8-cell stage. The third wave peaks at the morula stage followed by the fourth wave, which peaks at the blastocyst stage. Protein synthesis is apparent around day 2 in mice (Blerkom & Brockway, 1975). The different waves of gene activation may be used for specific classes of proteins at a specific time to support embryonic development. In addition, some early activated genes complete their function at early stages, and their expressions later may be harmful to the embryonic develop. This suggests that gene activation and inactivation at a specific time are critical to early embryo development (Hamatani et al., 2004). The embryo switches from pyruvate-dependence to glucose-dependence during the

preimplantation period is an example showing proper gene activation or inactivation at specific stages of embryonic development is important to maintain embryo survival.

2.4.2 Development of the Decidua in the Uterus

Proper decidualization is critical to pregnancy success and many pregnancy complications are associated with impaired decidualization. Human endometrial stromal cells (HESC) isolated from preeclampsia patients failed to decidualize *in vitro* (Garrido-Gomez et al., 2017). Recurrent pregnancy loss patients also exhibit impaired decidualization (Salker et al., 2010). In addition, decidualization in repeated implantation failure patients is poorly developed, including decreased decidual prolactin secretion and impaired transformation (Huang et al., 2017).

The depth of decidualization varies among different species, depending on the invasiveness of placental trophoblasts. In rodents, the initiation of the decidualization process is triggered by implantation. Decidualization can also be modeled in rodents through stimulation in the E2 and P4-primed uterus or mating with a vasectomized male following by physical stimulation of the uterine lumen. In contrast, humans are one of a few mammalian species that undergo spontaneous decidualization in the secretory phase (Tong et al., 2022). Interestingly, decidualization occurring without the presence of an embryo is also observed in some bats, higher primates and elephant shrews (Gellersen & Brosens, 2014). Mammals that exhibit decidualization without the presence of embryos share several reproductive similarities. These similarities include spontaneous ovulation, development of the hemochorial placenta, and fewer altricial offspring (Brosens et al., 2009; Emera et al., 2012).

During decidualization, the endometrium undergoes a biological and physiological transformation. The decidua, a temporary but critical structure in the uterus, is composed of differentiated endometrial stromal cells and maternal vascular cells (Mori et al., 2016). The undifferentiated stromal cells are fibroblast-like, but the appearance becomes large and round when decidualized. The decidual cells surrounding the embryo proliferate and terminally differentiate, and become multinuclear (Ansell et al., 1974). Glycogen and lipids are abundant in the decidual cells. Moreover, unique functional cell markers such as prolactin (PRL), insulin growth factor binding protein-1 (IGFBP-1), FOXO1, and Interleukin 6 family (IL6) are produced by decidual cells (Mori et al., 2016; Tong et al., 2022). Down-regulation of snail and vimentin (mesenchymal markers) and up-regulation of E-cadherin and cytokeratin (epithelial markers) was also observed in decidualized murine and human stromal cells (Zhang et al., 2013). Homeobox (Hox) genes are also important for maintaining the proper function of the uterus and establishing a decidua. Female *Hoxa10* mutant mice, generated via homologous recombination in embryonic stem cells, had a decreased decidual response to artificial decidualization stimulus compared to wild-type mice. Moreover, the transfer of wild-type murine embryos to the *Hoxa10* mutant murine uterus failed to rescue embryo survival, suggesting Hoxa-10 is important for the proper morphogenesis of the uterus (Benson et al., 1996).

Among the decidual markers, PRL and IGFBP-1 are widely used to monitor the decidualization process of HESC (Tong et al., 2022). In humans, the PRL concentration in the amniotic fluid produced by decidua can exceed circulating PRL concentration by up to 100-fold (Golander et al., 1979; Riddick et al., 1978). In rats, decidual PRL inhibits 20α-hydroxysteroid dehydrogenase (20α-HSD), which converts P4 to its inactive form, in the CL. Suppression of 20α -HSD expression is important since P4 is crucial to maintaining pregnancy. Decidual PRL

also represses the cytokine IL-6, which can trigger an inflammation response and may negatively affect the pregnancy (Bao et al., 2007).

The decidua contains a handful of immune cells such as natural killer (NK) cells and macrophages. These cells maintain a tolerant environment at the maternal-fetal interface and thus are crucial to embryo survival. The uterine decidual NK cells constitute more than half of the decidual lymphocytes (Koopman et al., 2003). These cells are important in maintaining the decidualization process as uterine NK cell deficient mice exhibit abnormal differentiation of fibroblasts compared to wild-type mice (Croy et al., 2002). Decidual macrophages play roles in spiral artery remodeling and secrete angiogenic growth factors (Lash et al., 2016). In mice and humans, angiogenesis and arterial remodeling were observed during decidualization, which suggested that maternal nutrition supplied through the blood vessels was important for early implantation (Mori et al., 2016).

In mice, differentiation of the primary decidual zone starts from the antimesometrial region and moves forward to the mesometrial region (Figure 5). Unlike humans that directly develop a chorioallantoic placenta, mice initially develop a choriovitelline placenta around days 6 to 11 and later develop into the chorioallantoic placenta. The antimesometrial decidua plays an important role in supporting the development of the choriovitelline placenta, whereas the decidualization of the mesometrial region contributes to the formation of the chorioallantoic placenta (Favaro et al., 2014). The avascular characterization of the primary decidual zone (PDZ) suggests that the PDZ may serve as a temporary permeable barrier between the maternal side and the developing embryo (Herington et al., 2009). It is also considered to be the first protective layer for the embryo and this layer may protect the embryo from infections by microorganisms or maternal immune system recognition (Parr & Parr, 1986). As the primary decidua extends and

decidualization continues to the secondary decidua up to the myometrium, dramatic remodelling of the decidua occurs leading to physical and humoral changes in the immune system (Mori et al., 2016). Polyploidy of the decidual cells was observed in the secondary decidual zone, and it is speculated that the limited lifetime of polyploidy cells may allow for the growth of the implanting embryo (Sroga et al., 2012).

2.4.3 Implantation

The implantation process varies across species, but the main purpose of implantation is to facilitate interactions between the trophoblast and the endometrial stroma. Implantation can be divided into three stages. The apposition stage is the start of implantation when trophoblastic cells become apposed to the endometrial epithelium. The adhesion stage, either subsequently or simultaneously occurring with the apposition stage, starts when the trophoblasts sufficiently interact with the endometrium. Separation of the blastocyst from the endometrium at the adhesion stage may cause damage to the uterus (Wang & Dey, 2006). In mammals with non-superficial implantation, the trophoblasts will then penetrate the uterine epithelium and reach the stroma.

The mechanisms of trophoblasts penetration appear different among species. In guinea pigs, the trophoblasts become syncytial and form an implantation cone intruding through the luminal epithelium (Enders & Schlafke, 1969). In mice and rats, the attachment of the blastocyst to the uterine luminal epithelium leads to apoptosis of the epithelial cells, resulting in penetration of trophoblasts through the epithelium into the uterine stroma (Wang & Dey, 2006). Closure of the uterine lumen was observed after the blastocyst hatched from the zona pellucida in rodents (Yoshinaga, 2013; Figure 5). In humans, shortly after hatching of the blastocyst from the zona

pellucida, trophoblast invasion begins between day 7 to day 10 of pregnancy and the blastocyst is completely invaded under the endometrial lining. To achieve invasion, first the microvilli on syncytiotrophoblast interact with pinopodes, microprotrusions that extend from the luminal epithelium. Then, during stable adhesion, the physical interaction between the blastocyst and the uterine epithelium increases, resulting in the beginning of invasion. In this stage, syncytiotrophoblast penetrates the epithelium (Norwitz et al., 2001).

Before the development of the placenta, the blastocyst is dependent on the secretions from histotroph under hypoxic and hypoglycemic conditions as high levels of oxygen and glucose are detrimental to embryonic development. At 8-10 weeks, placental extravillous cytotrophoblast cells invade decidua and myometrium in humans (Harris et al., 2019).

The extent of placental trophoblastic cell invasion is also different among species, ranging from no invasion to highly invasive. The intact-remaining uterine epithelium is the feature of epitheliochorial placentation and there is an obvious barrier between the uterus and the placenta. Strepsirrhine primates, some hoofed animals such as swine, cows, deer, and sheep share the epitheliochorial placenta (Carter & Enders, 2013). A high invasion of the placental trophoblast results in the hemochorial placenta, which penetrates through the uterine blood vessel and reaches the maternal circulation. The uterine tissue transforms into decidua, which does not occur in epitheliochorial placentation. Higher primates, lab animals such as rabbits, rats, and mice have the a hemochorial placenta (Moffett & Loke, 2006).

2.5 Carbohydrate Metabolism in Female Reproductive Tract

2.5.1 Carbohydrate in the Uterus during the Reproductive Cycle

Concentrations of carbohydrates such as glucose, pyruvate and lactate are different along the female reproductive tract and during the reproductive cycle. This leads to a changing environment for the developing embryo. In humans, pyruvate remained constant throughout the cycle in the oviduct, whereas lactate increased from the follicular phase to midcycle. In the uterine luminal fluid, pyruvate and lactate remained constant, but their concentrations were much lower than the concentrations found in the oviduct at midcycle (Gardner et al., 1996). The higher pyruvate and lactate in the oviduct during midcycle correlated with the indispensable need for these nutrients in the early embryonic development, when pyruvate and lactate are the main energy sources (Brinster, 1969; Leese & Barton, 1984; Martin & Leese, 1995). However, Harris et al. (2015) found that lactate concentration was similar throughout the reproductive tract in mice. The discrepancy in results may because of a species difference. Moreover, the study of mice was after superovulation whereas the study of humans was during natural cycles.

In contrast to pyruvate and lactate, glucose concentration in the oviduct was lowest around the time of ovulation (0.5 mM). The glucose concentration in the oviduct then increased during the luteal phase. The low glucose concentration in the oviduct around mid-cycle showed that the early preimplantation embryos were exposed to a low concentration of glucose (Gardner et al., 1996). It also suggested that low glucose level was required for early embryonic growth. In support of that, several *in vitro* studies in different mammalian species showed that glucose in the medium is responsible for the developmental arrest of the cleavage embryo (Schini & Bavister, 1988; Chatot et al., 1989; Thompson et al., 1992; Conaghan et al., 1993; Gardner et al.,

1993; Quinn, 1995; Fraser et al., 2007). Conversely to the glucose concentration in the oviduct, the concentration of glucose in the uterine fluid remains constant during the midcycle (3.15 mM). The higher glucose concentration in the uterine fluid matched the significant increase in glucose requirement during the morula and blastocyst stages. In addition, it is worth noting that the presence or absence of cumulus cells can affect glucose concentration in the oviduct. In mice, the presence of cumulus cells decreased glucose concentration in the oviduct. It is possible that the glucose was utilized by cumulus cells to form pyruvate, a preferred energy source in the early developing embryo (Gardner & Leese, 1990; Harris et al., 2005).

Comparing the glucose concentrations between the serum and the reproductive tract, many species appeared to have a higher glucose concentration in serum than in the reproductive tract. In mice, the glucose concentration in the oviduct is reported at 1-5 mM and 3.15 mM in the uterine luminal fluid (Gardner & Leese, 1990; Harris et al., 2005). These concentrations are lower than the reported glucose concentration in the blood, which ranges from 3.5 to 9.7 mM. Similarly in humans, the glucose concentration ranges from 0.5-3.1 mM in the reproductive tract, whereas in the plasma the glucose concentration is around 5 mM (Harris et al., 2005). In cattle, glucose concentration is around 2-4 mM in the oviduct fluid and uterine fluid, lower than 6-7 mM found in the plasma (Hugentobler et al., 2008). These differences between glucose concentrations in the serum and reproductive tract suggested that the high glucose concentration may not be suitable for embryonic growth. In support of that, glucose concentration at or higher than 5.56 mM impaired blastocyst hatching and development (Fraser et al., 2007).

2.5.2 Carbohydrate Metabolism in the Decidua of Early Pregnancy

At the implantation site, fibroblasts differentiate into decidual cells. Glucose metabolism through PPP is increased during decidualization and inhibition of glucose metabolism through PPP is detrimental to decidualization (Frolova et al., 2011; Tsai et al., 2013). In addition, increased glucose uptake and GLUT1 were observed both in the human and mouse decidualized endometrial stromal cells (Frolova et al., 2009). The increase of glucose flux during decidualization may be explained by the adaption of decidual cells to Warburg-like glycolysis (Zuo et al., 2015). In support of that, pyruvate kinase M2 (PKM2), which is a rate-limiting enzyme in glycolysis found strongly expressed in the decidua after implantation in mice. mRNA and protein levels of PKM2 increased significantly during the decidualization process both *in vivo* and *in vitro* in mice. The increase of PKM2 during decidualization suggested that Warburg metabolism was increased in the decidua (Su et al., 2020).

2.5.3 Carbohydrates Usage of Embryos during Early Pregnancy

Glucose uptake of human and mice embryos remained low during preimplantation development until the blastocyst stage (Brinster, 1969; Leese & Barton, 1984; Brown & Whittingham, 1991; Conaghan et al., 1993; Martin & Leese, 1995; Gardner et al., 1996). Excess glucose present in the medium retarded early embryonic development in many species. *In vitro* growth of human embryos showed that as glucose concentrations (0 mM, 0.5 mM, 1 mM) increased in the medium, the number of embryos developed to the blastocyst stage decreased. Embryos in glucose-free medium resulted in significantly more trophectoderm cells than in those grown in the medium containing 1 mM glucose (Conaghan et al., 1993). In mice embryos, the removal of glucose in the medium significantly improved the 1-cell stage to the 4-cell stage

differentiation (Chatot et al., 1989). In hamsters, 2-cell stage embryos can grow to the 4-cell stage in glucose-free medium, suggesting glucose was not required during early cleavage (Schini & Bavister, 1988). Furthermore, in sheep, the higher concentrations of glucose (>3 mM) without the addition of pyruvate and lactate resulted in inhibition of embryonic development to morula stage (Thompson et al., 1992).

Glucose was not preferred during early cleavage, but pyruvate and lactate were essential energy sources until the morula and blastocyst stages. Mouse embryos were able to develop from the 1-cell stage to the morula stage with only pyruvate and lactate as exogenous energy sources, suggesting pyruvate and lactate were the predominant energy sources before the blastocyst stage (Brown & Whittingham, 1991). In support of that, the presence of pyruvate alone in the medium was able to support human embryonic development until the blastocyst stage, and the absence of pyruvate lead to developmental arrest and a decrease in metabolic activities (Conaghan et al., 1993). Not only pyruvate, but lactate alone is also able to support first and second cleavages in mice embryos (Brown & Whittingham, 1991). In addition, when culturing mouse embryos in medium containing glucose, pyruvate uptake decreased from the morula stage to the blastocyst stage. Whereas culturing embryos in glucose-free medium, pyruvate uptake increased (Martin & Leese, 1995). This study showed a switch from pyruvate preference to glucose preference around the blastocyst stage. However, proper concentrations and interactions between these components can enhance embryo survival. With the presence of pyruvate and lactate, glucose can be beneficial to embryonic development from the 1-cell and the 2-cell stage to the blastocyst stage in the range of 0-3 mM (Thompson et al., 1992).

As embryos develop to the morula and blastocyst stage, glucose starts to become an important energy source. In human embryos, glucose consumption on day 4 and day 5 was

significantly higher in those embryos that resulted in pregnancy than those that failed to develop post-transfer to the uterus. The overall mean consumption of glucose from day 3 to day 5 was around 130 pmol/embryo/h for the embryos that successfully generated pregnancies. In contrast, glucose consumption was only 72 pmol/embryo/h for embryos that did not result in pregnancies. The higher glucose consumption in embryos established pregnancies showed that glucose is related to the success of pregnancies (Gardner et al., 2011). In addition, in the sheep embryos, glucose uptake was constant around 15 pmol/embryo/h during the 8-cell stage to the 16-cell stage, whereas in the blastocyst stage glucose uptake increased to 54 pmol/embryo/h (Gardner et al., 1993). This observation indicated that glucose was more important in the blastocyst stage than during cleavage development.

Glycogen was also found stored in the developing embryo. In mice, glycogen increased from 0.14 ng glycogen/embryo at the 1-cell stage to a peak of 2.24 ng glycogen/embryo at the 8-cell stage. At the blastocyst stage, glycogen accumulation was lower than the 2-cell, the 8-cell and the morula stage (Stern & Biggers, 1968). The accumulation of glycogen found in the early developing embryo was supported by the increasing glycogenesis and limited glycogenolysis during the early preimplantation period (Hsieh et al., 1979). The stored glycogen in the embryo may be utilized to control glucose concentration, preventing the embryo from being exposed to high concentrations of glucose. In addition, converting glucose to glycogen can drive passive glucose transport from the maternal side to the preimplantation embryo. In support of glucose transport in early developing embryos, the preimplantation embryos expressed GLUT1 in all stages, GLUT2 until the 8-cell stage, and first appeared GLUT3 in the morula stage (Purcell & Moley, 2009). The increased GLUTs in the later development of the preimplantation embryo suggests more glucose is uptake by the embryo. As more glucose is incorporated into the

embryo, more glycogen can be stored within the embryo. In agreement, an increase of glycogen accumulation was observed from the 1-cell stage to the morula stage (Pike & Wales, 1982). Since glucose is the main energy source for the blastocyst, it is possible that the stored glycogen is broken down and provides glucose for the development of embryos at later stages.

2.6 Uterine Glycogen Metabolism

2.6.1 Glucose can be Stored as Glycogen in the Uterus

Fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase were not detected in neither endometrium nor myometrium, showing that gluconeogenesis was unlikely to occur in the uterus (Yánez et al., 2003). However, expressions of GLUTs and SGLTs were found in the uterus, suggesting that glucose consumed by the uterus may come from the circulation (Frolova & Moley, 2011; Zhang et al., 2021). In addition, glucose can be stored as macromolecule glycogen, which may be utilized to maintain optimal glucose concentration for embryonic development (Dean, 2019).

2.6.2 Endometrial Glycogen Metabolism during the Reproductive Cycle

Glycogen was found in the endometrium of the uterus. An ultrastructural study in human showed that glycogen-bearing cells were detected in the endometrium during the entire estrous cycle. These cells were associated with subluminal stroma and glandular epithelium. At estrus, the number of glycogen-bearing cells increased, especially the glandular epithelial cells. At metestrus, glycogen in these cells decreased (Rosenbaum & Goolsby, 1957). Demir (2002) also

reported that glycogen particles were occasionally observed in the luminal epithelial cells, and some glandular epithelial cells were rich in glycogen.

Endometrial glycogen fluctuates in primates during the menstrual cycle. Endometrial biopsies of *Macaca arctoides* showed that glycogen stored during the secretory phase was 3-fold higher than that in the follicular phase. Glycogen deposition was low during the follicular phase, reached a peak around day 16 of the menstrual cycle and then decreased. In addition, the activities of GYS and PYG elevated during the follicular phase and reached a peak around day 16. The fluctuation of glycogen metabolizing enzymes was similar to the pattern of glycogen stored in the endometrium, suggesting that these enzymes may be responsible for the glycogen changes. The fluctuation of glycogen was also similar to the pattern of serum P4 concentration, which showed that P4 may be important for the regulation of glycogen deposition during the reproductive cycle (Demers et al., 1973). In humans, endometrial glycogen was highest around day 16 to day 23 of the cycle and was 5-to 10-fold higher than that in the proliferative phase (Maeyama et al., 1977). The activity of GYS increased gradually from the proliferative phase to the secretory phase and reached a peak around day 16 to day 23 of the cycle, which coincident with the higher glycogen deposition in the uterus (Hughes et al., 1969; Matsuo, 1978). Similar to GYS, the activity of PYG also increased from the proliferative phase to the secretory phase and the peak activity occurred between day 20 to day 25 of the cycle (Hughes et al., 1969).

Glycogen content also changes in a cyclic manner in the estrous cycle. In rats, glycogen was high during proestrus and estrus and decreased significantly in diestrus (Boettiger, 1946; Greenstreet & Fotherby, 1973a). The decreased glycogen content from proestrus and estrus to diestrus correlated with the activities of glycogen metabolizing enzymes. GYS activity was dramatically higher in rats at proestrus than diestrus (Rubulis et al., 1965). Activities of HK and

G6PC exhibited an inverse relationship. At estrus, HK showed the highest activity while G6PC activity was at the lowest. At diestrus, the increase of G6PC activity was associated with higher glucose in the uterus (Greenstreet & Fotherby, 1973a). In cattle, endometrial glycogen content was higher on day 1 than on day 11 of the estrous cycle. The decrease of glycogen from estrus to diestrus was mainly in the glandular epithelium and luminal epithelium whereas stroma stored little glycogen during the cycle. PYG was found higher on day 11, which was associated with lower glycogen deposit during the diestrus stage (Sandoval et al., 2021).

2.6.3 Uterine Glycogen Metabolism during Early Pregnancy

Endometrial glycogen may play a role in maintaining pregnancy. In support of that, the storage of glycogen and adequate glycogen turnover in the endometrium is associated with fertility in humans. Infertile patients exhibited significantly lower endometrial glycogen compared to the fertile group (Hughes et al., 1969; Maeyama et al., 1977). In addition, the activities of glycogen metabolizing enzymes, GYS and PYG, were statistically lower in the endometrium of infertile patients (Hughes et al., 1969). These observations showed that proper glycogen deposit is essential for successful pregnancies.

In humans, large quantities of glycogen were observed in the cytoplasm of most epithelial cells during early pregnancy. Glycogen secretions from the glandular epithelium and luminal epithelium were also reported (Burton et al., 2002; Demir et al., 2002). These studies suggested that the secretions of glycogen from both glandular epithelium and luminal epithelium may be important energy sources for the development of embryos during early pregnancy. In addition, glycogen catabolism releases glucose, which is the major nutrient for blastocyst and further embryonic development. As a result, breaking down stored glycogen may be essential to provide

glucose for the developing embryo during early pregnancy. In support of that, PYG was found in the apical surface of the glandular epithelium during early pregnancy (Jones et al., 2015).

In rats, glycogen content decreased constantly from day 1 to day 6 of pregnancy. A slight increase was observed on day 4, which correlated with the elevation of active forms of PYG and GYS around the implantation period (Demers et al., 1972). Greenstreet & Fotherby (1973b) found a similar pattern, namely that glycogen was highest on day 1, decreased on days 2 and 3, and gradually increased from day 4 until day 7. They also reported that G6PC was highest on day 3 to day 5 of pregnancy. G6PC can be used to liberate glucose from G6P, and the elevation of G6PC may associate with decreased glycogen during early pregnancy. The increase of glycogen around day 4 may be due to the development of decidua as glycogen accumulated in the decidual cells.

The decidua is rich in glycogen and proper development of decidua is critical to support successful implantation. Glycogen content increased dramatically in the rat uterine horn with developed decidua compared to undecidualized uterine horn (Cecil et al., 1962). Glycogen metabolizing enzymes were also reported in the decidual cells. PYG activity was detected in the decidual cells even though was not extensive (Bo et al., 1964). A similar result was found by Matsuo (1978) that the activity of PYG in the decidua was lower than the activity in the uterus during the secretory phase. In addition, moderate staining for G6PC was observed in the primary decidua of rats (Christie, 1966). These results indicated that the decidua could break down glycogen and yield free glucose through dephosphorylating G6P.

2.7 Effect of Steroid Hormones on the Endometrium

2.7.1 Concentrations of Estradiol and Progesterone

The endometrium is highly responsive to steroid hormones. The concentrations of E2 and P4 fluctuate throughout the menstrual and estrous cycles**.** In humans, the endometrial E2 concentration increased from the proliferative phase to the secretory phase, while endometrial P4 concentration was high during the secretory phase and low in the proliferative phase (Guerrero et al., 1975). Interestingly, the endometrial steroid hormone concentrations did not reflect plasma E2 and P4 concentrations. The endometrial E2 concentration was significantly higher than the circulating E2 concentration during the proliferative phase (Guerrero et al., 1975; Huhtinen et al., 2012). The endometrial P4 concentration was higher in both the proliferative phase and secretory phase compared to the serum P4 concentration. Moreover, the ratio of endometrial P4 concentration to serum endometrium concentration was significantly higher in the proliferative phase than in the secretory phase, suggesting P4 secreted during the secretory phase is retained by the endometrium to a limited extent (Guerrero et al., 1975).

In rats, the plasma E2 peaked at proestrus while P4 had two peaks during the estrous cycle. A larger peak was found around the time of proestrus. The other peak was during metestrus when P4 was secreted from the CL (Butcher et al., 1974). In mice, plasma P4 showed a similar pattern as in the rats in that a large peak was around proestrus and a smaller peak was at metestrus (Kosaka et al., 1988; Michael, 1976). The pre-ovulatory E2 surge stimulates the proliferation of the luminal and glandular epithelial cells, whereas P4 secreted from the newly formed CL enhances stromal cells proliferation. However, the duration of P4 treatment influences the proliferation of different cell types. In mice, one-day treatment of P4 decreased

E2-induced epithelium proliferation but did not greatly increase stroma proliferation. In contrast, a three-day treatment of P4 caused a maximal proliferation response of the stroma (Martin & Finn, 1968). These results suggested that the proper interaction of E2 and P4 is important to maintain the function of the uterus.

2.7.2 Effect of Estradiol and Progesterone on Fertility

Under influence of E2 and P4, the endometrium undergoes marked changes to prepare for embryo implantation and decidualization (Fox et al., 2016). The coordination of proper E2 and P4 is critical to establishing and maintaining the window of implantation (Liang et al., 2018). Lower doses of E2 (3 ng) extended the time of P4-primed uterine receptivity while higher doses of E2 (10 and 25 ng) decreased the duration time of the window of implantation in mice (Ma et al., 2003). Moreover, excess P4 was harmful to mice implantation and can impair *in vitro* and *in vivo* decidualization. HESC decidualization process was also compromised under excess P4 treatment. These results suggested that an optimal P4 level was necessary for establishing pregnancies. In addition, excess P4 also inhibited LIF expression (Liang et al., 2018). LIF is strongly expressed in the glandular epithelium. Deletion of LIF resulted in implantation failure in mice, indicating LIF is required for implantation (Jeong et al., 2010; Stewart et al., 1992). As a result, proper P4 concentrations are critical at the time of implantation since excess P4 impaired the LIF pathway.

The establishment of steroid hormone receptors in knockout mice also provided insights into the necessities of E2 and P4 and their downstream signalling pathways for maintaining pregnancies. Estrogen receptors (ERs) exhibit two isoforms, ERα and ERβ. ERα-knockout mice resulted in hypoplasia of the uterus and were infertile due to failure in the blastocyst attachment

preparation (Lubahn et al., 1993). Interestingly, P4 alone was sufficient to stimulate decidualization artificially in the ERα-knockout mice, which indicated that ERα was not critical to the decidualization process (Paria et al., 1999). This result further supported that ERα was important in blastocyst attachment preparation. Furthermore, ERβ-knockout mice were fertile, suggesting that E2 signaling during implantation was primarily through the ERα isoform. However, the ERβ-knockout mice exhibited poor reproductive performance and enhanced proliferation response to E2 (Weihua et al., 2000). This suggested that ERβ had a suppressive role on ERα and the coordination between ERα and ERβ was critical during pregnancy.

Progesterone receptors (PRs) also have two isoforms, PR-A and PR-B. Administration of PR antagonists lead to failure of decidualization in mice, which showed that PR was crucial to the decidualization process (Parandoosh et al., 1995). In addition, a null mutation of the PR gene mice exhibited reproductive defects, including hyperplasia and inflammation in the uterus, indicating PRs were also important to maintaining pregnancies (Lydon et al., 1995). A subsequent study on PR-A knockout mice showed that these mice were infertile and decidualization was impaired. This indicated that infertility was associated with defective at the implantation site (Mulac-Jericevic et al., 2000). In contrast, PR-B knockout mice were fertile (Mulac-Jericevic et al., 2003). As a result, PR-A, not PR-B, was required to establish pregnancies in mice. Interestingly, administration of P4 to PR-A knockout mice led to the proliferation of epithelial cells, which suggested that PR-B mediated proliferation whereas PR-A played a role in repressing the epithelial cells (Mulac-Jericevic et al., 2000). In humans, however, both PR-A and PR-B were important for establishing pregnancy, while PR-B played a predominant role in the decidualization process. (Kaya et al., 2015).

The expression of PR exhibits spatiotemporal differences in the epithelium and the stroma in the endometrium. In the epithelium, PR mRNA expression and protein level increased significantly during day 3 and day 4 of pregnancy but was undetectable at the time of implantation in wild-type mice (Tan et al., 1999). Loss of PR in the epithelium during implantation was also detected in humans. These observations suggested that reduction of PR in the epithelium was indispensable for the embryo implantation. In support of that, forced expression of PR in the uterine epithelium disrupted embryo implantation (Li et al., 2021). In contrast to decreased expression in the epithelium, PR increased significantly in the stroma at the time of implantation (Tan et al., 1999).

2.7.3 Effect of Estradiol and Progesterone on Endometrial Glycogen Content

Higher glycogen accumulation was found in the intact mice compared with ovariectomized mice, suggesting steroid hormones played important roles in glycogen metabolism (Cecil et al., 1962). E2 has a glycogenic effect in the rodent uterus. Glycogen content increased 2.6-fold when treating ovariectomized rats with 25 μg /kg E2 for 3 days (Gregoire et al., 1967). In addition, one single injection of E2 to the ovariectomized rats also increased glycogen deposit for 2 days (Boettiger, 1946). Similarly, E2 treatment (5 μg) given to ovariectomized rats induced a significant increase in glycogen content and GYS activity (Demers & Jacobs, 1973a). These findings indicated that E2 can induce glycogen deposit in the uterus of rodents. The glycogenic effect of E2 in the uterus was also observed in other species such as rabbits, guinea pigs, and mink (Bowman & Rose, 2016; Demers & Jacobs, 1973b).

P4 also affects glycogen content in various species. In monkeys and humans, glycogen content increased during the secretory phase, suggesting that P4 was the main drive for glycogenesis in the uterus (Demers et al., 1973; Gupta et al., 2013). *In vitro*, P4 increased glycogen content in the human endometrial cells collected from the mid-secretory phase, which was due to the increase of GYS activity concomitant with a decrease of PYG activity (Ishihara et al., 1988). However, epithelial cells and stromal cells were not separated in the study and it was difficult to differentiate the P4 effect on the epithelial cells and stromal cells. To distinguish the P4 effect on different cell types, human endometrial epithelial cells treated with medroxyprogesterone (MPA) did not alter glycogen content, yet insulin increased glycogen content (Flannery et al., 2018). Thus, the constant glycogen content in epithelial cells under the effect of progestin suggested that P4 has a more glycogenic effect on the endometrial stromal cells than epithelial cells. In support of P4 increased glycogen content in the stromal cells, human endometrial stromal cells were able to decidualize under MPA and cyclic adenosine monophosphate (cAMP) treatment, suggesting P4 played an important role in differentiating stromal cells to glycogen-rich decidual cells (Teklenburg et al., 2010). Since glycogen is stored in spontaneously decidualized cells in primates during the secretory phase, the overall glycogenesis effect of P4 on the uterus may be due to the development of decidua.

In contrast to humans, most mammals animals do not spontaneously form decidua during the estrous cycle. Therefore, P4 has a glycogenolytic effect on those animals in the cycling uterus. In mice, administrations of 1 mg progesterone for 3 days tended to decrease glycogen content in the uterus (Gregoire et al., 1967). A combination of E2 and P4 treatment showed a decrease in glycogen deposit, suggesting P4 antagonized the glycogenic effect of E2 in mice (Carrington & Bailey, 1985). In cattle, endometrial glycogen decreased from estrus to P4-

dominated diestrus (Sandoval et al., 2021). In mink, glycogen content was lower in E2 treatment following P4 treatment compared to E2 treatment alone, suggesting P4 may be responsible for breaking down the glycogen (Bowman & Rose, 2016).

2.8 Figures

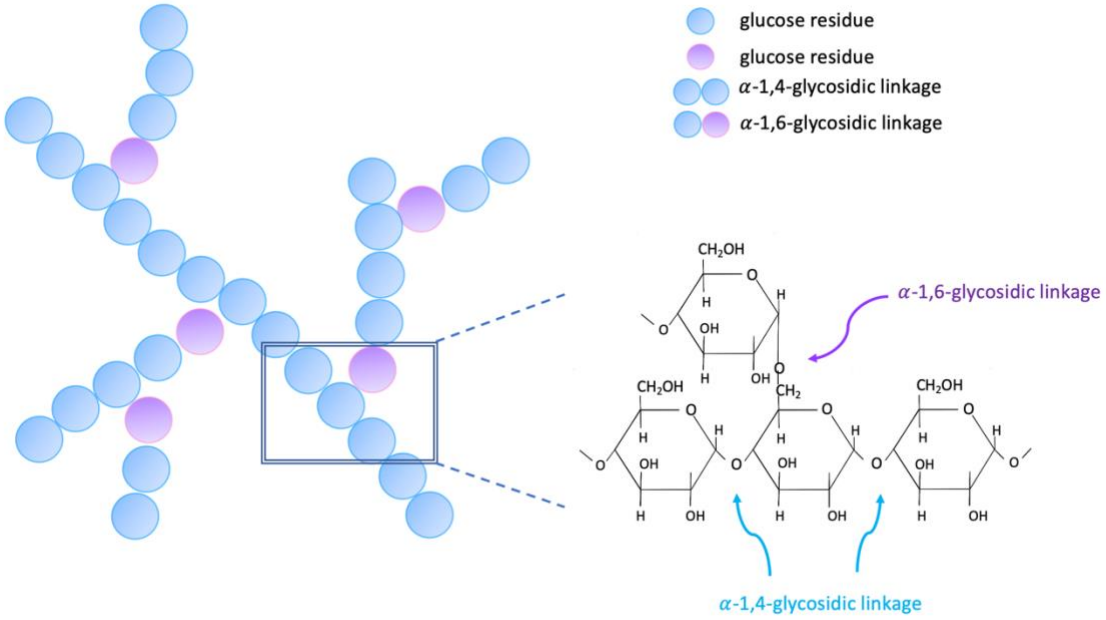


Figure 1: Glycogen Structure. Glucose can be stored as macromolecule glycogen. The α-1,4-glycosidic bonds form the backbone of glycogen whereas α-1,6-glycosidic bonds form the branches.

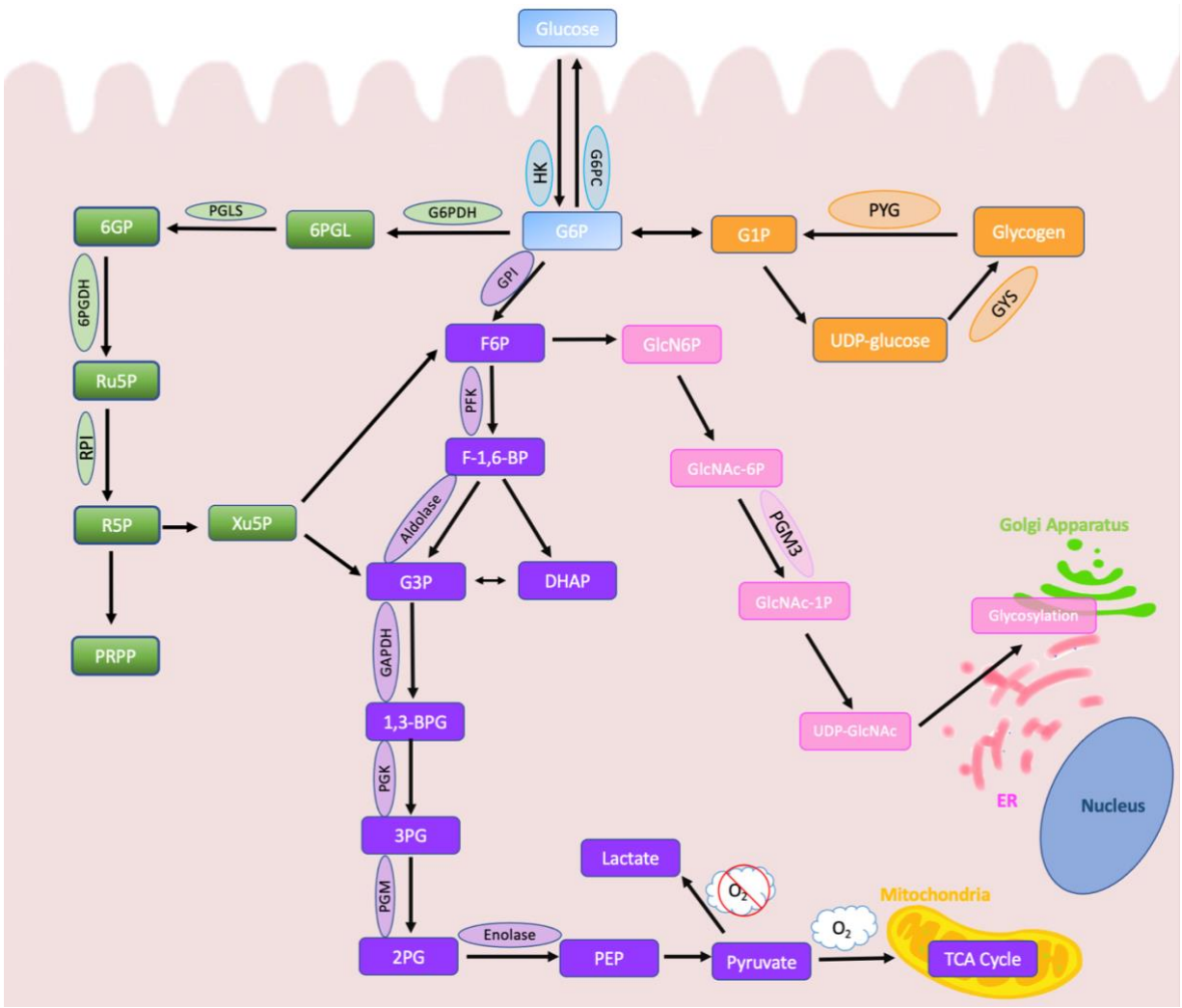


Figure 2: Glucose Metabolic Pathways. After glucose enters the cell, it is converted to G6P. G6P can enter four main pathways, the pentose phosphate pathway (green), the glycolytic pathway (purple), the hexosamine biosynthetic pathway (pink), the glycogenic and glycogenolytic pathway (orange). In glycolytic pathway, with the presence of oxygen, pyruvate can enter TCA cycles in the mitochondria, producing loads of ATP. In the absence of oxygen, pyruvate converts to lactate. In the hexosamine biosynthetic pathway, the product UDP-GlcNAc can be used for glycosylation in the Golgi apparatus and endoplasmic reticulum.

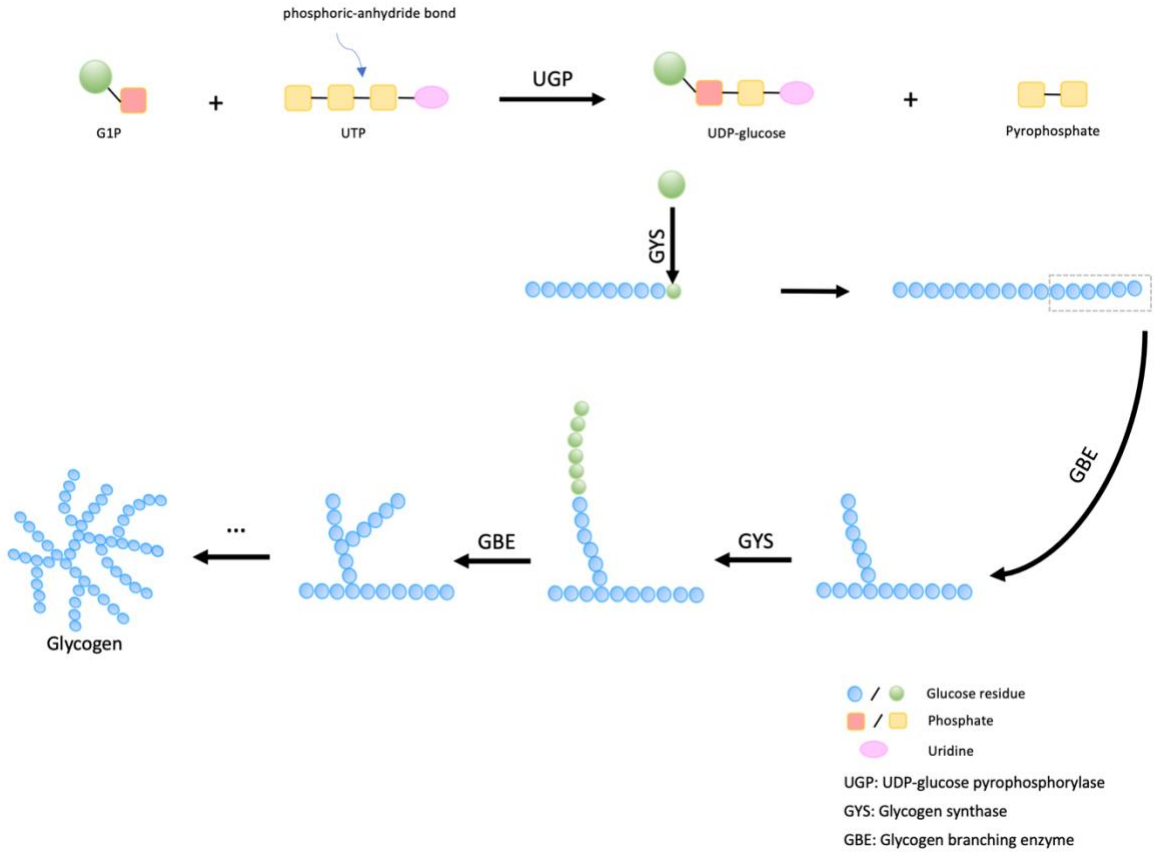


Figure 3: Glycogen Synthesis Steps. The phosphate group on G1P replaced the phosphoric-anhydride bond in UTP catalyzed by UGP. Thus, UDP-glucose and pyrophosphate were produced. GYS linked the glucose residue from UDP-glucose to the linear chain while GBE created the branches of glycogen. Through the corporation of GYS and GBE, the macromolecule glycogen was formed.

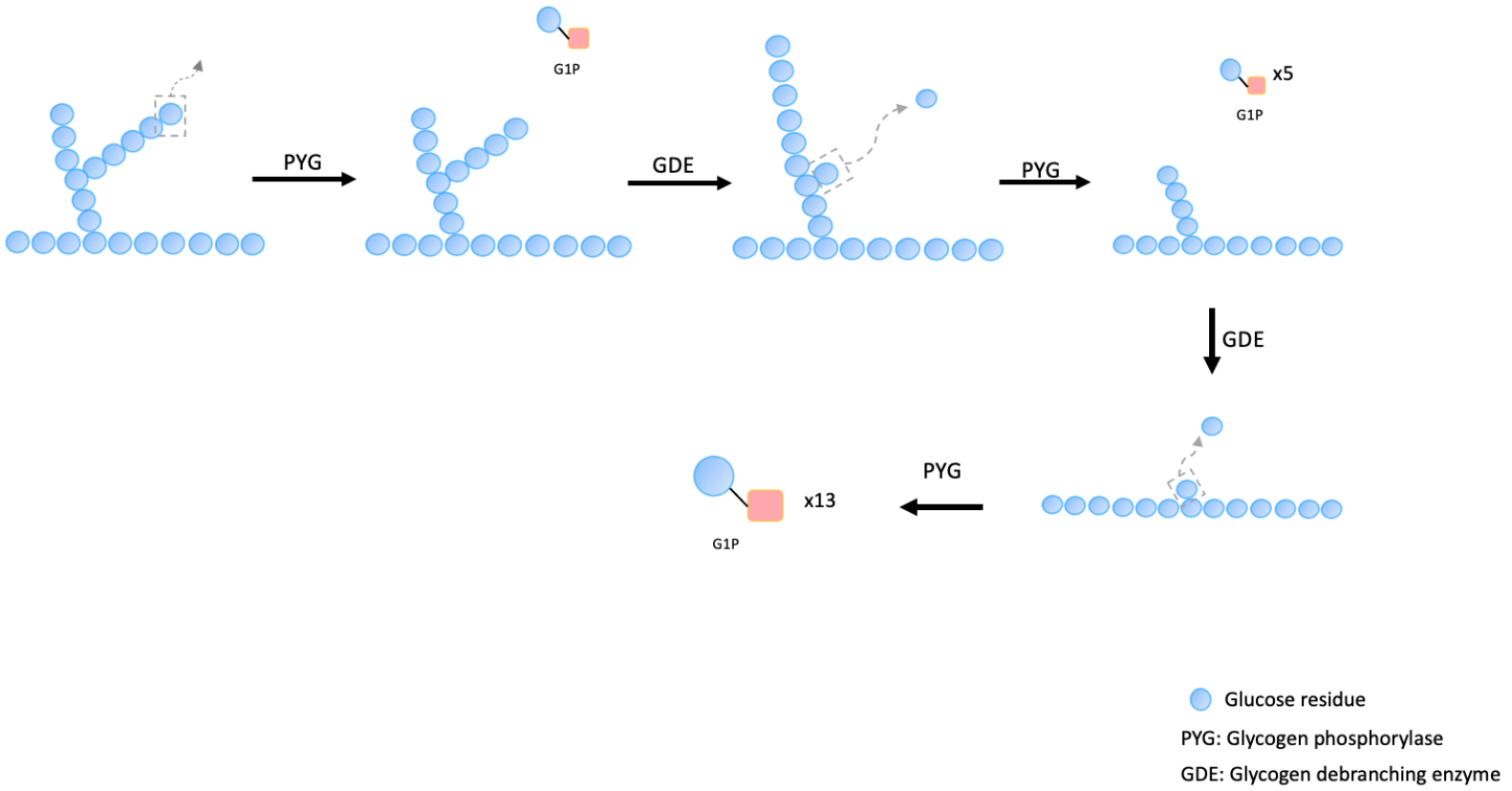


Figure 4: Glycogen Catabolism Steps in the Cytoplasm. PYG first releases G1P from the branches of glycogen, leaving four glycosyls on that branch. GDE transfers three glycosyls to another branch, creating a linear chain of glycosyls. GDE then releases the glucose residue on that branch. With several steps of PYG and GDE actions, multiple G1Ps can be released.

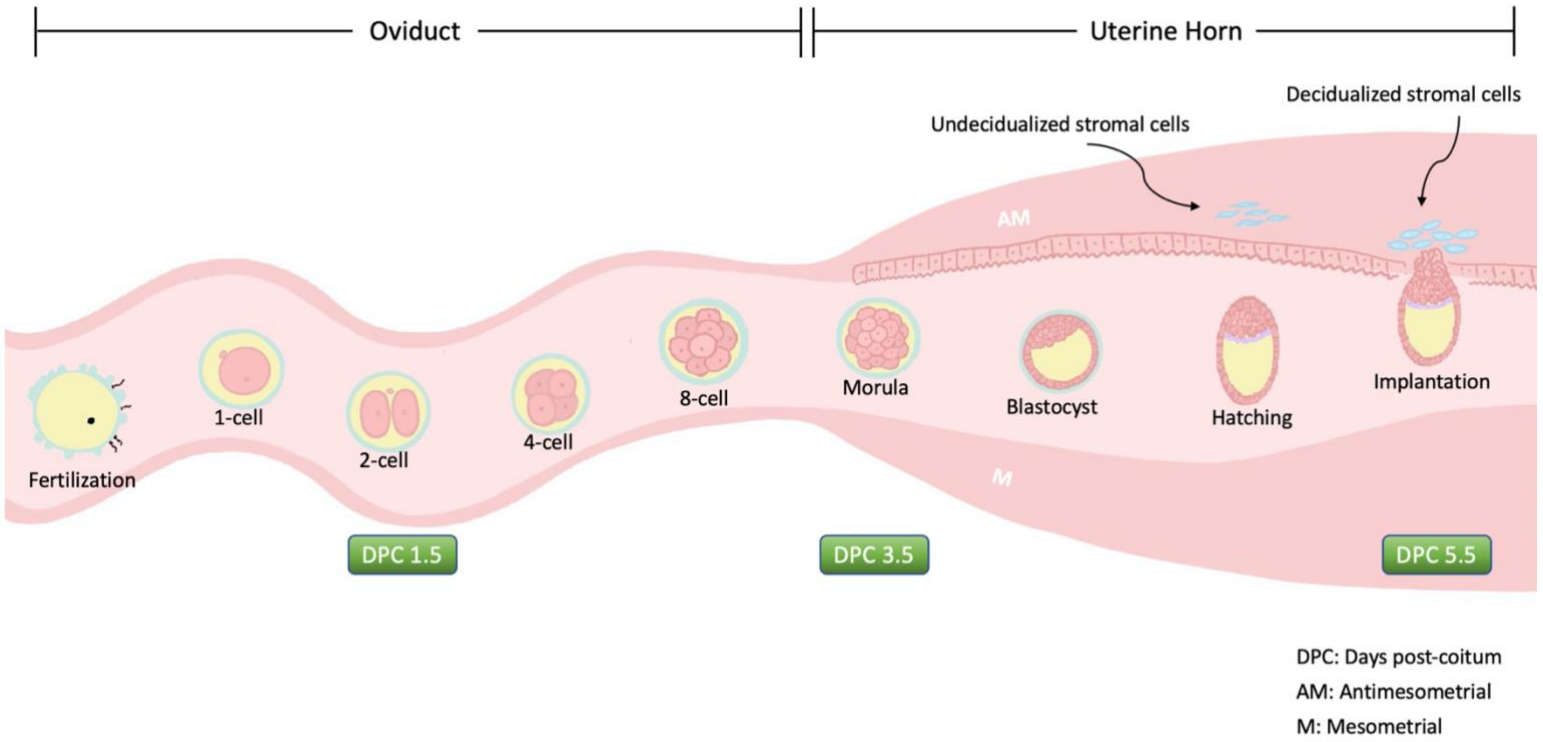


Figure 5: Pre-and Peri-Implantation in Murine Uterus. After fertilization, the embryo undergoes cleavages to 1-cell stage, 2-cell stage, 4-cell stage and 8-cell stage in the oviduct. DPC 1.5 is around the 2-cell stage. The embryo enters the uterine horn around the morula stage, which is about DPC 3.5. As the embryo develops to blastocyst, cells are differentiated to the inner cell mass and the trophoblastic cells. The embryo then hatches from zona pellucida and is prepared for implantation. At the implantation site, luminal closure occurs and the decidualization process of endometrial stromal cells starts from the antimesometrial region and moves to the mesometrial region in mice. DPC 5.5 is around the time of peri-implantation.

CHAPTER 3: ENDOMETRIAL GLYCOGEN METABOLISM DURING EARLY PREGNANCY IN MICE

3.1 Abstract

Glucose is critical for endometrial function and embryo survival. The uterus can store glucose as glycogen but glycogen metabolism during early pregnancy is poorly understood. This study was conducted to analyze glycogen storage and localization of glycogen metabolizing enzymes from proestrus until implantation in the murine uterus. Quantification of diastase-labile periodic acid-Schiff (PAS) staining showed glycogen in the glandular epithelium decreased 71.4% at 1.5 days post-coitum (DPC) and 62.13% at DPC 3.5 compared to proestrus. In the luminal epithelium, glycogen was the highest at proestrus, decreased 46.2% at DPC 1.5 and 63.2% at DPC 3.5. Immunostaining showed that prior to implantation, glycogen metabolizing enzymes were primarily localized to the glandular and luminal epithelium. Stromal glycogen was low from proestrus to DPC 3.5. However, at the DPC 5.5 implantation sites, stromal glycogen levels increased 7-fold. Similarly, artificial decidualization resulted in a 5-fold increase in glycogen levels. In both models, decidualization increased the expression of glycogen synthesizing and catabolizing enzymes. In conclusion, glycogen levels decreased in the uterine epithelium before implantation, indicating that released glucose could be used to support preimplantation embryos. Decidualization resulted in a dramatic increase in stromal glycogen levels, suggesting it could be used by the decidual cells or secreted.

3.2 Introduction

Pregnancy loss is quite common in humans, with most losses occurring very early in pregnancy (Annual Capri Workshop Group, 2020; Zinaman et al., 1996). Prior to implantation, embryos are dependent on nutrients secreted into the uterine lumen. Of the nutrients in uterine secretions, glucose is one of the most important. Glucose uptake by embryos is low from fertilization until the 8-cell stage, and before compaction too much glucose is toxic. Around the morula stage glucose uptake starts to increase and is dramatically higher by the blastocyst stage (Dan-Goor et al., 1998; Leese & Barton, 1984). In human embryos produced via IVF or ICSI, glucose consumption was higher in the embryos that resulted in a live birth (Gardner et al., 2011). Matching the increased glucose needs of the blastocyst, the glucose concentrations are higher in fluid from the uterus than those in fluid from the oviduct (Gardner et al., 1996; Hugentobler et al., 2010).

At the implantation site, the stromal fibroblasts undergo a morphological and physiological change into decidual cells. Decidualization results in increased flux through the pentose phosphate pathway, and blocking this pathway impairs the decidual response in mice and human endometrial stromal cells (Frolova et al., 2011; Tsai et al., 2013). After decidualization, glucose uptake increases due to a shift to a Warburg-like metabolism (Zuo et al., 2015). Hence, the glucose needs of both the embryo and uterus change in a spatiotemporal manner during early pregnancy.

The uterus lacks the enzymes to make glucose de novo; therefore, all glucose used by the endometrium or secreted into the uterine lumen must come from the maternal circulation (Yánez et al., 2003; Zimmer & Magnuson, 1990). The facilitative glucose transporters (GLUTs, gene

family *Slc2a*) and sodium-glucose linked transporter 1 (SGLT1, gene symbol *Slc5a1*) are both expressed in the endometrium (Frolova & Moley, 2011a; Zhang et al., 2021). Thus, the uterus may take up glucose from maternal circulation as needed; however, the endometrium can also transiently store glucose as the macromolecule glycogen.

After glucose enters a cell, it is phosphorylated by hexokinase (HK) to produce glucose-6-phosphate. Glucose-6-phosphate can be metabolized by many different pathways. To be stored as glycogen, the glucose-residue is isomerized to glucose-1-phosphate and then transferred to UTP, yielding UDP-glucose. From there, glycogen synthase (GYS) transfers the glucose to a pre-existing glycogen molecule. Glucose-1-phosphate is liberated from glycogen by the enzyme glycogen phosphorylase (PYG). Glucose-1-phosphate is isomerized back to glucose-6-phosphate, which is trapped in the cell. To be secreted, the glucose moiety must be dephosphorylated by glucose-6-phosphatase (G6PC).

In humans, endometrial glycogen concentrations peak during the luteal phase and are correlated with fertility (Maeyama et al., 1977). In rats, uterine glycogen concentrations are high on day 1 of pregnancy and then decrease over preimplantation. Glycogen concentrations begin to increase after implantation (Greenstreet & Fotherby, 1973b). However, it is unclear which tissues store the glycogen or where the glycogen metabolizing enzymes are expressed. Mice are important biomedical research models, yet uterine glycogen metabolism has never been characterized in this species.

Our objectives were to 1) characterize glycogen stores in the murine uterus from proestrus through implantation in the glandular epithelium, luminal epithelium, and stroma; 2) localize key glycogen metabolizing enzymes during the same period; and 3) determine if

decidualization is sufficient to drive glycogen accumulation in the endometrial stroma independently of pregnancy.

3.3 Materials and Methods

3.3.1 Animals

CD-1 mice were purchased from Charles River Laboratories and maintained at the University of Illinois animal facility. Mice were kept on a 12L:12D light cycle and were fed a standard chow diet. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol #190624). To obtain uteri at proestrus, vaginal lavages were examined daily to monitor the estrous cycle in mice. After at least two normal estrous cycles, mice were sacrificed at proestrus and uterine horns were collected. To collect uteri after mating, female mice were housed with males of proven fertility and examined every morning for the presence of a vaginal plug. Observance of a vaginal plug was designated as day post-coitum (DPC) 0.5. The female mice were sacrificed at DPC 1.5, 3.5 or 5.5 accordingly. Uterine horns were collected and fixed in 4% PFA (Fisher Scientific, ICN15014601).

To induce artificial decidualization of the uterus, CD-1 female mice (Charles River Laboratories) were ovariectomized via 2 mid-dorsal incisions and given two weeks to heal and to eliminate circulating steroid hormones. Next 100 ng estradiol in 0.1 ml corn oil was given subcutaneously every 24 h for 3 consecutive days. Then after 2 days of rest, 10 ng estradiol (Sigma-Aldrich, E2758) and 1 mg progesterone (Sigma-Aldrich, P8783) in 0.1 ml corn oil were injected subcutaneously daily for 3 consecutive days. Decidualization was initiated 4 hours after the last injection. Corn oil (15 μl) was injected into the lumen of the left uterine horn through a

flank incision. The right uterine horn was left unstimulated and served as an internal control. The mice were treated with 1 mg progesterone in 0.1 ml corn oil daily for 4 days and were euthanized 96 h post-inducing decidualization (Appendix B). Both uterine horns were collected, weighed, and fixed in 4% PFA.

3.3.2 Periodic acid-Schiff Staining

Tissues were sectioned at 5 μm. Two slides were used for PAS and PAS with diastase (PASD) staining separately. Slides were deparaffined in xylene (Avantor, 8668-16) for 10 minutes and rehydrated with graded concentrations of ethanol. Slides for PAS staining were incubated in PBS, and slides for PASD staining were immersed in PBS with 0.5% diastase (Sigma-Aldrich, 09962) at 37℃ for 60 minutes. After incubation, slides were incubated in 0.5% periodic acid solution (Fisher Scientific, AC453171000) for 5 minutes at room temperature and washed 3 times with distilled water. Sections were immersed in Schiff's reagent (Sigma-Aldrich, 3952016) for 15 minutes at room temperature, followed by 5 minutes of wash in lukewarm running tap water. Then slides were counterstained with hematoxylin (Fisher Scientific, 22-050-206) and dipped in ammonium hydroxide buffer (Thermo Fisher, A669S) for 20 seconds. Slides were dehydrated and incubated in xylene overnight. Slides were mounted with Permount mounting media (Fisher Scientific, SP15100). Images were captured using Zeiss Axioskop with an Axoicam 305 color camera.

Images were analyzed with ImageJ (https://imagej.nih.gov/ij/). Within each image, the glandular epithelium, luminal epithelium, and stroma were separated. The total area of each tissue and the area positive for PAS were measured. Next, the percent area of positive staining in

each tissue was calculated. To account for non-glycogen PAS staining, the area positive in PASD images was subtracted from the area stained in the corresponding PAS images.

3.3.3 Immunohistochemistry

Tissues were sectioned at 5 μm, added to slides, deparaffinized, and rehydrated. Slides were boiled in sodium citrate buffer (Fisher Scientific, S271-3) and then cooled to room temperature. Then the slides were incubated in 3% hydrogen peroxide (Fisher Scientific, H325-500) for 15 minutes. Non-specific blocking was inhibited with a block containing 10% goat serum (Vector Laboratory, S-1000-20) and 5% bovine serum albumin (BSA; Fisher Scientific, BP9706100) in Tris-buffered saline (TBS) for 1 hour at room temperature. After the serum block, previously validated primary antibodies (Appendix A) were diluted in the block, added to tissue sections, and incubated at 4℃ overnight (Sandoval et al., 2021). All incubations were performed in hydrated chamber. The next day, slides were washed in TBS with tween (TBS-T) three times and incubated with secondary antibody (Vector Laboratories, BA-5000-1.5) diluted in block for 30 minutes at room temperature. Then slides were washed three times and incubated with avidin-biotin complex (ABC) reagent (Vector Laboratory, SP-2001) for 30 minutes at room temperature. After 3 washes in TBS-T, 3, 3′-Diaminobenzidine (DAB) (Vector Laboratory, SK-4100) was applied. Slides were counterstained with hematoxylin for 2 minutes. Then the tissues were dehydrated, mounted, and imaged with a Zeiss Axioskop with an Axoicam 305 color camera. Negative controls were treated as described above, except that the primary antibody was replaced with an isotype control (anti-GFP) antibody.

3.3.4 Western Blots

Uterine tissues were homogenized in radioimmunoprecipitation assay buffer (RIPA) supplemented with phosphatase and protease inhibitors (Sigma-Aldrich, P0044-1ML and Thermo Fisher, A32953; respectively), and the protein concentration was determined by BCA assay. Protein (25 μg) were separated in a 10 % SDS-PAGE gel using constant voltage. Then proteins were transferred onto PVDF membranes and were blocked for 1 hour with either 5% BSA in TBS-T or 5% non-fat dry milk in TBS-T depending on the primary antibody. The membranes were incubated in primary antibody (Appendix A) overnight at 4℃. The next day, the membranes were washed 3 times with TBS-T and incubated in a block in TBS-T containing anti-rabbit secondary antibody (Cell Signalling, 7074S) for 30 minutes. SuperSignal West Pick PLUS chemiluminescent substrate (Thermo Scientific, 34577) was used for developing signals, and images were obtained using an ImageQuant LAS 4000 (GE Healthcare)

3.3.5 Statistical Analysis

Statistical calculations were performed using GraphPad Prism version 8.3.1. Data collected during early pregnancy were analyzed by a one-way ANOVA followed by a Dunnett's analysis. For the artificial decidualization experiments, glycogen content and uterine weight were analyzed using a paired t-test. Results are presented as mean ± SEM and differences were considered significant when $P<0.05$.

3.4 Results

3.4.1 Endometrial Glycogen Levels during Early Pregnancy

Uteri were collected from mice at proestrus and days post-coitum (DPC) 1.5, DPC 3.5, and DPC 5.5 and stained with Periodic acid–Schiff (PAS), with or without diastase (D) pretreatment to localize glycogen. PAS staining indicated the presence of glycogen in the epithelium at proestrus and in the decidua after implantation (Figure 6A). Quantification of the diastase-liable staining showed that in the glandular epithelium, glycogen content was highest at proestrus, decreased 71.4% at DPC 1.5 ($P<0.01$), and decreased 62.13% at DPC 3.5 ($P<0.01$; Figure 6A). By DPC 5.5, the glycogen content in the glandular epithelium at the inter-implantation site (IIS) increased and was similar to proestrus (Figure 6B). Similar results were found in the luminal epithelium, where glycogen content was highest at proestrus, 46.2% lower at DPC 1.5 ($P=0.061$), and 63.2% lower at DPC 3.5 ($P<0.05$; Figure 6C). At DPC 5.5-IIS, the glycogen content of the luminal epithelium was 32% lower than that of proestrus, but this was not significant ($P=0.37$; Figure 6C).

In contrast, the stroma stored little glycogen during the preimplantation period. Glycogen content did not change significantly from proestrus through DPC 3.5. At DPC 5.5, glycogen content was still low in the stroma at the IIS; however, the glycogen level increased 7-fold at the implantation site (IS) compared to the stroma of proestrus or the IS ($P<0.0001$; Figure 6D).

3.4.2 Glycogen Metabolizing Enzymes during Early Pregnancy

The levels of glycogen metabolizing enzymes (HK1, GYS, pGYS, PYG) in the uterus of mice at proestrus and pregnant mice were analyzed by western blot. There were no significant

differences in the levels of the glycogen synthesizing enzymes HK1, GYS, and pGYS (Figure 7A-C). Similarly, no difference in the level of the glycogen catabolizing enzyme PYG was detected during early pregnancy (Figure 7D).

Immunohistochemistry demonstrated that glycogen synthesizing enzymes (HK and GYS) were highly expressed in the uterine epithelium. HK1 was localized to the glandular and lumen epithelium and was undetectable in the stroma. Immunostaining in the epithelium was consistent from proestrus to DPC 5.5 IIS (Figure 8 top). GYS was present in the luminal and glandular epithelium. Immunostaining was higher on DPC 1.5 and 3.5 compared to proestrus or DPC 5.5 IIS. Some immunostaining for GYS was observed in the stroma on DPC 3.5 (Figure 8 bottom).

Similar to glycogen synthesizing enzymes, glycogen catabolizing enzymes (PYG and G6PC) were also found primarily in the glandular and luminal epithelium. PYG immunostaining was higher after mating (DPC 1.5-5.5) than at proestrus (Fig. 9 top). There was moderate PYG immunostaining in the stroma at DPC 3.5 (Figure 9 top). G6PC was localized to the uterine epithelium and the expression was higher from DPC 1.5 to DPC 5.5 when compared to proestrus (Figure 9 bottom).

HK1 expression was undetectable in the stroma at the DPC 5.5 IS by IHC, similar to the stroma at the IIS. In contrast, there was a dramatic increase in immunostaining for GYS at the DPC 5.5 IS compared to the stroma at the DPC 5.5 IIS, which agrees with the increased glycogen levels at the IS (Figure 10 top). Interestingly, there were modest increases in immunostaining for PYG and G6PC at DPC 5.5 IS compared to DPC 5.5 IIS in the stroma (Figure 10 bottom).

3.4.3 Endometrial Glycogen Metabolism After Artificial Decidualization

Next, we induced decidualization artificially to determine if decidualization increased glycogen storage. Mice were ovariectomized, primed with ovarian steroids, and the left uterine horn was stimulated to initiate the decidual reaction. The right uterine horn was unstimulated and served as an internal control. The stimulated uterine horn appeared larger and weighed significantly more than the non-stimulated horn, confirming successful decidualization (Figure 11A). Quantification of PAS and PASD staining showed that the glycogen content was 5-times higher in the stimulated horn than that in the unstimulated horn (Figure 11B; $P<0.05$). Similar to the data from the DPC 5.5 IIS and IS, HK1 immunostaining was undetectable in the stroma of the unstimulated horn and barely detectable in the stimulated horn. GYS immunostaining was absent in the stroma of the unstimulated horn and was markedly increased in the stimulated horn (Figure 12 top). In addition, immunostaining for both PYG and G6PC was moderately increased in the stimulated horn compared to the stroma of the unstimulated horn (Figure 12 bottom).

3.5 Discussion

The early embryo prefers pyruvate and lactate as energy substrates but has switched to glucose by the blastocyst stage (Gardner & Leese, 1990; Leese & Barton, 1984). Too much glucose during cleavage development is toxic to the embryo (Cagnone et al., 2012; Pantaleon et al., 2010). As a result, preimplantation embryos require optimal glucose concentrations to survive. Given the near-ubiquitous expression of GLUTs in the endometrium and their facilitated diffusion mechanism of action (Frolova & Moley, 2011a), GLUTs themselves are unlikely to adequately regulate glucose secretion into the uterine lumen.

Endometrial glycogen content peaks during estrus and then declines during the luteal phase or pregnancy (Dean et al., 2014; Demers et al., 1972; Sandoval et al., 2021). This has led to the theory that glycogen acts as an energy reservoir for preimplantation embryos (Dean, 2019). In support of that, we show that glycogen mobilized during the preimplantation period is coming from the uterine epithelium, the cells that secrete histotroph. We also showed that the epithelium expresses G6PC, which is necessary for the secretion of glucose liberated from glycogen. G6PC has also been localized to the uterine epithelium of cyclic cows (Sandoval et al., 2021). Global knockout of G6PC leads to a 50% decrease in litter size in mice, suggesting that G6PC is important for pregnancy (Jun et al., 2012), though systemic effects of G6PC knockout cannot be ruled out.

Prior to implantation, all four enzymes detected by immunohistochemistry (HK1, GYS, PYG, and G6PC) were primarily localized in the glandular and luminal epithelium, which is consistent with the significant change of glycogen content in the epithelium instead of the stroma. The expression of GYS, PYG, and G6PC in the uterine epithelium appeared to increase during the preimplantation period (DPC 1.5 and 3.5). These results agree with a study in mink that found uterine expression of *Gys*, *Pyg*, and *G6pc* mRNA increased after progesterone treatment with estradiol priming (Bowman & Rose, 2016). It might seem paradoxical to have increased expression of glycogen synthesizing and catabolizing enzymes at the same time. However, glycogen synthesis and catabolism occur concurrently within the cell, and the increased glycogen synthesis may facilitate the continued transport of glucose from maternal blood to the uterine lumen even as glycogen levels are decreasing.

We also observed a substantial increase of glycogen in decidualized stromal cells at the IS and after artificial decidualization. In agreement, electron microscope studies showed that

glycogen deposition was detected in the decidual cells in hamsters (Blankenship et al., 1990). In humans, endometrial glycogen concentration peaks in the luteal phase, presumably due to glycogen in the decidua, though glycogen has also been observed in the epithelium during the luteal phase (Jones et al., 2015). Endometrial glycogen deficiency during the luteal phase is correlated to infertility in humans (Gupta et al., 2013; Maeyama et al., 1977). Collectively, these results suggest that glycogen synthesis may be an inherent feature of decidualization and might be critical for maintaining a successful pregnancy.

The increase in GYS expression at the IS and the decidualized endometrium agrees with the dramatic increase of glycogen in the same tissues. The purpose of this glycogen reserve is currently unclear. Decidualization is a glucose-intensive process, but the increased glycogen levels observed here are after the completion of decidualization. Surprisingly there were also modest increases in PYG and G6PC levels in the decidua, suggesting concomitant synthesis and catabolism. It is possible that glycogen in the decidua is used to regulate the supply of glucose to decidual cells themselves, preventing negative effects of hyperglycemia, or to supply glucose to the developing embryo (Favaro et al., 2013; Zuo et al., 2015). More work is needed to elucidate the role of glycogen in the decidua and to determine if it is required for a successful pregnancy.

In conclusion, we show that the glycogen content of the glandular and luminal epithelium decreased during early pregnancy. This decrease was accompanied by increased expression of PYG and G6PC, suggesting glycogen is broken down into glucose and possibly secreted into the uterine lumen. The increased levels of glycogen in the decidual cells correlated with increased expression of GYS, PYG, and G6PC, suggesting concurrent synthesis and breakdown of glycogen in the decidua. Currently, the role of glycogen in the decidua is unclear, but it could be utilized by the uterus or secreted to supply nutrients for the embryo.

3.6 Figures

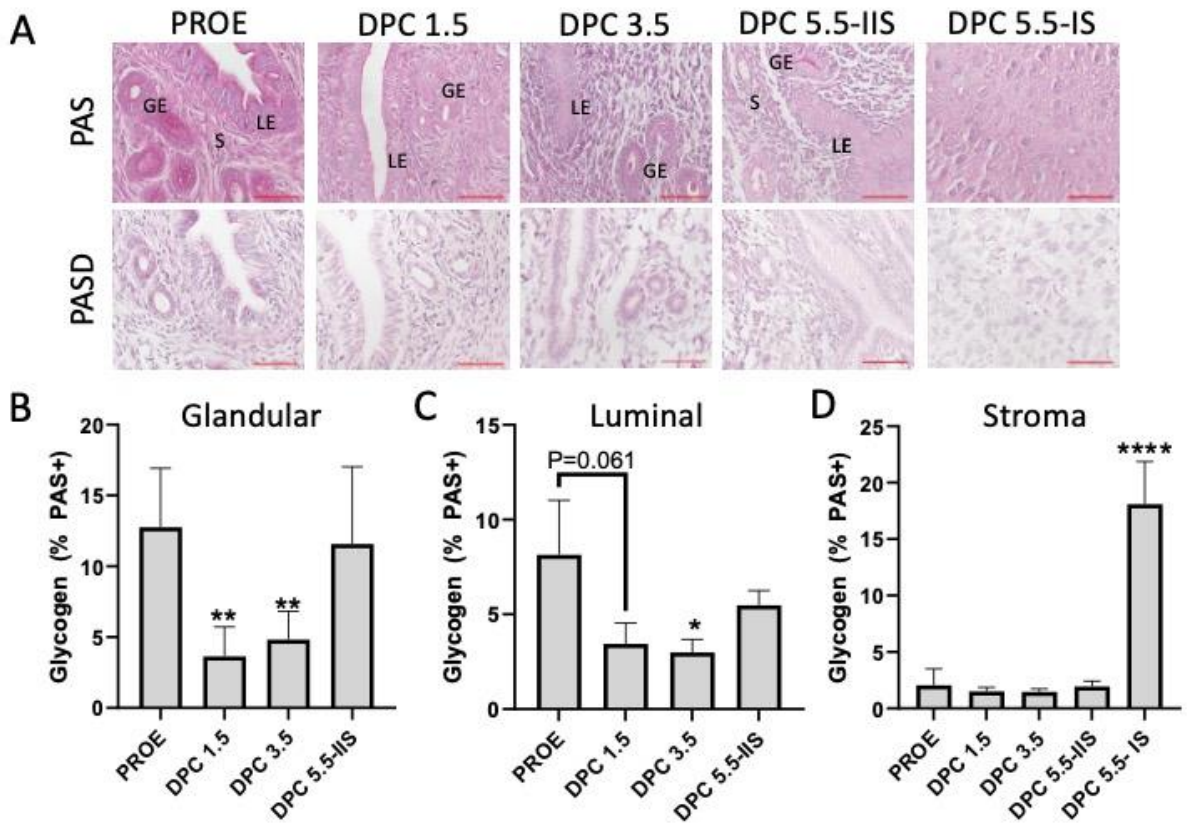


Figure 6: **Glycogen Levels in the Murine Endometrium during the First 6 Days of Pregnancy**. A) Representative images from the murine uterus collected at proestrus (PROE), days post-coitum (DPC) 1.5, DPC 3.5, and DPC 5.5. Sections were stained with Periodic acid–Schiff (PAS, top). Other slides were pretreated with diastase (D) to digest glycogen prior to PAS staining (bottom). B-D) Glycogen content of the glandular epithelium (B), the luminal epithelium (C), and stroma (D) was determined by subtraction of the PAS positive area in the PASD slides from the PAS slides. LE, luminal epithelium; GE, glandular epithelium; S, stroma; IS, implantation site; IIS, inter-implantation site. *P<0.05, **P<0.01, ****P<0.0001 relative to PROE. n=6. scale bar = 50 µm.

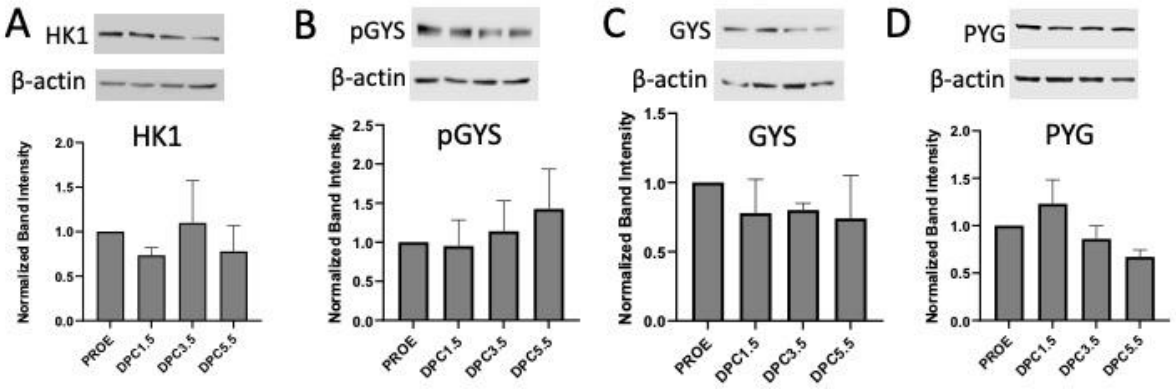


Figure 7: **Levels of Glycogen Metabolizing Enzymes in Uterine Homogenates**. Levels of glycogen metabolizing enzymes remain constant during early pregnancy. A-D) Western blots for hexokinase 1 (HK1, A), phospho-glycogen synthase (pGYS, B), glycogen synthase (GYS, C), and glycogen phosphorylase (PYG, D) in uteri collected from mice at proestrus (PROE) and days post-coitum (DPC) 1.5, 3.5, and 5.5. n=4.

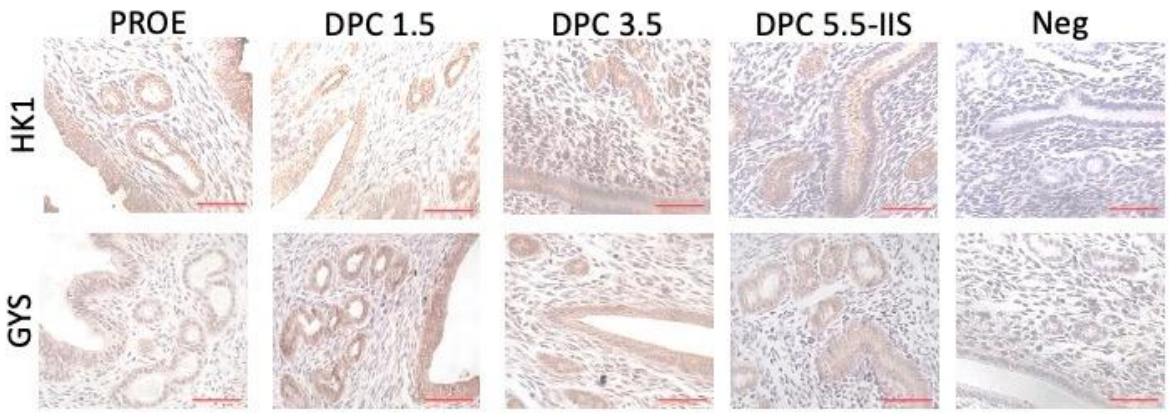


Figure 8: **Localization of Glycogen Synthesizing Enzymes.** Glycogen synthesizing enzymes localized to the uterine epithelium brfore implantation. Immunohistochemistry for hexokinase 1 (HK1) and glycogen synthase (GYS) in uteri collected at proestrus (PROE) or days post-coitum (DPC) 1.5, DPC 3.5, and DPC 5.5. IIS, inter-implantation site. Neg, negative control. n=4. scale bar = 50 µm.

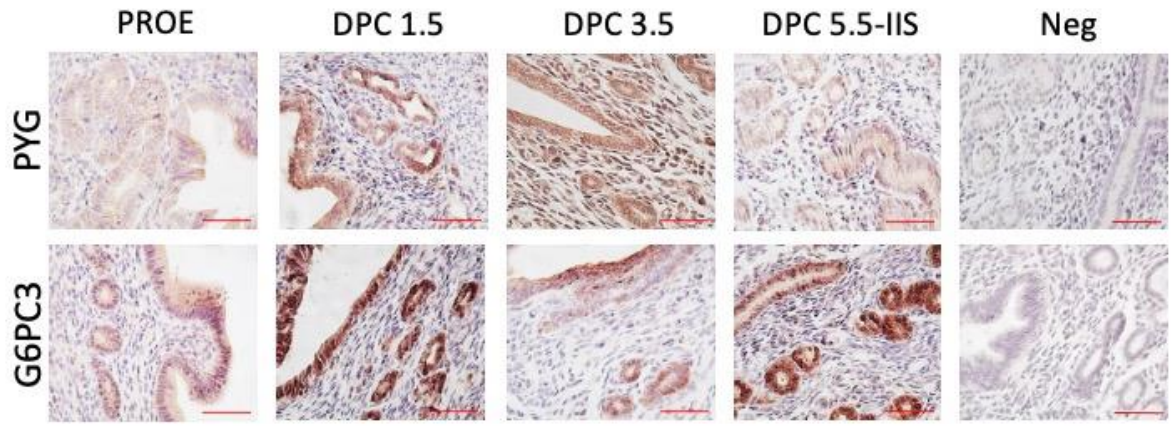


Figure 9: Localization of Glycogen Catabolizing Enzymes. Glycogen catabolizing enzymes primarily localized to the uterine epithelium during the preimplantation period. Immunohistochemistry for glycogen phosphorylase (PYG) and glucose-6-phosphatse (G6PC) in uteri collected at proestrus (PROE) or days post-coitum (DPC) 1.5, DPC 3.5, and DPC 5.5. IIS, inter-implantation site. Neg, negative control. n=4. scale bar = 50 µm.

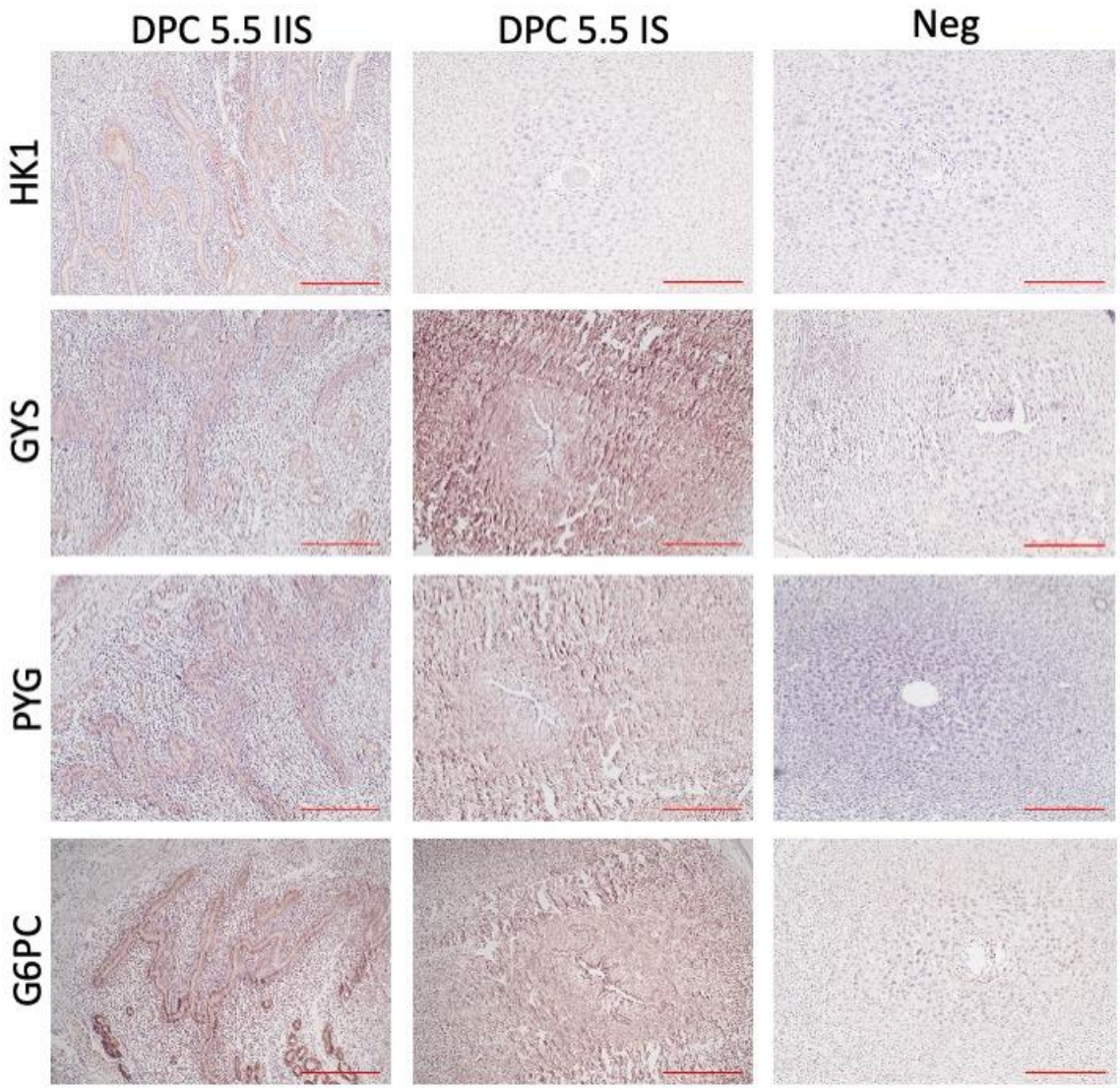


Figure 10: Glycogen Metabolizing Enzymes at Implantation Site. Most glycogen metabolizing enzymes are higher at the implantation site (IS) than at the inter-implantation site (IIS). Immunohistochemistry for glycogen synthesizing enzymes (hexokinase 1 [HK1] and glycogen synthase [GYS]) and glycogen catabolizing enzymes (glycogen phosphorylase [PYG] and glucose-6-phosphatase [G6PC]) in uteri at DPC 5.5 IIS and IS. Neg, negative control. n=4. scale bar = 200 μm.

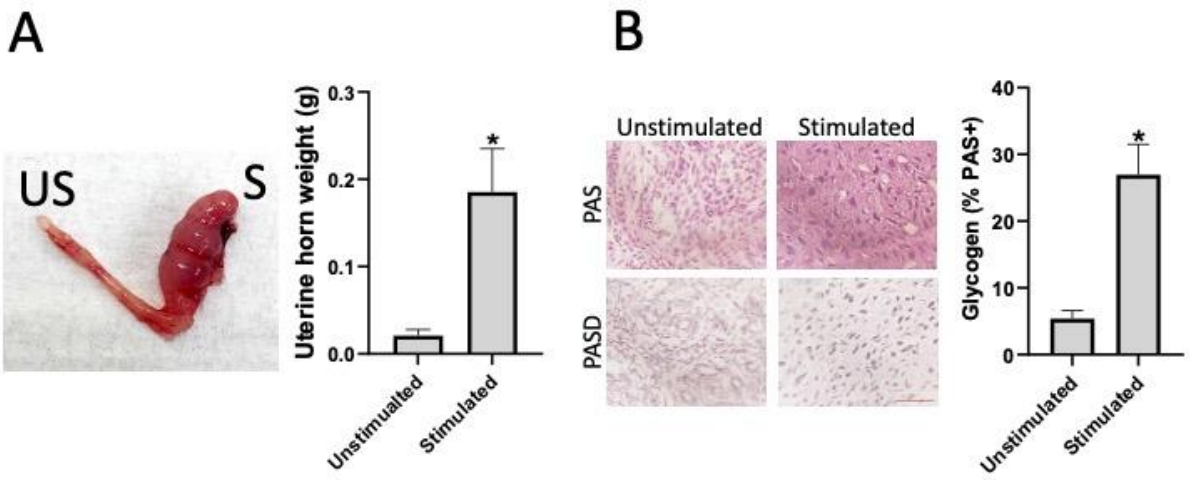


Figure 11: **Artificial Decidualization Resulted in Increased Levels of Glycogen.** A) Representative image showing the stimulated (S) and unstimulated (US) uterine horns, and corresponding uterine horn weight. B) PAS and PASD staining showed an increase of glycogen detected in the stimulated uterine horn compared to the unstimulated uterine horn. *P<0.05. n=4. scale bar = 50 µm.

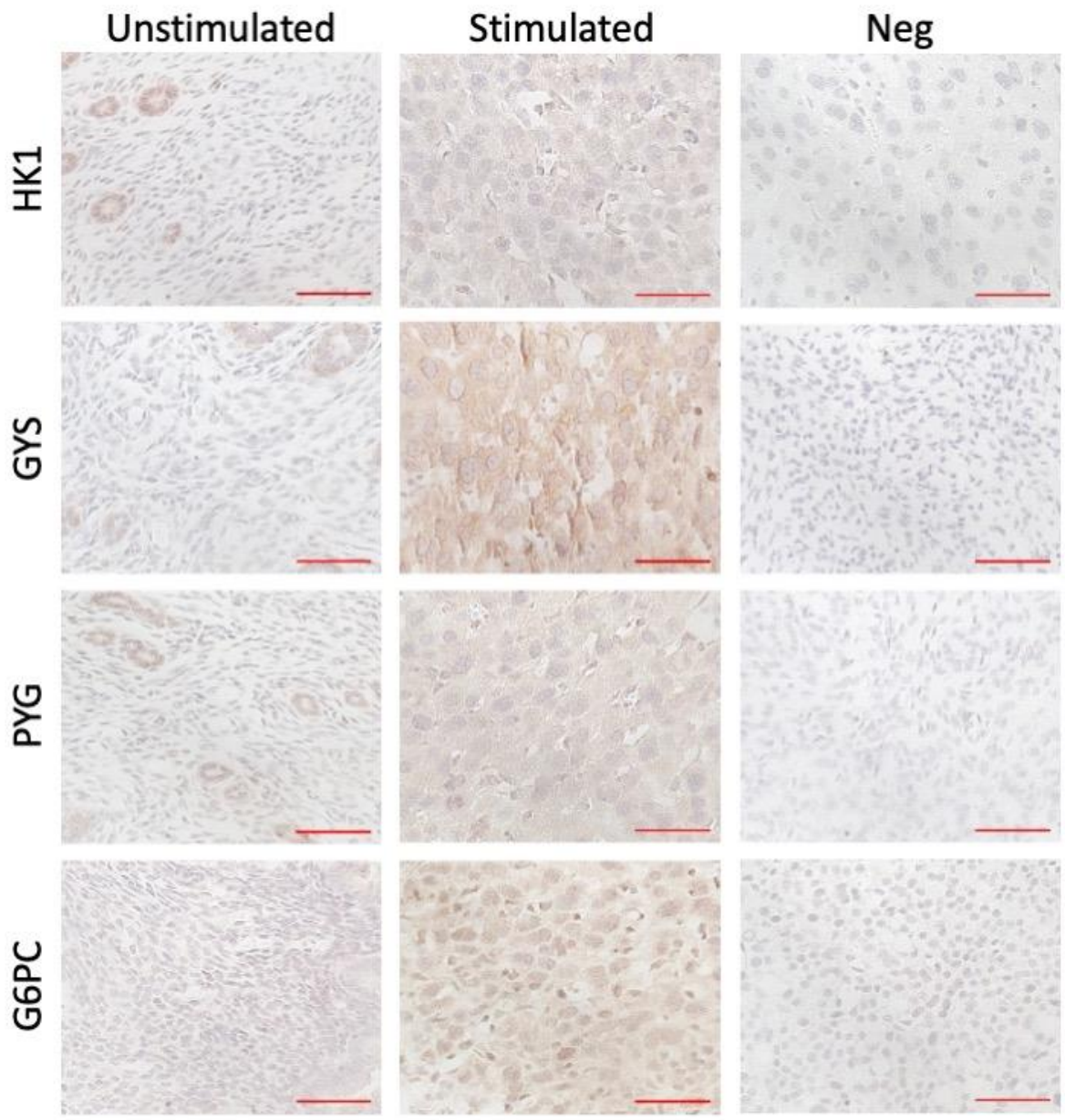


Figure 12: Glycogen Metabolizing Enzyme Levels in the Decidual Stromal Cells. Glycogen metabolizing enzyme levels are elevated in decidualized stromal cells. Representative images of immunohistochemistry for glycogen synthesizing enzymes (hexokinase 1 [HK1] and glycogen synthase [GYS]) and glycogen catabolizing enzymes (glycogen phosphorylase [PYG] and glucose-6-phosphatase [G6PC]) in the stimulated and unstimulated uterine horns. Neg, negative control. n=4. scale bar = 50 µm

CHAPTER 4: CONCLUSION AND FUTURE DIRECTIONS

4.1 Summary

The maximum pregnancy rate is about 30% per menstrual cycle in humans. Contributing to the low pregnancy rate, pregnancy failure is quite common in humans. Loss of pregnancies can occur at any time from fertilization until term, but most occur during early pregnancy. Embryos are dependent on nutrients secreted from the uterus during the preimplantation period. Of the secreted nutrients, glucose is one of the important ones. Glucose uptake by the embryo is low from fertilization until the 8-cell stage and too much glucose is toxic to the developing embryo before compaction. Nevertheless, glucose uptake increases dramatically during the blastocyst stage. As a result, regulation of glucose concentrations in the uterus is critical to embryonic development, and optimal glucose is required for maintaining embryo differentiation during early pregnancy. In support of that, glucose concentration is higher in the uterine fluid than in the oviduct, which matches the glucose needs for proper embryo development.

Glucose can enter the glycogenesis pathway and be stored as glycogen. We found that glycogen content decreased on DPC 1.5 and DPC 3.5 in the luminal and glandular epithelium compared to proestrus. Moreover, it is worth noting that increases in GYS, PYG, and G6PC were detected from proestrus to the preimplantation period. This suggests that glycogen synthesis and breakdown may happen simultaneously to regulate glycogen content and the decrease of glycogen in the glandular and luminal epithelium may be due to the elevation of glycogen catabolizing enzymes PYG and G6PC. G6PC is important to liberate glucose from the cells, and the presence of G6PC indicated that glucose liberated from glycogen can be secreted from the glandular epithelium and luminal epithelium to provide nutrients to the embryo.

Glycogen storage in the stroma exhibited different patterns than in the glandular and luminal epithelium. Our results demonstrated that only little glycogen was stored in the stroma during the preimplantation period or in the undecidualized stromal cells around the time of implantation. However, glycogen increased significantly in the decidua. In addition, to determine if glycogen is stored independently from the presence of embryos, we performed artificial decidualization in the E2 and P4-primed uterus of the ovariectomized mice. Not surprisingly, glycogen content was dramatically increased in the stimulated uterine horn compared with unstimulated internal control. These experiments showed that decidualization can lead to increased glycogen storage without interaction with the embryo. IHC staining detected high expression of GYS and moderate amounts of PYG and G6PC in the decidualized stromal cells in both pregnant and artificial decidualized models. These observations suggest that the decidua can store glycogen, which may be later secreted as glucose to support uterus metabolism and embryo development.

4.2 Future Directions

Even though we found that decidua stores glycogen during early pregnancy, whether glycogen drives decidualization or vice versa is unclear. In vitro decidualization of HESC may be useful to understand the correlation between glycogen synthesis and decidualization. The cells will be grown in DMEM medium until confluence. As FBS contains insulin, which stimulates glycogenesis, serum-free DMEM will be used for 2 days. Day 0 will be collected as a control. From there, HESC will be cultured in the serum-free medium with the addition of 1 μM medroxyprogesterone-17-acetate (MPA) and 1 mM cAMP for 1, 2, and 4 days to decidualize. Real-time polymerase chain reaction (qPCR) can be performed on decidualization markers such

as PRL and IGFBP to determine the process of decidualization. In addition, a glycogen assay can measure the glycogen stored in different treatments. This experiment will allow us to closely monitor the glycogen levels during decidualization and determine if glycogen metabolism is required for proper decidualization.

It might seem that as embryos implant in the uterus, glycogen in the decidua should be catabolized to glucose to provide nutrients for the embryo or the uterus itself, yet the opposite result was found. The role of glycogen in the decidua remains a mystery. To resolve the question, generating a GYS1 knockout mice model can be useful. Since we are focusing on the effect of uterine glycogen on fertility, generating tissue-specific models can eliminate other factors contributing to pregnancy failure due to global knockout. To conditionally knockout GYS1 in the uterus, we can breed PR-Cre mice with homozygous GYS1 floxed mice. With those mice, many experiments can be conducted to understand the importance of glycogen in the uterus. The number of implantation sites, resorbed embryos, and litter size can provide insights into whether glycogen affects fetal development and to what extent glycogen contributes to fertility. Most importantly, attempting artificial decidualization may build a step to understanding the role of glycogen in the decidua. If the uterine horn failed to decidualize, we can infer that glycogen synthesized by GYS1 is required for initiating the decidualization process. On the other hand, if the uterine horn is successfully decidualized, glycogen catabolizing enzymes can be analyzed to understand if GYS1 or glycogen influences the amount and activities of PYG and G6PC in the decidua. If the changes in PYG and G6PC affect embryonic development, we can suggest that glycogen breakdown and releasing glucose in the decidua are important to the developing embryo. Therefore, to what extent does glycogen in the decidua contributing to fertility may be characterized.

In the future, a comprehensive understanding of glycogen metabolism in the uterus during the pre-and peri-implantation period may significantly contribute to unveiling the "black box" of early pregnancy loss. It may also build a solid foundation for unraveling the mysteries of pregnancy failure. By implementing the knowledge of endometrial glycogen storage, more people and animals can benefit from the reduction of pregnancy loss and higher fertility rate.

REFERENCES

Annual Capri Workshop Group. (2020). Early pregnancy loss: The default outcome for fertilized human oocytes. *Journal of Assisted Reproduction and Genetics*, 35(5), 1057–1063. https://doi.org/10.1007/s10815-020-01749-y

Adastra, K. L., Frolova, A. I., Chi, M. M., Cusumano, D., Bade, M., Carayannopoulos, M. O., & Moley, K. H. (2012). Slc2a8 deficiency in mice results in reproductive and growth impairments. *Biology of Reproduction*, *87*(2), 1–11. https://doi.org/10.1095/biolreprod.111.097675

Adeva-Andany, M. M., González-Lucán, M., Donapetry-García, C., Fernández-Fernández, C., & Ameneiros-Rodríguez, E. (2016). Glycogen metabolism in humans. *BBA Clinical*, *5*, 85–100. https://doi.org/10.1016/j.bbacli.2016.02.001

Akella, N. M., Ciraku, L., & Reginato, M. J. (2019). Fueling the fire: Emerging role of the hexosamine biosynthetic pathway in cancer. *BMC Biology*, *17*(1), 1–14. https://doi.org/10.1186/s12915-019-0671-3

Akram, M. (2013). Mini-review on glycolysis and cancer. *Journal of Cancer Education*, *28*(3), 454–457. https://doi.org/10.1007/s13187-013-0486-9

Ansell, J. D., Barlow, P. W., & McLaren, A. (1974). Binucleate and polyploid cells in the decidua of the mouse. *Journal of Embryology and Experimental Morphology*, *31*(1), 223–227. https://doi.org/10.1242/dev.31.1.223

Bao, L., Tessier, C., Prigent-Tessier, A., Li, F., Buzzio, O. L., Callegari, E. A., Horseman, N. D., & Gibori, G. (2007). Decidual prolactin silences the expression of genes detrimental to pregnancy. *Endocrinology*, *148*(5), 2326–2334. https://doi.org/10.1210/en.2006-1643

Bazer, F. W., & Words, K. (1975). Uterine protein secretions: relationship to development of the

conceptus. *Journal of Animal Science*, *41*(5), 1376–1382.

Bellofiore, N., Ellery, S. J., Mamrot, J., Walker, D. W., Temple-Smith, P., & Dickinson, H. (2017). First evidence of a menstruating rodent: the spiny mouse (Acomys cahirinus). *American Journal of Obstetrics and Gynecology*, *216*(1), 40.e1-40.e11. https://doi.org/10.1016/j.ajog.2016.07.041

Benson, G. V, Lim, H., Paria, B. C., Satokata, I., Dey, S. K., & Maas, R. L. (1996). Mechanisms of reduced fertility in Hoxa-10 mutant mice: uterine homeosis and loss of maternal Hoxa-10 expression. *Development*, *12*(9), 2687–2696.

Bhagavan, N. V. (2002). Carbohydrate metabolism II: gluconeogenesis, glycogen synthesis and breakdown, and alternative pathways. In *Medical Biochemistry* (pp. 275–305). Elsevier. https://doi.org/10.1016/B978-012095440-7/50017-2

Biggers, J. D., Whittingham, D. G., & Donahue, R. P. (1967). The pattern of energy metabolism in the mouse oöcyte and zygote. *Proceedings of the National Academy of Sciences of the United States of America*, *58*(2), 560–567. https://doi.org/10.1073/pnas.58.2.560

Blankenship, T. N., Given, R. L., & Parkening, T. A. (1990). Blastocyst implantation in the chinese hamster (Cricetulus griseus). *American Journal of Anatomy*, *187*(2), 137–157. https://doi.org/10.1002/aja.1001870203

Bo, W. J., Smith, M. S., & Colborn, G. (1964). Histochemical studies on phosphorylase and uridinediphosphoglucose-glycogen transferase in decidual cells of the rat. *The Anatomical Record*, *150*(2), 173–177. https://doi.org/10.1002/ar.1091500208

Boettiger, E. G. (1946). Changes in the glycogen and water content of the rat uterus. *Journal of Cellular and Comparative Physiology*, *27*(1), 9–14. https://doi.org/10.1002/jcp.1030270103

Bowman, K., & Rose, J. (2016). Estradiol stimulates glycogen syntheis whereas progesterone

promotes glycogen catabolism in the uterus of the American mink. *Physiology & Behavior*, *176*(3), 139–148. https://doi.org/10.1111/asj.12564.Estradiol

Braude, P., Bolton, V., & Stephen, M. (1988). Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature, 332*, 459–461.

Brinster, R. L. (1969). Incorporation of carbon from glucose and pyruvate into the preimplantation mouse embryo. *Experimental Cell Research*, *58*(1), 153–158. https://doi.org/10.1016/0014-4827(69)90125-6

Brosens, J. J., Parker, M. G., McIndoe, A., Pijnenborg, R., & Brosens, I. A. (2009). A role for menstruation in preconditioning the uterus for successful pregnancy. *American Journal of Obstetrics and Gynecology*, *200*(6), 615.e1-615.e6. https://doi.org/10.1016/j.ajog.2008.11.037

Brown, J. J. G., & Whittingham, D. G. (1991). The roles of pyruvate, lactate and glucose during preimplantation development of embryos from F1 hybrid mice in vitro. *Development*, *112*(1), 99–105. https://doi.org/10.1242/dev.112.1.99

Browner, M. F., Nakano, K., Bang, A. G., & Fletterick, R. J. (1989). Human muscle glycogen synthase cDNA sequence: A negatively charged protein with an asymmetric charge distribution. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(5), 1443–1447. https://doi.org/10.1073/pnas.86.5.1443

Burton, G. J., Watson, A. L., Hempstock, J., Skepper, J. N., & Jauniaux, E. (2002). Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. *Journal of Clinical Endocrinology and Metabolism*, *87*(6), 2954–2959. https://doi.org/10.1210/jcem.87.6.8563

Burwinkel, B., Bakker, H. D., Herschkovitz, E., Moses, S. W., Shin, Y. S., & Kilimann, M. W.

(1998). Mutations in the liver glycogen phosphorylase gene (PYGL) underlying glycogenosis type VI (Hers disease). *American Journal of Human Genetics*, *62*(4), 785–791. https://doi.org/10.1086/301790

Butcher, R. L., Collins, W. E., & Fugo, N. W. (1974). Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-Day estrous cycle of the rat. *Endocrinology*, *94*(6), 1704–1708. https://doi.org/10.1210/endo-94-6-1704

Byers, S. L., Wiles, M. V., Dunn, S. L., & Taft, R. A. (2012). Mouse estrous cycle identification tool and images. *PLoS ONE*, *7*(4), 2–6. https://doi.org/10.1371/journal.pone.0035538

Cagnone, G. L. M., Dufort, I., Vigneault, C., & Sirard, M. A. (2012). Differential gene expression profile in bovine blastocysts resulting from hyperglycemia exposure during early cleavage stages. *Biology of Reproduction*, *86*(2), 1–12. https://doi.org/10.1095/biolreprod.111.094391

Caligioni, C. S. (2009). Assessing Reproductive Status/Stages in Mice. *Current Protocols in Neuroscience*, *48*(1), 1–11. https://doi.org/10.1002/0471142301.nsa04is48

Carayannopoulos, M. O., Chi, M. M. Y., Cui, Y., Pingsterhaus, J. M., McKnight, R. A., Mueckler, M., Devaskar, S. U., & Moley, K. H. (2000). GLUT8 is a glucose transporter responsible for insulin-stimulated glucose uptake in the blastocyst. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(13), 7313–7318. https://doi.org/10.1073/pnas.97.13.7313

Carrington, L. J., & Bailey, C. J. (1985). Effects of natural and synthetic estrogens and progestins on glycogen deposition in female mice. *Hormone Research in Paediatrics*, *21*(3), 199–203. https://doi.org/10.1159/000180045

Carter, A. M., & Enders, A. C. (2013). The evolution of epitheliochorial placentation. *Annual*

Review of Animal Biosciences, *1*, 443–467. https://doi.org/10.1146/annurev-animal-031412-103653

Catalini, L., & Fedder, J. (2020). Characteristics of the endometrium in menstruating species: Lessons learned from the animal kingdom. *Biology of Reproduction*, *102*(6), 1160–1169. https://doi.org/10.1093/biolre/ioaa029

Cecil, H. C., Bitman, J., Connolly, M. R., & Wrenn, T. R. (1962). Glycogen in the decidual tissue of the rat uterus. *Journal of Endocrinology*, *25*(1), 69–76. https://doi.org/10.1677/joe.0.0250069

Chatot, C. ., Ziomek, C. A., Bavister, B. D., Lewis, J. L., & Torres, I. (1989). An improved culture medium supports development of random-bred 1-cell mouse Embryos in iitro. *Journal of Reproduction and Fertility*, *86*, 679–688.

Christie, G. A. (1966). Implantation of the rat embryo: glycogen and alkaline phosphatases. *Journal of Reproduction and Fertility*, *12*(2), 279–294. https://doi.org/10.1530/jrf.0.0120279

Conaghan, J., Handyside, A. H., Winston, R. M. ., & Leese, J. (1993). Effects of pyruvate and glucose on the development of human preimplantation embryos in vitro. *Journal of Reproduction and Fertility*, 87–96.

Crowe, M. A. (2022). Reproduction, events and management: estrous cycles: characteristics. In *Encyclopedia of Dairy Sciences* (Third Edit, Vol. 1). Elsevier. https://doi.org/10.1016/b978-0-12-818766-1.00079-9

Croy, B. A., Chantakru, S., Esadeg, S., Ashkar, A. A., & Wei, Q. (2002). Decidual natural killer cells: Key regulators of placental development (a review). *Journal of Reproductive Immunology*, *57*(1–2), 151–168. https://doi.org/10.1016/S0165-0378(02)00005-0

Dan-Goor, M., Sasson, S., Davarashvili, A., & Almagor, M. (1998). Expression of glucose transporter and glucose uptake in human oocytes and preimplantation embryos. *Human Reproduction*, *12*(11), 2508–2510. https://doi.org/10.1093/humrep/12.11.2508

Dean, M. (2019). Glycogen in the uterus and fallopian tubes is an important source of glucose during early pregnancy. *Biology of Reproduction*, *101*(2), 297–305. https://doi.org/10.1093/biolre/ioz102

Dean, M., Hunt, J., McDougall, L., & Rose, J. (2014). Uterine glycogen metabolism in mink during estrus, embryonic diapause and pregnancy. *Journal of Reproduction and Development*, *60*(6), 438–446. https://doi.org/10.1262/jrd.2014-013

Demers, L. M., & Jacobs, R. D. (1973a). Hormonal regulation of rat uterine glycogen metabolism. *Biology of Reproduction*, *9*(3), 272–278. https://doi.org/10.1093/biolreprod/9.3.272

Demers, L. M., & Jacobs, R. D. (1973b). Comparative effects of ovarian steroids on glycogen metabolism of rat, rabbit and guinea pig uterine tissue. *Experimental Biology and Medicine*, *143*(4), 1158–1163. https://doi.org/10.3181/00379727-143-37491

Demers, L. M., Yoshinaga, K., & Greep, R. O. (1972). Uterine glycogen metabolism of the rat in early pregnancy. *Biology of Reproduction*, *7*(2), 297–304. https://doi.org/10.1093/biolreprod/7.2.297

Demers, Laurence M., MacDonald, G. J., & Greep, R. O. (1973). Hormonal control of endometrial glycogen metabolism in the Macaca arctoides. *American Journal of Physical Anthropology*, *38*(2), 183–187. https://doi.org/10.1002/ajpa.1330380207

Demir, R., Kayisli, U. A., Celik-Ozenci, C., Korgun, E. T., Demir-Weusten, A. Y., & Arici, A. (2002). Structural differentiation of human uterine luminal and glandular epithelium during

early pregnancy: An ultrastructural and immunohistochemical study. *Placenta*, *23*(8–9), 672–684. https://doi.org/10.1053/plac.2002.0841

Dey, S. K., Lim, H., Das, S. K., Reese, J., Paria, B. C., Daikoku, T., & Wang, H. (2004). Molecular cues to implantation. *Endocrine Reviews*, *25*(3), 341–373. https://doi.org/10.1210/er.2003-0020

Diril, M. K., Schmidt, S., Krauß, M., Gawlik, V., Joost, H. G., Schürmann, A., Haucke, V., & Augustin, R. (2009). Lysosomal localization of GLUT8 in the testis - The EXXXLL motif of GLUT8 is sufficient for its intracellular sorting via AP1- and AP2-mediated interaction. *FEBS Journal*, *276*(14), 3729–3743. https://doi.org/10.1111/j.1742-4658.2009.07089.x

Dockery, P., Li, T. C., Rogers, A. W., Cooke, I. D., & Lenton, E. A. (1988). The ultrastructure of the glandular epithelium in the timed endometrial biopsy. *Human Reproduction*, *3*(7), 826–834. https://doi.org/10.1093/oxfordjournals.humrep.a136793

Dockery, P., Pritchard, K., Taylor, A., Li, T. C., Warren, M. A., & Cooke, I. D. (1993). The fine structure of the human endometrial glandular epithelium in cases of unexplained infertility: A morphometric study. *Human Reproduction*, *8*(5), 667–673. https://doi.org/10.1093/oxfordjournals.humrep.a138117

Emera, D., Romero, R., & Wagner, G. (2012). The evolution of menstruation: A new model for genetic assimilation: Explaining molecular origins of maternal responses to fetal invasiveness. *BioEssays*, *34*(1), 26–35. https://doi.org/10.1002/bies.201100099

Enders, A. C., & Schlafke, S. (1967). A morphological analysis of the early implantation stages in the rat. *American Journal of Anatomy*, *120*(2), 185–225. https://doi.org/10.1002/aja.1001200202

Enders, A. C., & Schlafke, S. (1969). Cytological aspects of trophoblast-uterine interaction in

early implantation. *American Journal of Anatomy*, *125*(1), 1–29. https://doi.org/10.1002/aja.1001250102

Ercan-Fang, N., Gannon, M. C., Rath, V. L., Treadway, J. L., Taylor, M. R., & Nuttall, F. Q. (2002). Integrated effects of multiple modulators on human liver glycogen phosphorylase a. *American Journal of Physiology - Endocrinology and Metabolism*, *283*(1 46-1), 29–37. https://doi.org/10.1152/ajpendo.00425.2001

Favaro, R., Abrahamsohn, P. A., & Zorn, M. T. (2014). Decidualization and Endometrial Extracellular Matrix Remodeling. In *The Guide to Investigation of Mouse Pregnancy* (pp. 125–142). Elsevier. https://doi.org/10.1016/B978-0-12-394445-0.00011-4

Favaro, R. R., Salgado, R. M., Covarrubias, A. C., Bruni, F., Lima, C., Fortes, Z. B., & Zorn, T. M. (2013). Long-term type 1 diabetes impairs decidualization and extracellular matrix remodeling during early embryonic development in mice. *Placenta*, *34*(12), 1128–1135. https://doi.org/10.1016/j.placenta.2013.09.012

Finn, C. A., & Lawn, A. M. (1967). Specialized junctions between decidual cells in the uterus of the pregnant mouse. *Journal of Ultrasructure Research*, *20*(5–6), 321–327. https://doi.org/10.1016/S0022-5320(67)80102-3

Flannery, C. A., Choe, G. H., Cooke, K. M., Fleming, A. G., Radford, C. C., Kodaman, P. H., Jurczak, M. J., Kibbey, R. G., & Taylor, H. S. (2018). Insulin regulates glycogen synthesis in human endometrial glands through increased GYS2. *Journal of Clinical Endocrinology and Metabolism*, *103*(8), 2843–2850. https://doi.org/10.1210/jc.2017-01759

Fox, C., Morin, S., Jeong, J. W., Scott, R. T., & Lessey, B. A. (2016). Local and systemic factors and implantation: What is the evidence? *Fertility and Sterility*, *105*(4), 873–884. https://doi.org/10.1016/j.fertnstert.2016.02.018

Fraser, R. B., Waite, S. L., Wood, K. A., & Martin, K. L. (2007). Impact of hyperglycemia on early embryo development and embryopathy: in vitro experiments using a mouse model. *Human Reproduction*, *22*(12), 3059–3068. https://doi.org/10.1093/humrep/dem318

Frolova, A., Flessner, L., Chi, M., Kim, S. T., Foyouzi-Yousefi, N., & Moley, K. H. (2009). Facilitative glucose transporter type 1 is differentially regulated by progesterone and estrogen in murine and human endometrial stromal cells. *Endocrinology*, *150*(3), 1512–1520. https://doi.org/10.1210/en.2008-1081

Frolova, A. I., & Moley, K. H. (2011a). Glucose transporters in the uterus: an analysis of tissue distribution and proposed physiological roles. *Physiology & Behavior*, *176*(3), 139–148. https://doi.org/10.1530/REP-11-0114.Glucose

Frolova, A. I., & Moley, K. H. (2011b). Quantitative analysis of glucose transporter mRNAs in endometrial stromal cells reveals critical role of GLUT1 in uterine receptivity. *Endocrinology*, *152*(5), 2123–2128. https://doi.org/10.1210/en.2010-1266

Frolova, A. I., O'Neill, K., & Moley, K. H. (2011). Dehydroepiandrosterone inhibits glucose flux through the pentose phosphate pathway in human and mouse endometrial stromal cells, preventing decidualization and implantation. *Molecular Endocrinology*, *25*(8), 1444–1455. https://doi.org/10.1210/me.2011-0026

Gardner, D. K., Lane, M., Calderon, I., & Leeton, J. (1996). Environment of the preimplantation human embryo in vivo: Metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. *Fertility and Sterility*, *65*(2), 349–353. https://doi.org/10.1016/s0015-0282(16)58097-2

Gardner, D. K., Lane, M., & Schoolcraft, W. B. (2000). Culture and transfer of viable blastocysts: A feasible proposition for human IVF. *Human Reproduction*, *15*(SUPPL. 6), 9–

23.

Gardner, D. K., & Leese, H. J. (1990). Concentrations of nutrients in mouse oviduct fluid and their effects on embryo development and metabolism in vitro. *Journal of Reproduction and Fertility*, *88*(1), 361–368. https://doi.org/10.1530/jrf.0.0880361

Gardner, David K., Lane, M., & Batt, P. (1993). Uptake and metabolism of pyruvate and glucose by individual sheep preattachment embryos developed in vivo. *Molecular Reproduction and Development*, *36*(3), 313–319. https://doi.org/10.1002/mrd.1080360305

Gardner, David K., Wale, P. L., Collins, R., & Lane, M. (2011). Glucose consumption of single post-compaction human embryos is predictive of embryo sex and live birth outcome. *Human Reproduction*, *26*(8), 1981–1986. https://doi.org/10.1093/humrep/der143

Garrido-Gomez, T., Dominguez, F., Quiñonero, A., Diaz-Gimeno, P., Kapidzic, M., Gormley, M., Ona, K., Padilla-Iserte, P., McMaster, M., Genbacev, O., Perales, A., Fisher, S. J., & Simón, C. (2017). Defective decidualization during and after severe preeclampsia reveals a possible maternal contribution to the etiology. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(40), E8468–E8477. https://doi.org/10.1073/pnas.1706546114

Gellersen, B., & Brosens, J. J. (2014). Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocrine Reviews*, *35*(6), 851–905. https://doi.org/10.1210/er.2014-1045

Gibbons, B. J., Roach, P. J., & Hurley, T. D. (2002). Crystal structure of the autocatalytic initiator of glycogen biosynthesis, glycogenin. *Journal of Molecular Biology*, *319*(2), 463–477. https://doi.org/10.1016/S0022-2836(02)00305-4

Golander, A., Hurley, T., Barrett, J., Hizi, A., & Handwerger, S. (1979). Prolactin synthesis by

human chorion-decidual tissue: A possible source of prolactin in the amniotic fluid. *Obstetrical and Gynecological Survey*, *34*(3), 225–226. https://doi.org/10.1097/00006254-197903000-00017

Gray, C. A., Taylor, K. M., Ramsey, W. S., Hill, J. R., Bazer, F. W., Bartol, F. F., & Spencer, T. E. (2001). Endometrial glands are required for preimplantation conceptus elongation and survival. *Biology of Reproduction*, *64*(6), 1608–1613. https://doi.org/10.1095/biolreprod64.6.1608

Greenstreet, R., & Fotherby, K. (1973a). Carbohydrate metabolism in rat uterus during the oestrous cycle. *Steroids and Lipids Research*, *4*(5), 341–350. http://www.ncbi.nlm.nih.gov/pubmed/4368470

Greenstreet, R., & Fotherby, K. (1973b). Carbohydrate metabolism in the rat uterus during early pregnancy. *Steroids and Lipids Research*, *4*(1), 48–64. http://www.ncbi.nlm.nih.gov/pubmed/4353464

Gregoire, A. T., Ramsey, H., & Adams, A. (1967). The Effect of Various Doses of Oestradiol 17-β on Glycogen Deposition in the Rat Uterus, Cervis and Vagina. *Reproduction*, *14*(2), 231–234. https://doi.org/10.1530/jrf.0.0140231

Grossbard, L., & Schimke, R. T. (1966). Multiple hexokinases of rat tissues. Purification and comparison of soluble forms. *Journal of Biological Chemistry*, *241*(15), 3546–3560. https://doi.org/10.1016/S0021-9258(18)99866-2

Guerrero, R., Landgren, B.-M., Montiel, R., Cekan, Z., & Diczfalusy, E. (1975). Unconjugated steroids in the human endometrium. *Contraception*, *11*(2), 169–177. https://doi.org/10.1016/0010-7824(75)90028-1

Gupta, A., Mathur, S. K., & Gupta, A. (2013). Co-orelation of histological dating and glycogen

content by histochemical stain during various phases of menstrual cycle in primary infertility. *Open Journal of Pathology*, *03*(02), 65–68. https://doi.org/10.4236/ojpathology.2013.32012

Hamatani, T., Carter, M. G., Sharov, A. A., & Ko, M. S. H. (2004). Dynamics of global gene expression changes during mouse preimplantation development. *Developmental Cell*, *6*(1), 117–131. https://doi.org/10.1016/S1534-5807(03)00373-3

Hamlett, G. W. D. (1934). Uterine bleeding in a bat, Glossophaga soricina. *The Anatomical Record*, *60*(1), 9–17. https://doi.org/10.1002/ar.1090600104

Harris, L. K., Benagiano, M., D'Elios, M. M., Brosens, I., & Benagiano, G. (2019). Placental bed research: II. Functional and immunological investigations of the placental bed. *American Journal of Obstetrics and Gynecology*, *221*(5), 457–469. https://doi.org/10.1016/j.ajog.2019.07.010

Harris, R. A. (2013). Glycolysis Overview. In *Encyclopedia of Biological Chemistry: Second Edition* (2nd ed., Vol. 1). Elsevier Inc. https://doi.org/10.1016/B978-0-12-378630-2.00044-X

Harris, S. E., Gopichandran, N., Picton, H. M., Leese, H. J., & Orsi, N. M. (2005). Nutrient concentrations in murine follicular fluid and the female reproductive tract. *Theriogenology*, *64*(4), 992–1006. https://doi.org/10.1016/j.theriogenology.2005.01.004

Herington, J. L., Underwood, T., McConaha, M., & Bany, B. M. (2009). Paracrine signals from the mouse conceptus are not required for the normal progression of decidualization. *Endocrinology*, *150*(9), 4404–4413. https://doi.org/10.1210/en.2009-0036

Horecker, B. L. (2002). The pentose phosphate pathway. *The Journal of Biological Chemistry*, *277*(50), 47965–47971. https://doi.org/10.1074/jbc.X200007200

Horst, C. J. van der. (1954). Elephantulus going into anoestrus; menstruation and abortion. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *238*(653), 27–61. https://doi.org/10.1098/rstb.1954.0005

Hsieh, B., Chi, M. M. Y., Knor, J., & Lowry, O. H. (1979). Enzymes of glycogen metabolism and related metabolites in preimplantation mouse embryos. *Developmental Biology*, *72*(2), 342–349. https://doi.org/10.1016/0012-1606(79)90123-4

Huang, C., Jiang, Y., Zhou, J., Yan, Q., Jiang, R., Cheng, X., Xing, J., Ding, L., Sun, J., Yan, G., & Sun, H. (2017). Increased Krüppel-like factor 12 in recurrent implantation failure impairs endometrial decidualization by repressing Nur77 expression. *Reproductive Biology and Endocrinology*, *15*(1), 1–14. https://doi.org/10.1186/s12958-017-0243-8

Hugentobler, S. A., Humpherson, P. G., Leese, H. J., Sreenan, J. M., & Morris, D. G. (2008). Energy substrates in bovine oviduct and uterine fluid and blood plasma during the oestrous cycle. *Molecular Reproduction and Development*, *75*(3), 496–503. https://doi.org/10.1002/mrd.20760

Hugentobler, S. A., Sreenan, J. M., Humpherson, P. G., Leese, H. J., Diskin, M. G., & Morris, D. G. (2010). Effects of changes in the concentration of systemic progesterone on ions, amino acids and energy substrates in cattle oviduct and uterine fluid and blood. *Reproduction, Fertility and Development*, *22*(4), 684–694. https://doi.org/10.1071/RD09129

Hughes, E. C., Demers, L. M., Csermely, T., & Jones, D. B. (1969). Organ culture of human endometrium. Effect of ovarian steroids. *American Journal of Obstetrics and Gynecology*, *105*(5), 707–720. https://doi.org/10.1016/0002-9378(69)90007-6

Huhtinen, K., Desai, R., Stahle, M., Salminen, A., Handelsman, D. J., Perheentupa, A., & Poutanen, M. (2012). Endometrial and endometriotic concentrations of estrone and estradiol

are determined by local metabolism rather than circulating levels. *Journal of Clinical Endocrinology and Metabolism*, *97*(11), 4228–4235. https://doi.org/10.1210/jc.2012-1154

Hutton, J. C., & O'Brien, R. M. (2009). Glucose-6-phosphatase catalytic subunit gene family. *Journal of Biological Chemistry*, *284*(43), 29241–29245. https://doi.org/10.1074/jbc.R109.025544

Iino, K., Fukuhara, R., Yokota, M., & Yokoyama, Y. (2022). Fertility awareness and subclinical infertility among women trying to get pregnant at home. *BMC Women's Health*, *22*(1), 1–9. https://doi.org/10.1186/s12905-022-01626-z

Ishihara, S., Taketani, Y., & Mizuno, M. (1988). Inhibitory effects of estradiol on glycogen synthesis in primary cell cultures of human endometrium. *Endocrinol Japon*, *35*(5), 691–696.

Jeong, J. W., Kwak, I., Lee, K. Y., Kim, T. H., Large, M. J., Stewart, C. L., Kaestner, K. H., Lydon, J. P., & DeMayo, F. J. (2010). Foxa2 is essential for mouse endometrial gland development and fertility. *Biology of Reproduction*, *83*(3), 396–403. https://doi.org/10.1095/biolreprod.109.083154

Jones, C. J. P., Choudhury, R. H., & Aplin, J. D. (2015). Tracking nutrient transfer at the human maternofetal interface from 4 weeks to term. *Placenta*, *36*(4), 372–380. https://doi.org/10.1016/j.placenta.2015.01.002

Jun, H. S., Cheung, Y. Y., Lee, Y. M., Mansfield, B. C., & Chou, J. Y. (2012). Glucose-6-phosphatase-β, implicated in a congenital neutropenia syndrome, is essential for macrophage energy homeostasis and functionality. *Blood*, *119*(17), 4047–4055. https://doi.org/10.1182/blood-2011-09-377820

Kaya, H. S., Hantak, A. M., Stubbs, L. J., Taylor, R. N., Bagchi, I. C., & Bagchi, M. K. (2015).

Roles of progesterone receptor A and B isoforms during human endometrial decidualization. *Molecular Endocrinology*, *29*(6), 882–895. https://doi.org/10.1210/me.2014-1363

Kelleher, A. M., Milano-Foster, J., Behura, S. K., & Spencer, T. E. (2018). Uterine glands coordinate on-time embryo implantation and impact endometrial decidualization for pregnancy success. *Nature Communications*, *9*(1), 1–12. https://doi.org/10.1038/s41467-018-04848-8

Kim, S. T., & Moley, K. H. (2009). Regulation of facilitative glucose transporters and AKT/MAPK/PRKAA signaling via estradiol and progesterone in the mouse uterine epithelium. *Biology of Reproduction*, *81*(1), 188–198. https://doi.org/10.1095/biolreprod.108.072629

Koopman, L. A., Kopcow, H. D., Rybalov, B., Boyson, J. E., Orange, J. S., Schatz, F., Masch, R., Lockwood, C. J., Schachter, A. D., Park, P. J., & Strominger, J. L. (2003). Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *Journal of Experimental Medicine*, *198*(8), 1201–1212. https://doi.org/10.1084/jem.20030305

Korgun, E. T., Demir, R., Hammer, A., Dohr, G., Desoye, G., Skofitsch, G., & Hahn, T. (2001). Glucose transporter expression in rat embryo and uterus during decidualization, implantation, and early postimplantation. *Biology of Reproduction*, *65*(5), 1364–1370. https://doi.org/10.1095/biolreprod65.5.1364

Kosaka, T., Saito, T., & Takahashi, K. (1988). Changes in plasma progesterone levels during the estrous cycle and preganacy in 4-day cyclic mice. *Experimental Animals*, *37*(3), 351–353.

Lash, G. E., Pitman, H., Morgan, H. L., Innes, B. A., Agwu, C. N., & Bulmer, J. N. (2016).

Decidual macrophages: key regulators of vascular remodeling in human pregnancy. *Journal of Leukocyte Biology*, *100*(2), 315–325. https://doi.org/10.1189/jlb.1a0815-351r

Leese, H. J., & Barton, A. M. (1984). Pyruvate and glucose uptake by mouse ova and preimplantation embryos. *Reproduction*, *72*(1), 9–13. https://doi.org/10.1530/jrf.0.0720009

Li, R., Wang, X., Huang, Z., Balaji, J., Kim, T. H., Wang, T., Zhou, L., Deleon, A., Cook, M. E., Marbrey, M. W., Wu, S. P., Jeong, J. W., Arora, R., & DeMayo, F. J. (2021). The role of epithelial progesterone receptor isoforms in embryo implantation. *IScience*, *24*(12), 103487. https://doi.org/10.1016/j.isci.2021.103487

Liang, Y. X., Liu, L., Jin, Z. Y., Liang, X. H., Fu, Y. S., Gu, X. W., & Yang, Z. M. (2018). The high concentration of progesterone is harmful for endometrial receptivity and decidualization. *Scientific Reports*, *8*(1), 1–12. https://doi.org/10.1038/s41598-017-18643-w

Lubahn, D. B., Moyer, J. S., Golding, T. S., Couse, J. F., Korach, K. S., & Smithies, O. (1993). Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(23), 11162–11166. https://doi.org/10.1073/pnas.90.23.11162

Lydon, J. P., DeMayo, F. J., Funk, C. R., Mani, S. K., Hughes, A. R., Montgomery, C. A., Shyamala, G., Conneely, O. M., & O'Malley, B. W. (1995). Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes and Development*, *9*(18), 2266–2278. https://doi.org/10.1101/gad.9.18.2266

Ma, W. ge, Song, H., Das, S. K., Paria, B. C., & Dey, S. K. (2003). Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(5),

2963–2968. https://doi.org/10.1073/pnas.0530162100

Macklon, N. S., Geraedts, J. P. M., & Fauser, B. C. J. M. (2002). Conception to ongoing pregnancy: The “black box” of early pregnancy loss. *Human Reproduction Update*, *8*(4), 333–343. https://doi.org/10.1093/humupd/8.4.333

Maeyama, M., Sudo, I., Saito, K., Matsuo, I., & Nakahara, K. (1977). Glycogen estimation by a rapid enzymic method in very small samples of human endometrium: Glycogen content in the endometrium of infertile patients during the menstrual cycle. *Fertility and Sterility*, *28*(2), 159–162. https://doi.org/10.1016/s0015-0282(16)42375-7

Malfatti, E., Nilsson, J., Hedberg-Oldfors, C., Hernez-Lain, A., Michel, F., Dominguez-Gonzalez, C., Viennet, G., Orhan Akman, H., Kornblum, C., Van Bergh, P. Den, Romero, N. B., Engel, A. G., DiMauro, S., & Oldfors, A. (2014). A new muscle glycogen storage disease associated with glycogenin-1 deficiency. *Annals of Neurology*, *76*(6), 891–898. https://doi.org/10.1002/ana.24284

Marcolongo, P., Fulceri, R., Gamberucci, A., Czegle, I., Banhegyi, G., & Benedetti, A. (2013). Multiple roles of glucose-6-phosphatases in pathophysiology: State of the art and future trends. *Biochimica et Biophysica Acta - General Subjects*, *1830*(3), 2608–2618. https://doi.org/10.1016/j.bbagen.2012.12.013

Martin, K. L., & Leese, H. J. (1995). Role of glucose in mouse preimplantation embryo development. *Molecular Reproduction and Development*, *40*(4), 436–443. https://doi.org/10.1002/mrd.1080400407

Martin, L., & Finn, C. A. (1968). Hormonal regulation of cell division in epithelial and connective tissues of the mouse uterus. *The Journal of Endocrinology*, *41*(3), 363–371. https://doi.org/10.1677/joe.0.0410363

Matsuo, I. (1978). Studies on the Activities of Glycogen Synthetase and Glycogen Phosphorylase in the Human Endometrium. *Folia Endocrinologica Japonica*, *54*(6), 759–769. https://doi.org/10.1507/endocrine1927.54.6_759

Michael, S. D. (1976). Plasma prolactin and progesterone during the estrous cycle in the mouse. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)*, *153*(2), 254–257. https://doi.org/10.3181/00379727-153-39522

Moffett, A., & Loke, C. (2006). Immunology of placentation in eutherian mammals. *Nature Reviews Immunology*, *6*(8), 584–594. https://doi.org/10.1038/nri1897

Moore, E. L., Wang, S., & Larina, I. V. (2019). Staging mouse preimplantation development in vivo using optical coherence microscopy. *Journal of Biophotonics*, *12*(5), 167–182. https://doi.org/10.1002/jbio.201800364

Mori, M., Bogdan, A., Balassa, T., Csabai, T., & Szekeres-Bartho, J. (2016). The decidua—the maternal bed embracing the embryo—maintains the pregnancy. *Seminars in Immunopathology*, *38*(6), 635–649. https://doi.org/10.1007/s00281-016-0574-0

Mulac-Jericevic, B., Mullinax, R. A., DeMayo, F. J., Lydon, J. P., & Conneely, O. M. (2000). Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. *Science*, *289*(5485), 1751–1754. https://doi.org/10.1126/science.289.5485.1751

Mulac-Jericevic, Biserka, Lydon, J. P., DeMayo, F. J., & Conneely, O. M. (2003). Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(17), 9744–9749. https://doi.org/10.1073/pnas.1732707100

Munger, R., Temler, E., Jallut, D., Haesler, E., & Felber, J.-P. (1993). Correlations of glycogen

synthase and phosphrylase activities with glycogen concentration in human muscle biopsies. Evidence for a Double-Feedback mechanism regulation glycogen synthesis and breakdown. *Metabolism*, *42*(1), 36–43.

Murphy, C. R. (1993). The plasma membrane of uterine epithelial cells: structure and histochemistry. *Progress in Histochemistry and Cytochemistry*, *27*(3), 3–5. https://doi.org/10.1016/S0079-6336(11)80004-5

Navale, A. M., & Paranjape, A. N. (2016). Glucose transporters: physiological and pathological roles. *Biophysical Reviews*, *8*(1), 5–9. https://doi.org/10.1007/s12551-015-0186-2

Ng, S. W., Norwitz, G. A., Pavlicev, M., Tilburgs, T., Simón, C., & Norwitz, E. R. (2020). Endometrial decidualization: The primary driver of pregnancy health. *International Journal of Molecular Sciences*, *21*(11), 1–20. https://doi.org/10.3390/ijms21114092

Niakan, K. K., Han, J., Pedersen, R. A., Simon, C., & Pera, R. A. R. (2012). Human pre-implantation embryo development. *Development*, *139*(5), 829–841. https://doi.org/10.1242/dev.060426

Norwitz, E. R., Schust, D. J., & Fisher, S. J. (2001). Implantation and the survival of early pregnancy. *New England Journal of Medicine*, *345*(19), 1400–1408. https://doi.org/10.1056/nejmra000763

Nuttall, F. Q., Gannon, M. C., Bai, G., & Lee, E. Y. C. (1994). Primary structure of human liver glycogen synthase deduced by cDNA cloning. *Archives of Biochemistry and Biophysics*, *311*(2), 443–449. https://doi.org/10.1006/abbi.1994.1260

Pantaleon, M., Tan, H. Y., Kafer, G. R., & Kaye, P. L. (2010). Toxic effects of hyperglycemia are mediated by the hexosamine signaling pathway and O-linked glycosylation in early mouse embryos. *Biology of Reproduction*, *82*(4), 751–758.

https://doi.org/10.1095/biolreprod.109.076661

Parandoosh, Z., Crombie, D. L., Tetzke, T. A., Hayes, J. S., Heap, R. B., & Wang, M. (1995). Progesterone and oestrogen receptors in the decidualized mouse uterus and effects of different types of anti-progesterone treatment. *Reproduction*, *105*(2), 215–220. https://doi.org/10.1530/jrf.0.1050215

Paria, B. C., Tan, J., Lubahn, D. B., Dey, S. K., & Das, S. K. (1999). Uterine decidual response occurs in estrogen receptor-α-deficient mice. *Endocrinology*, *140*(6), 2704–2710. https://doi.org/10.1210/endo.140.6.6825

Parr, M. B., & Parr, E. L. (1986). Permeability of the primary decidual zone in the rat uterus: Studies using fluorescein-labeled proteins and dextrans. *Biology of Reproduction*, *34*(2), 393–403. https://doi.org/10.1095/biolreprod34.2.393

Patra, K. C., & Hay, N. (2014). The pentose phosphate pathway and cancer. *Trends in Biochemical Sciences*, *39*(8), 347–354. https://doi.org/10.1016/j.tibs.2014.06.005

Pike, I. L., & Wales, R. G. (1982). Uptake and incorporation of glucose especially into the glycogen pools of preimplantation mouse embryos during culture in vitro. *Australian Journal of Biological Sciences*, *35*(2), 195–206. https://doi.org/10.1071/BI9820195

Pohler, K. G., Franco, G. A., Reese, S. T., & Smith, M. F. (2019). Physiology and pregnancy of beef cattle. In *Animal Agriculture: Sustainability, Challenges and Innovations*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-817052-6.00003-3

Potts, D. M. (1968). The ultrastructure of implantation in the mouse. *Journal of Anatomy*, *103*(1), 77–90.

Printz, R. L., Koch, S., Potter, L. R., O'Doherty, R. M., Tiesinga, J. J., Moritz, S., & Granner, D. K. (1993). Hexokinase II mRNA and gene structure, regulation by insulin, and evolution.

Journal of Biological Chemistry, *268*(7), 5209–5219. https://doi.org/10.1016/s0021-9258(18)53521-3

Purcell, S. H., & Moley, K. H. (2009). Glucose transporters in gametes and preimplantation embryos. *Trends in Endocrinology & Metabolism*, *20*(10), 483–489. https://doi.org/10.1016/j.tem.2009.06.006

Quinn, P. (1995). Enhanced results in mouse and human embryo culture using a modified human tubal fluid medium lacking glucose and phosphate. *Journal of Assisted Reproduction and Genetics*, *12*(2), 97–105. https://doi.org/10.1007/BF02211377

Rasweiler, J. J. (1991). Spontaneous decidual reactions and menstruation in the black mastiff bat, Molossus ater. *American Journal of Anatomy*, *191*(1), 1–22. https://doi.org/10.1002/aja.1001910102

Riddick, D. H., Luciano, A. A., Kusmik, W. F., & Maslar, I. A. (1978). De Novo synthesis of prolactin by human decidua. *Life Sciences*, *23*(19), 1913–1921. https://doi.org/10.1016/0024-3205(78)90557-X

Roach, P. J., Depaoli-Roach, A. A., Hurley, T. D., & Tagliabracci, V. S. (2012). Glycogen and its metabolism: Some new developments and old themes. In *Biochemical Journal* (Vol. 441, Issue 3, pp. 763–787). https://doi.org/10.1042/BJ20111416

Rosenbaum, R. M., & Goolsby, C. M. (1957). The histochemical demonstration of hormonally controlled, intracellular glycogen in the endometrium of the rat. *Journal of Histochemistry & Cytochemistry*, *5*(1), 33–46. https://doi.org/10.1177/5.1.33

Rubulis, A., Jacobs, R. D., & Hughes, E. C. (1965). Glycogen synthetase in mammalian uterus. *Biochimica et Biophysica Acta*, *99*(3), 584–586. https://doi.org/10.1016/s0926-6593(65)80222-3

Ryu, J. H., Drain, J., Kim, J. H., McGee, S., Gray-Weale, A., Waddington, L., Parker, G. J., Hargreaves, M., Yoo, S. H., & Stapleton, D. (2009). Comparative structural analyses of purified glycogen particles from rat liver, human skeletal muscle and commercial preparations. *International Journal of Biological Macromolecules*, *45*(5), 478–482. https://doi.org/10.1016/j.ijbiomac.2009.08.006

Salker, M., Teklenburg, G., Molokhia, M., Lavery, S., Trew, G., Aojanepong, T., Mardon, H. J., Lokugamage, A. U., Rai, R., Landles, C., Roelen, B. A. J., Quenby, S., Kuijk, E. W., Kavelaars, A., Heijnen, C. J., Regan, L., Macklon, N. S., & Brosens, J. J. (2010). Natural selection of human embryos: Impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. *PLoS ONE*, *5*(4). https://doi.org/10.1371/journal.pone.0010287

Sandoval, K., Berg, M. D., Guadagnin, A. R., Cardoso, F. C., & Dean, M. (2021). Endometrial glycogen metabolism on days 1 and 11 of the reproductive cycle in dairy cows. *Animal Reproduction Science*, *233*(December 2020), 106827. https://doi.org/10.1016/j.anireprosci.2021.106827

Schaftingen, E., & Gerin, I. (2002). The glucose-6-phosphatase system. *Biochemical Journal*, *362*(3), 513. https://doi.org/10.1042/0264-6021:3620513

Schini, S. A., & Bavister, B. D. (1988). Two-cell block to development of cultured hamster embryos is caused by phosphate and glucose. *Biology of Reproduction*, *39*(5), 1183–1192. https://doi.org/10.1095/biolreprod39.5.1183

Simpson, I. A., Dwyer, D., Malide, D., Moley, K. H., Travis, A., & Vannucci, S. J. (2008). The facilitative glucose transporter GLUT3: 20 Years of distinction. *American Journal of Physiology - Endocrinology and Metabolism*, *295*(2), 242–253.

https://doi.org/10.1152/ajpendo.90388.2008

Skurat, A. V., Dietrich, A. D., & Roach, P. J. (2006). Interaction between glycogenin and glycogen synthase. *Archives of Biochemistry and Biophysics*, *456*(1), 93–97. https://doi.org/10.1016/j.abb.2006.09.024

Spencer, T. E., Johnson, G. A., Bazer, F. W., Burghardt, R. C., & Palmarini, M. (2007). Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. *Reproduction, Fertility and Development*, *19*(1), 65. https://doi.org/10.1071/RD06102

Sroga, J. M., Ma, X., & Das, S. K. (2012). Developmental regulation of decidual cell polyploidy at the site of implantation. *Frontiers in Bioscience (Scholar Edition)*, *4*(1), 1475–1486. https://doi.org/10.2741/s347

Stern, S., & Biggers, J. D. (1968). Enzymatic estimation of glycogen in the cleaving mouse embryo. *Journal of Experimental Zoology*, *168*(1), 61–66. https://doi.org/10.1002/jez.1401680106

Stewart, C. L., Kaspar, P., Brunet, L. J., Bhatt, H., Gadi, I., Köntgen, F., & Abbondanzo, S. J. (1992). Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature*, *359*(6390), 76–79. https://doi.org/10.1038/359076a0

Su, Y., Guo, S., Liu, C., Li, N., Zhang, S., Ding, Y., Chen, X., He, J., Liu, X., Wang, Y., & Gao, R. (2020). Endometrial pyruvate kinase M2 is essential for decidualization during early pregnancy. *Journal of Endocrinology*, *245*(3), 357–368. https://doi.org/10.1530/JOE-19-0553

Tachi, S., Tachi, C., & Lindner, H. R. (1970). Ultrastructural features of blastocyst attachment and trophoblastic invasion in the rat. *Journal of Reproduction and Fertility*, *21*(1), 37–56.

https://doi.org/10.1530/jrf.0.0210037

Tan, J., Paria, B. C., Dey, S. K., & Das, S. K. (1999). Differential uterine expression of estrogen and progesterone receptors correlates with uterine preparation for implantation and decidualization in the mouse. *Endocrinology*, *140*(11), 5310–5321. https://doi.org/10.1210/endo.140.11.7148

Teklenburg, G., Salker, M., Molokhia, M., Lavery, S., Trew, G., Aojanepong, T., Mardon, H. J., Lokugamage, A. U., Rai, R., Landles, C., Roelen, B. A. J., Quenby, S., Kuijk, E. W., Kavelaars, A., Heijnen, C. J., Regan, L., Brosens, J. J., & Macklon, N. S. (2010). Natural selection of human embryos: Decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. *PLoS ONE*, *5*(4), 2–7. https://doi.org/10.1371/journal.pone.0010258

Testoni, G., Duran, J., García-Rocha, M., Vilaplana, F., Serrano, A. L., Sebastián, D., López-Soldado, I., Sullivan, M. A., Slebe, F., Vilaseca, M., Muñoz-Cánoves, P., & Guinovart, J. J. (2017). Lack of Glycogenin Causes Glycogen Accumulation and Muscle Function Impairment. *Cell Metabolism*, *26*(1), 256-266.e4. https://doi.org/10.1016/j.cmet.2017.06.008

Thompson, J. G., Simpson, A. C., Pugh, P. A., & Tervit, H. R. (1992). Requirement for glucose during in vitro culture of sheep preimplantation embryos. *Molecular Reproduction and Development*, *31*(4), 253–257. https://doi.org/10.1002/mrd.1080310405

Tong, J., Lv, S., Yang, J., Li, H., Li, W., & Zhang, C. (2022). Decidualization and related pregnancy complications. *Maternal-Fetal Medicine*, *4*(1), 24–35. https://doi.org/10.1097/fm9.0000000000000135

Tsai, J. H., Schulte, M., O'Neill, K., Chi, M. M. Y., Frolova, A. I., & Moley, K. H. (2013).

Glucosamine inhibits decidualization of human endometrial stromal cells and decreases litter sizes in mice. *Biology of Reproduction*, *89*(1), 1–10. https://doi.org/10.1095/biolreprod.113.108571

Van Blerkom, J., & Brockway, G. O. (1975). Qualitative patterns of protein synthesis in the preimplantation mouse embryo. II. During release from facultative delayed implantation. *Developmental Biology*, *46*(2), 446–451. https://doi.org/10.1016/0012-1606(75)90123-2

Verma, V. (1983). Ultrastructural changes in human endometrium at different phases of the menstrual cycle and their functional significance. *Gynecologic and Obstetric Investigation*, *15*(4), 193–212. https://doi.org/10.1159/000299412

Von Wolff, M., Ursel, S., Hahn, U., Steldinger, R., & Strowitzki, T. (2003). Glucose transporter proteins (GLUT) in human endometrium: Expression, regulation, and function throughout the menstrual cycle and in early pregnancy. *Journal of Clinical Endocrinology and Metabolism*, *88*(8), 3885–3892. https://doi.org/10.1210/jc.2002-021890

Wang, H., & Dey, S. K. (2006). Roadmap to embryo implantation: Clues from mouse models. *Nature Reviews Genetics*, *7*(3), 185–199. https://doi.org/10.1038/nrg1808

Wang, T., Wang, J., Hu, X., Huang, X., & Chen, G.-X. (2020). Current understanding of glucose transporter 4 expression and functional mechanisms. *World Journal of Biological Chemistry*, *11*(3), 76–98. https://doi.org/10.4331/wjbc.v11.i3.76

Weihua, Z., Saji, S., Mäkinen, S., Cheng, G., Jensen, E. V., Warner, M., & Gustafsson, J. Å. (2000). Estrogen receptor (ER) β, a modulator of ERα in the uterus. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(11), 5936–5941. https://doi.org/10.1073/pnas.97.11.5936

Weinhouse, S. (1976). Regulation of glucokinase in liver. In *Current Topics in Cellular*

Regulation (Vol. 11, Issue C). ACADEMIC PRESS, INC. https://doi.org/10.1016/B978-0-12-152811-9.50008-0

Wilcox, A. J., Weinberg, C. R., O'Connor, J. F., Baird, D. D., Schlatterer, J. P., Canfield, R. E., Armstrong, E. G., & Nisula, B. C. (1988). Incidence of early loss of pregnancy. *The New England Journal of Medicine*, *319*(4), 189–194. https://doi.org/10.1056/NEJM198807283190401

Yamaguchi, M., Sakata, M., Ogura, K., & Miyake, A. (1996). Gestational changes of glucose transporter gene expression in the mouse placenta and decidua. *Journal of Endocrinological Investigation*, *19*(8), 567–569. https://doi.org/10.1007/BF03349018

Yánez, A. J., Nualart, F., Droppelmann, C., Bertinat, R., Brito, M., Concha, I. I., & Slebe, J. C. (2003). Broad expression of fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase provide evidence for gluconeogenesis in human Tissues other than liver and kidney. *Journal of Cellular Physiology*, *197*(2), 189–197. https://doi.org/10.1002/jcp.10337

Yoshinaga, K. (2013). A sequence of events in the uterus prior to implantation in the mouse. *Journal of Assisted Reproduction and Genetics*, *30*(8), 1017–1022. https://doi.org/10.1007/s10815-013-0093-z

Zhai, L., Schroeder, J., Skurat, A. V., & Roach, P. J. (2001). Do rodents have a gene encoding glycogenin-2, the liver isoform of the self-glucosylating initiator of glycogen synthesis? *IUBMB Life*, *51*(2), 87–91. https://doi.org/10.1080/15216540152122076

Zhang, L. xue, Song, J. wei, Ma, Y. dan, Wang, Y. cheng, Cui, Z. hui, Long, Y., Yuan, D. zhi, Zhang, J. hu, Hu, Y., Yu, L. lin, Nie, L., & Yue, L. min. (2021). Expression of SGLT1 in the mouse endometrial epithelium and its role in early embryonic development and implantation. *Reproductive Sciences*, *28*(11), 3094–3108. https://doi.org/10.1007/s43032-

021-00480-y

Zhang, X. H., Liang, X., Liang, X. H., Wang, T. S., Qi, Q. R., Deng, W. B., Sha, A. G., & Yang, Z. M. (2013). The mesenchymal-epithelial transition during in vitro decidualization. *Reproductive Sciences*, *20*(4), 354–360. https://doi.org/10.1177/1933719112472738

Zimmer, D. B., & Magnuson, M. A. (1990). Immunohistochemical localization of phosphoenolpyruvate carboxykinase in adult and developing mouse tissues. *Journal of Histochemistry and Cytochemistry*, *38*(2), 171–178. https://doi.org/10.1177/38.2.1688895

Zimmermann, F. K. (2001). Glycolysis. *Encyclopedia of Genetics*, 885–888. https://doi.org/10.1006/rwgn.2001.0570

Zinaman, M. J., Clegg, E. D., Brown, C. C., O'Connor, J., & Selevan, S. G. (1996). Estimates of human fertility and pregnancy loss. *Fertility and Sterility*, *112*(4), e229–e235. https://doi.org/10.1016/j.fertnstert.2019.08.096

Zuo, R. J., Gu, X. W., Qi, Q. R., Wang, T. S., Zhao, X. Y., Liu, J. L., & Yang, Z. M. (2015). Warburg-like glycolysis and lactate shuttle in mouse decidua during early pregnancy. *Journal of Biological Chemistry*, *290*(35), 21280–21291. https://doi.org/10.1074/jbc.M115.656629

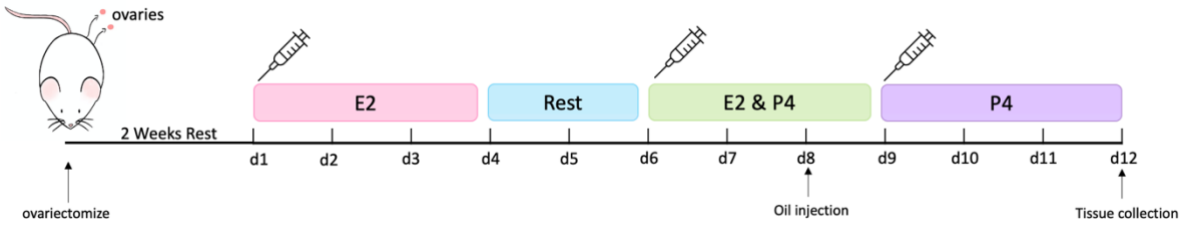
APPENDIX A: PRIMARY ANTIBODIES

Primary antibodies and summary of conditions used for western blot (WB) and immunohistochemistry (IHC).

Antigen	Catalog No.	Technique	Dilution	Block
Hexokinase 1	2024 Cell signaling	WB	1:500	BSA
		IHC	1:20	Goat serum
Glycogen Synthase	3886 Cell signaling	WB	1:500	BSA
		IHC	1:40	Goat serum
Phospho-glycogen synthase	47043 Cell signaling	WB	1:500	Milk
Glycogen phosphorylase	Ab231963 Abcam	WB	1:500	BSA
	A9392 ABclonal	IHC	1:100	Goat serum
Glucose-6-phosphatase	PA5-70653 Invitrogen	IHC	1:50	Goat serum
GFP	2956 Cell signaling	IHC	variable	Goat serum
β-actin	A2066 Sigma-Aldrich	WB	1:1000	Milk

Block for western bots (WB) consisted of 5% powdered milk or 5% BSA in TBS-T. Block for IHC was 3% BSA and 10% goat serum in TBS.

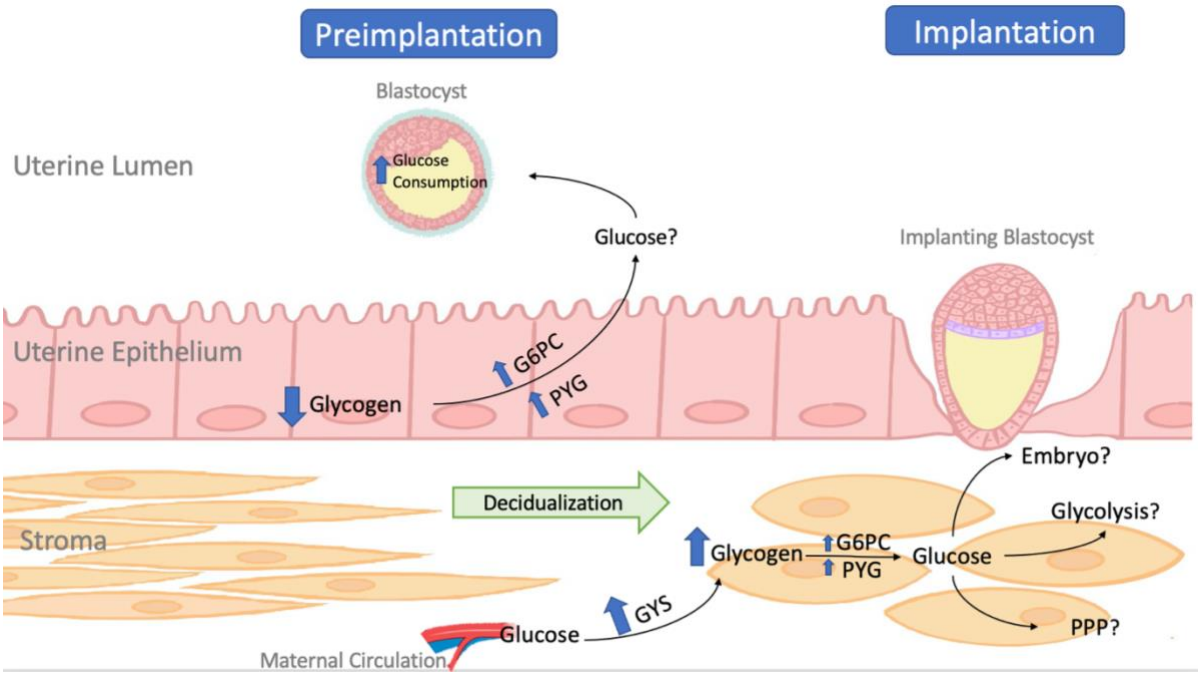
APPENDIX B: ARTIFICIAL DECIDUALIZATION



Days	Treatments	Stimulation and Tissue Collection
D1	100 ng E2 in 0.1 ml corn oil	
D2	100 ng E2 in 0.1 ml corn oil	
D3	100 ng E2 in 0.1 ml corn oil	
D4	Rest	
D5	Rest	
D6	10 ng E2 & 1mg P4 in 0.1 ml corn oil	
D7	10 ng E2 & 1mg P4 in 0.1 ml corn oil	
D8	10 ng E2 & 1mg P4 in 0.1 ml corn oil	15 ul corn oil injection to one uterine horn (2-4 h after hormone injection)
D9	1 mg P4 in 0.1 ml corn oil	
D10	1 mg P4 in 0.1 ml corn oil	
D11	1 mg P4 in 0.1 ml corn oil	
D12	1 mg P4 in 0.1 ml corn oil	Collect tissues 96 h after oil injection

Decidualization timeline. Mice were ovariectomized and rested for 2 weeks to clear out endogenous hormones. Injections of 100 ng E2 in 0.1ml corn oil were given for three days. Then the mice were rested for two days, followed by an injection of 10 ng E2 and 1 mg P4 in corn oil for three days. Oil injection to stimulate one of the uterine horns was given on day 8. The other uterine horn was left as the internal control. Next, 1 mg P4 in 0.1 ml corn oil was injected for four days and uterine horns were collected 96 h after oil stimulation.

APPENDIX C: OVERVIEW OF ENDOMETRIAL GLYCOGEN USES



Overview of glycogen stores in the endometrium of uterus and their potential uses. In the uterine epithelium, glycogen is broken down by G6PC and PYG, liberating glucose which could be secreted into uterine lumen. As high glucose consumption is observed during the blastocyst stage, the secreted glucose may be support preimplantation embryo development. After stromal cells decidualize at implantation, GYS expression increases and glycogen is stored in the decidua. The moderate increase in G6PC and PYG expression suggest that glycogen is broken down to release glucose, which may be used by the developing embryo, metabolized in the decidual cells via glycolysis or the pentose phosphate pathway (G6PC: glucose-6-phosphatase; PYG: glycogen phosphorylase; GYS: glycogen synthase; PPP: pentose phosphate pathway).