Trends in
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Review

Mechanisms of lipid metabolism in uterine receptivity and embryo development

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Metabolic regulation plays important roles in embryo development and uterine receptivity during early pregnancy, ultimately influencing pregnancy efficiency in mammals. The important roles of lipid metabolism during early pregnancy have not been fully understood. Here, we described the regulatory roles of phospholipid, sphingolipid, and cholesterol metabolism on early embryo development, implantation, and uterine receptivity through production of cannabinoids, prostaglandins, lysophosphatidic acid, sphingosine-1-phosphate, and steroid hormones. Moreover, the impacts of lipids and fatty acids on embryo development potential and the related epigenetic modifications are also discussed. This review aims to elucidate the modulations of lipid metabolism on uterine receptivity and embryo development, contributing to novel strategies to establish dietary balanced lipids and fatty acids for reducing early embryo loss.

Highlights

The quality of embryo implantation, depending on uterine receptivity and early embryo development during early pregnancy, determines ongoing pregnancy efficiency.

Lipid metabolism, including phospholipid, sphingolipid, and cholesterol metabolism, regulates embryo development, implantation, and uterine decidualization.

Maternal lipid metabolism signaling regulates the reproductive axis through hypothalamic neurons.

Lipid metabolism and fatty acids regulate early embryo development potential and modulate embryonic epigenetic modification.

Introduction

Embryo loss in mammals mostly occurs in the clinically recognized peri-implantation period, and embryo implantation failure in early pregnancy is the predominant cause of pregnancy failure (Box 1). Lipid metabolism plays important roles during early pregnancy; however, the exact mechanisms of lipid metabolism on early pregnancy success are not well understood [1–4]. Lipid metabolism in the ovary and uterus affects the microenvironment in which oocytes and embryos develop; in addition, lipid metabolism of oocytes and embryos is critically important for blastocyst development potential [2,5]. Early pregnancy events in mammals include embryo development, embryonic genomic activation, uterine receptivity [also known as **uterine decidualization** (see Glossary) in humans], and **embryo implantation** (Box 1) [6,7]. Therefore, the precise regulation of lipid mediators and signaling pathways to guarantee the proper progression of embryonic genomic activation, uterine decidualization, and embryo implantation is an effective strategy to reduce early embryo loss and improve fertility.

Diet-induced obesity before and during pregnancy has long been recognized as a hazard for mitochondrial oxidative stress, endoplasmic reticulum stress, and epigenetic disorders in oocytes and embryos [8]. The self-replication of mitochondrial DNA allows it to retain its characteristics during mother–fetus transmission, indicating that maternal obesity-induced mitochondrial damage could compromise the metabolism and development of the oocytes and embryos. Moreover, the non-esterified fatty acids (NEFA) exposure of oocytes can lead to disorders of mitochondrial metabolism and developmental epigenetic reprogramming in the subsequent blastocyst stage [9,10]. However, emerging evidence suggests that lipid metabolism and fat mobilization are critical for fertility in females [11]. Furthermore, regulation of lipid metabolic profiles may be used as a compensatory energy consumption strategy under conditions of maternal reproductive stress [12]. Recent evidence suggests that lipid metabolism is critical to signaling pathways for cell proliferation and differentiation, immune response, and vascular development through regulating energy homeostasis and epigenetic modifications, and converting to lipid mediators, such as

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cannabinoids, lysophospholipids, and sex hormones [13–17]. This review discusses the latest advances on how lipid metabolism contributes to early pregnancy success by intermediating uterine receptivity and regulating embryonic genomic activation and embryo implantation. Understanding the molecular mechanisms of lipid metabolism for regulation of early pregnancy efficiency is beneficial for appropriate utilization of metabolic regulation and reproductive technologies to improve fertility in mammals.

Lipid-derived hormones and lipid metabolism signaling

Cannabinoid signaling

Anandamide (*N*-arachidonoylethanolamide) is an endogenously produced cannabinoid-like lipid mediator termed endocannabinoid. The production of anandamide results from hydrolyzing *N*-acylphosphatidylethanolamine (NAPE) on the cell membrane by phospholipase D (NAPE-PLD) (Figure 1Aa), while the degradation of anandamide to arachidonic acid and ethanolamine is catalyzed by fatty acid amide hydrolase (FAAH) (Figure 1Ab). In humans, growing evidence highlights the impact of abnormal signaling of the endocannabinoid system on embryonic, endometrial, and placenta physiology in pregnancy failure, such as endometriosis, ectopic pregnancy, miscarriage, and pre-eclampsia [18,19]. Cannabinoid/endocannabinoid signaling exerts biological effects through activation of cannabinoid receptors (CB1 and CB2). Notably, both anandamide and CB1 are maintained at high levels in the nonreceptive uterus and dormant blastocyst, and elevated anandamide levels in peripheral circulation are correlated with spontaneous pregnancy failure [18,19]. Animal studies show that when mouse embryos are subjected to an abnormally high level of anandamide, the activation of CB1 could block the Ca^{2+} signal to inhibit blastocyst activation, resulting in early embryo developmental retardation and embryo retention in the fallopian tubes, as well as decreasing uterine receptivity, eventually leading to early embryo death and implantation failure [20]. Recently, the molecular mechanism of endocannabinoid signaling on oocyte maturation and subsequent embryo development was studied. The activation of CB1 during mouse oocyte maturation modulates the phosphorylation of Akt and ERK1/2 and enhances the subsequent embryo development, whereas the absence of CB1 impairs oocyte maturation and delays the subsequent embryo development, without rescue of the influences of CB2, which suggest that cannabinoid signaling drives CB1 to regulate oocyte maturation and subsequent embryo development through PI3K/Akt and MAPK pathways [19]. Moreover, a significantly reduced level of anandamide in the receptive uterus is necessary for early pregnancy success [21]. Therefore, a normal physiological setting of endocannabinoid tone renders an appropriate CB1 function that is critical to ensure early pregnancy events.

The underlying mechanism and metabolic regulation by which the endocannabinoid tone is established in a targeted-manner in reproductive tissues has gained interest. Animal studies show that the temporal and spatial different expression patterns of NAPE-PLD and FAAH in the fallopian tubes, uterus, and early embryo contribute to the on-demand nature of endocannabinoid signaling on embryo development in mice (Table 1) [21,22]. *Faah*-mutant embryos have significant elevated levels of anandamide and abnormal expression of transcription factors Cdx2, Nanog, and Oct3/4, which result in defective cell lineage pluripotency and delayed cellular development, ultimately leading to asynchronous and stunted early embryonic development [21]. In clinical practice endocannabinoids, such as anandamide and cannabidiol, are administered to help relieve pain, certain behaviors of cancer, neurodegenerative diseases, and anxiety [23,24]. Thus, the utilization of FAAH inhibitors and the study of single-nucleotide polymorphisms in the *Faah* gene have gained wide interest. However, enhanced endocannabinoid signaling as a result of FAAH functional intervention carries a significant risk to women's fertility, such as ectopic pregnancy and miscarriage [15,18]. Therefore, the target therapies and appropriate applications of the endocannabinoid system and CB1 signals during early pregnancy are

Glossary

Embryonic genome activation: in early embryo development, both the paternal and maternal genomes must undergo chromatin demethylation and zygote genomic methylation to initially establish the gene expression patterns for embryo development.

Embryo implantation: a dynamic developmental process that involves a series of physiological and biochemical reactions between the trophoblast cells of blastocysts and the luminal epithelium, glandular epithelium, and stromal cells of the uterus. There are three stages to implantation: localization, adhesion, and invasion. When the uterine cavity fluid is absorbed and causes closure of the uterine cavity, the embryonic trophoblastic layer approaches the endometrial epithelium, then the embryos adhere to the luminal epithelium, thereby invading into the uterine stromal cell layer, accompanied by increased permeability of endometrial vessels.

Reproductive axis: ovarian steroidogenesis is manipulated by the hypothalamic–pituitary–gonadal axis (reproductive axis), which is mainly modulated by hypothalamic gonadotropin-releasing hormone (GnRH) neurons synthesizing and releasing GnRH in a pulsed manner into the pituitary gland to generate luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and as a result of that, LH and FSH send signals to the gonad and trigger progesterone and estrogen production in the ovaries.

Uterine decidualization: a series of morphological changes of the uterus in early pregnancy in humans, which is essential for subsequent placenta formation and fetal development. The decidualization signal is generated through two types of cell–cell interactions, namely, embryo–endometrial epithelium interactions and endometrial epithelium–matrix interactions.

Box 1. Early pregnancy events

In mammals, the fertilized egg (embryo) undergoes multiple cell divisions to progress through the two-cell, four-cell, eight-cell, morula, and blastula stages (Figure I). The key period of embryonic genome activation is at the two-cell stage in mice and rats, the four-cell stage in pigs and humans, and the eight-cell stage in cattle (Table I). If the transcription of the zygote genome fails, the cell cycle will stop. The embryo implantation stage is also called the implantation window, which is day 5–9 of postovulation (i.e., day 20–24 of the menstrual cycle shown in Figure II) in humans, day 4.5–5 of gestation in mice, day 5.5–6 of gestation in rats, day 9–13 of gestation in pigs, and day 20–30 of gestation in cattle (Table I). In the decidualization process, the uterine epithelium is ruptured, and extensive cell proliferation and angiogenesis (decidual reaction) occur in the sub-epithelial stroma (Figure II).

According to statistics, the maximum probability of a spontaneously conceiving woman having a successful pregnancy and completing delivery is approximately 30%. In cases of unsuccessful pregnancy, the failure of embryo implantation is the main reason, accounting for approximately 60~75% [6]. In pigs, 30~50% of embryos are lost during early pregnancy, and 75% of these occur during the first 25 days of pregnancy [92]. In cattle, approximately 30% of embryo loss occurs during days 8~27 of pregnancy; due to the effects of oocytes quality and environmental factors, the embryonic mortality of cattle during the first week of gestation varies greatly, from 10% to 50% [93] (Table I). Therefore, embryo loss and implantation failure during early pregnancy are the main reasons leading to pregnancy failure. Successful embryo implantation into the uterus requires the blastocysts to acquire implantation capability and to synchronize with the development of the endometrial receptive status which are precisely regulated by sex hormones and signaling molecules, including cytokines, growth factors, homeobox transcription factors, and lipid mediators, synthesized by mother and embryo [94,95].

Table I. Early pregnancy events and embryo loss

Species	Embryo genome activation timing	Implantation timing (days post ovulation)	Gestation length (days)	Early pregnancy loss
Human	4-cell phase	5~9	280	42~52.5% (implantation failure)
Mouse	2-cell phase	4.5~5	20	
Rat	2-cell phase	5.5~6	21	
Pig	4-cell phase	9~13	114	30~50%
Cattle	8-cell phase	20~30	283	30 (8th~27th days of pregnancy)

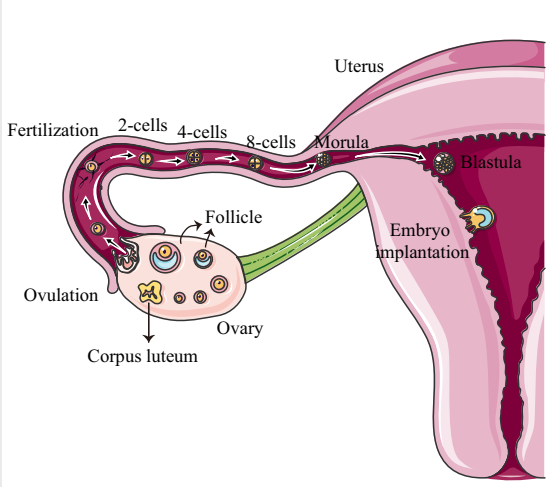

Figure I. Process of early embryo development.

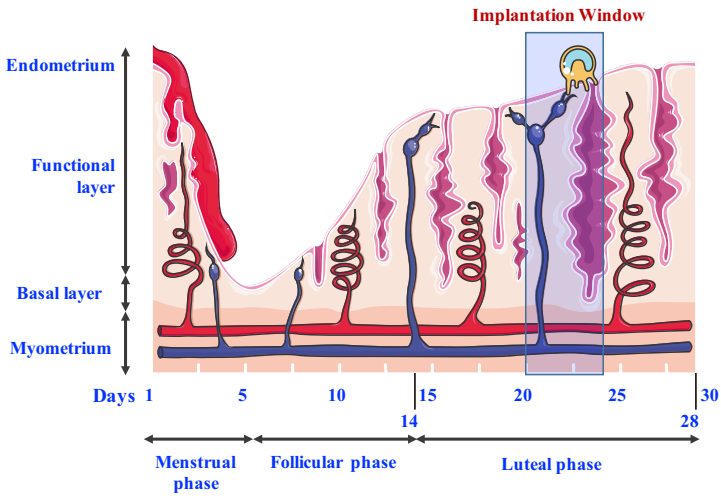

Figure II. Process of menstrual cycle and uterine decidualization in human.

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efficient to improve embryonic fate in pathophysiological aspects of uterine and pregnancy disorders.

Phospholipid metabolism and the production of prostaglandins

Prostaglandins (PGs) are produced through the hydrolysis of membrane phospholipids by cytoplasmic phospholipase A2 (cPLA2) to release arachidonic acid, which is converted to PGs by

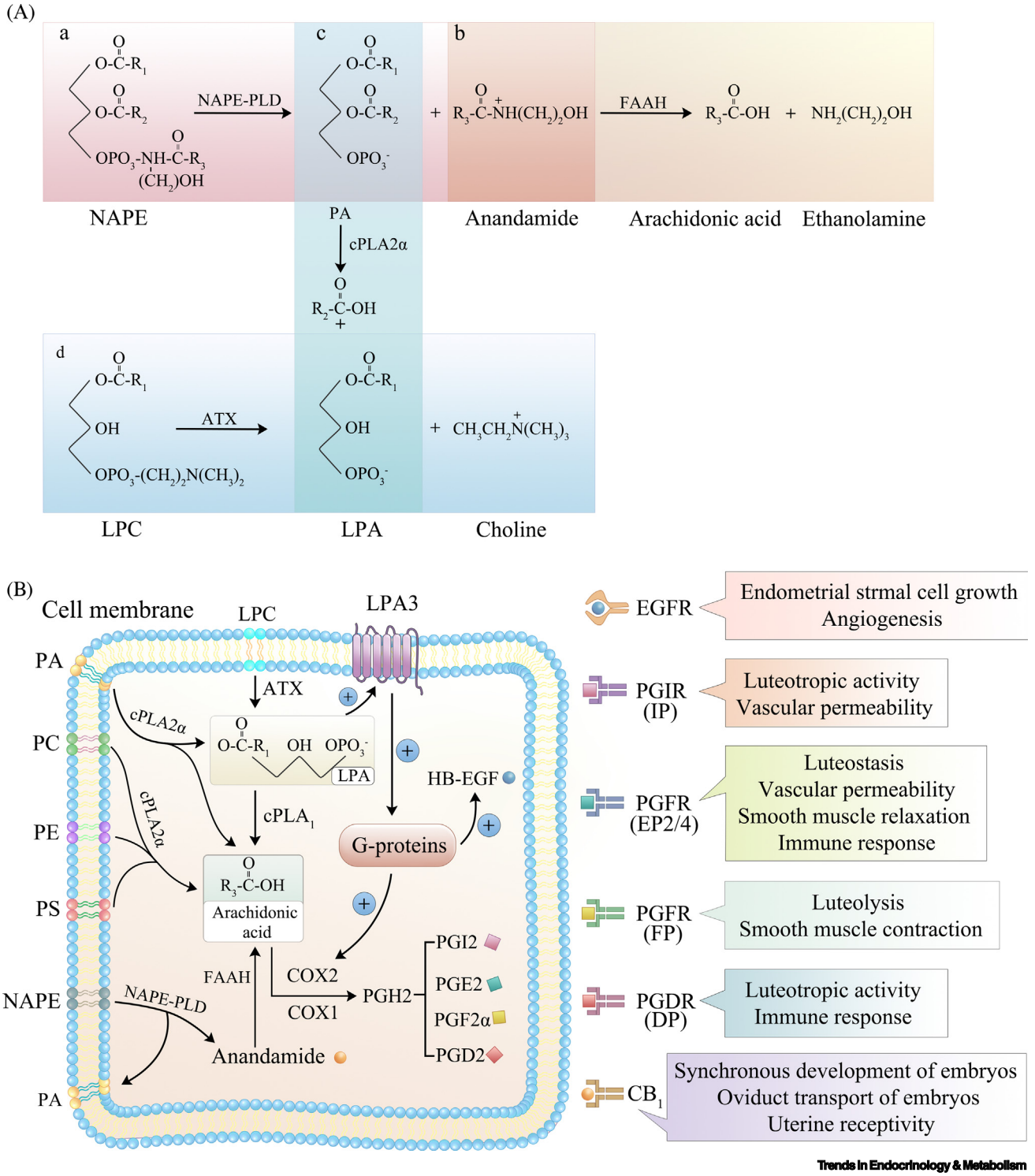

Figure 1. Regulatory effects of phospholipid metabolic pathways on embryo implantation and uterine receptivity. (A) Schematics of phospholipid metabolism. (a) The hydrolysis of *N*-acylphosphatidylethanolamine (NAPE) by its selective phospholipase D (NAPE-PLD) to release cannabinoid. (b) Cannabinoid is degraded by hydrolysis to arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH). (c) The hydrolysis of phosphatidic acid (PA) by cytoplasmic

(Figure legend continued at the bottom of the next page.)

Table 1. Effects of lipid metabolism related mediators, enzymes and receptors on early pregnancy events and ovarian steroidogenesis

Genes	Molecules encoded	Species	Knockout or deficiency phenotypes in females	Refs
NAPE-PLD	*N*-Acylphosphatidylethanolamine-hydrolyzing Phospholipase D	Mouse	Spontaneous pregnancy failure; defective uterine receptivity and blastocyst activation	[21]
FAAH	Fatty acid amide hydrolase	Mouse	Asynchronous development of preimplantation embryo; Defective oviductal embryo transport; deferred on-time implantation	[22]
CB1	Cannabinoid receptor 1	Mouse	Impaired oocyte maturation and delayed subsequent embryo development	[19]
Pla2g4a	Phospholipase A2, group IVA	Mouse	Deferred on-time implantation and decidualization; aberrant embryo spacing	[34]
Ptgs2	Prostaglandin-endoperoxide synthase 2	Mouse	Defective implantation and decidualization; Reduced angiogenic response	[35]
Ppard	Peroxisome proliferator-activated receptor-δ	Mouse	Delayed initiation of embryo attachment	[37]
LPA3	Lysophosphatidic acid receptor 3	Mouse	Deferred on-time implantation and decidualization; aberrant embryo spacing and reduced angiogenesis response	[39,40]
Sphk1 and Sphk2	Sphingosine kinase 1 and sphingosine kinase 2	Mouse	Uterine hemorrhage and early embryonic lethality; excessive neutrophil extracellular traps formation at the feto-maternal interface	[43,45]
Ldlr	Low-density lipoprotein receptor	Mouse	Reduced intracellular cholesterol and steroid hormone levels in luteal cells	[53]
Scarb1	Scavenger receptor class B member 1	Mouse	Reduced intracellular cholesterol and steroid hormone levels in luteal cells	[53]
PIBF	Progesterone-induced blocking factor	Mouse	Impaired T cell activation and Th1 differentiation, increased NK activity, resulting in impaired implantation and increased resorption rates	[47]
Pla2g10	Phospholipase A2, group X	Mouse	Reduced number of implantation sites	[51]
ACADL	Acyl-CoA dehydrogenase, long-chain	Mouse	Aberrant embryo development and lower blastocyst rate	[74]

COX2 and PG endoperoxide H synthase (Box 2). PGs intermediate the functions of the corpus luteum, participate in maternal–fetal interface immunomodulation and pregnancy identification, and stimulate angiogenesis during early pregnancy [25–28]. PGs can also regulate myometrium relaxation and contraction via PG transporters and receptors, thus affecting blastocyst transportation and adhesion reactions of the endometrium–trophoblast, ultimately regulating the distribution of the implanted embryos in the uterus [29–31] (Box 2).

However, the synergistic effect and priority of the roles of PGs in early pregnancy have not been clearly defined among different species. In pigs, PGE2 is abundantly synthesized and secreted by conceptuses and endometrial tissue, and PGE2 plays synergistic regulatory roles with estradiol on endometrial transcriptome of genes related to focal adhesion, vascularization, cell migration and proliferation, and immune response [25]. In addition, evidence indicate that PGF2α promotes angiogenesis in the porcine endometrium by activating the MAPK signaling pathway [26]. Moreover, both PGE2 and PGF2α can modulate the chemerin system in the porcine endometrium

phospholipase A2α (cPLA2α) to lysophosphatidic acid (LPA) and fatty acid. (d) The hydrolysis of lysophosphatidylcholine (LPC) by the secretase autotaxin (ATX) to lysophosphatidic acid (LPA) and choline. (B) Phospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), PA, and NAPE-PLD, catalyzed by the specific phospholipase to release bioactive metabolites (such as LPA, cannabinoid, and prostaglandins [PGs]). Binding of LPA to LPA receptor 3 (LPA3) in the endometrium activates specific G proteins to stimulate heparin binding-epidermal growth factor (HB-EGF) and cyclooxygenase 2 (COX2). HB-EGF in turn leads to endometrial stromal cell growth and angiogenesis. COX2 contributes to the synthesis of PGs, which act on the dissolution and maintenance of the corpus luteum in ovary and regulate vascular permeability and myocyte motility in uterus. Endocannabinoids, such as anandamide, transmit biosignals through the cannabinoid receptor CB1 to regulate embryonic synchronous development, oviduct transport of embryos, and uterine receptivity.

Box 2. The synthesis of prostaglandins

The fatty acid ester bond of phospholipids at the *sn*-2 position is hydrolyzed by cytoplasmic phospholipase A2 (cPLA2) to produce free fatty acids and lysophospholipids. The key regulatory step of prostaglandin (PG) biosynthesis is that cyclooxygenase (COX) converts arachidonic acid to prostaglandin G2 (PGG2), and then PGG2 is reduced by PG endoperoxide H synthase to an unstable peroxide intermediate prostaglandin H2 (PGH2), which is metabolized by a specific PG synthase into five structurally related active PGs, including PGE2, PGD2, PGF2, PGI2, and thromboxane A2. PGs bind to cell-surface receptors to exert their biological functions, including EP1–EP4, FP, DP, IP, and TP, which correspond to PGE2, PGF2, PGD2, PGI2, and thromboxane, respectively. After activation, the PG receptors EP2, EP4, IP, and DP couple with adenylate cyclase to produce cAMP, which can activate PKA signaling, so they are called 'relaxation' receptors; while the second messenger inositol triphosphate (IP3), produced by the coupling of TP, FP, or EP1 with phospholipase C, is involved in the intracellular release of calcium ions and diacylglycerol and activates protein kinase C, so these receptors are called 'contractile' receptors; additionally, EP3 reduces the level of cAMP and thus is called the 'inhibitory' receptor.

during early pregnancy [28]. In human trophoblast cells, PGF2α was reported to activate PGF2α receptor FP to stimulate cellular adhesion and the proliferation process through the MAPK pathway, as well as to increase invasiveness through inducing metalloproteinase proteolytic activity and mTOR signaling [31]. Moreover, using agonists and antagonists of PPARs and MAPK in porcine trophoblast cells, studies suggest that PPARγ and PPARδ positively regulate the synthesis of PGE2 and IFN-γ through the MAPK pathway [32]. In the ovine thymus, the expression of prostaglandin synthases is significantly improved during early pregnancy, and the PGD2 receptor DP2 is expressed in T-helper type 2 (Th2) immune cells, which is a chemoattractant receptor of the Th2 lymphocyte subset in the allergic inflammatory response [33]. Therefore, researchers are also attracted to investigate whether and how PGs participate in the maternal–embryo interface immunomodulation and pregnancy identification in different species at early pregnancy.

Previous studies have identified the critical roles of COX2 and cPLA2α in embryo implantation and uterine decidualization mostly in mice (Table 1). Maternal cPLA2α deficiency delays embryo implantation and results in an extremely crowded embryo distribution in the uterus and abnormal placental development [34], while COX2 deficiency shows abnormal ovulation and fertilization, embryo implantation failure, and uterine decidualization defects [35]. Studies reveal that PGs activate endometrial vascular development and decidualization by stimulating PPAR proteins and retinoic acid X receptor [36]. Recent studies suggest that PGI2 promotes the embryo development and blastocyst hatching through PPARδ-dependent pathway, which highlight a novel therapeutic target of interaction between PGI2 and PPARδ to improve *in vitro* fertilization (IVF) outcomes [37]. Furthermore, PPARγ and PPARδ are reported to affect decidual mTOR signaling and regulate embryo viability, uterine decidualization and feto-placental growth during early pregnancy [13]. In theory, PPAR family members not only serve as nuclear receptors for polyunsaturated fatty acids, PGs, and other esters, but also act as transcription factors to affect lipid metabolism and energy homeostasis to further regulate differentiation of decidual cells. However, the specific mechanisms of PPARs on differentiation of decidual cells remain obscure and need further validation, and some studies indicate that specific PPARs, such as PPARα, PPARγ, and PPARδ, have the synergistic or opposite effect on regulating transcription profile of genes related to lipid metabolism and cellular differentiation [38].

Lysophosphatidic acid

Lysophosphatidic acid (LPA) is derived from the hydrolysis of phosphatidic acids by cPLA2α (Figure 1Ac), or hydrolysis of lysophospholipids, such as lysophosphatidylcholine (LPC), by the secretase autotaxin (ATX) (Figure 1Ad). LPA signaling is found to be involved in angiogenesis, ovulation, fertilization, early embryo development, embryo implantation spacing and timing, and uterine decidualization. Recently, LPA is highlighted for its roles in vascular remodeling at the maternal–fetal interface [16]. Studies reveal that the LPA receptor LPA3 (encoded by *Lpar3*) is

highly expressed in peri-implantation uterine epithelial cells and is significantly reduced in patients with repeated IVF implantation failure [17].

Recent work confirmed that uterine vascular development and stromal cells proliferation were regulated by heparin-binding epidermal growth factor (HB-EGF) and COX2, induced by ATX-LPA-LPA3 signaling at the embryo–epithelial boundary during uterine decidualization in mice (Figure 1B) [39]. There are similar phenotypes between LPA3-deficient and cPLA2α-deficient mice: delayed embryo implantation, impaired fetal development, embryo crowding, multiple embryos sharing a placenta, and reduced litter size (Table 1) [34,40]. These similar abnormal reproductive phenotypes may be attributed to the fact that both LPA3 and cPLA2α deficiency lead to reduction of PGs synthesis by COX2 inhibition. Although the PG therapy could alleviate the delayed embryo implantation both in *Pla2g4a*$^{-/-}$ and *Lpar3*$^{-/-}$ female mice, the crowded distribution of embryos in the uterus of *Lpar3*$^{-/-}$ female mice is maintained, suggesting that the regulatory roles of LPA3 on uterine and placental vascular development regulated by the HB-EGF-EGFR-Bmp2/Wnt4 signaling pathway could not be compensated by PGs [39].

Sphingolipid

Sphingolipid metabolism can produce complex lipids and bioactive metabolites, such as phosphatidylethanolamine (PE) and sphingosine-1-phosphate (S1P). S1P acts on G protein-coupled receptors (S1P1-5) and has important physiological roles in feto-placental vascular growth [41], immune response [42], and uterine decidualization [43]. Sphingosine kinase (SphK) is a key enzyme in the metabolism of sphingosine to S1P. Pregnant mice lacking SphK (*Sphk1*$^{-/-}$ and *Sphk2*$^{+/-}$) accumulate dihydrosphingosine and sphingosine, but decrease PE levels in the uterus [43]. Uterine decidual cells lacking SphK mostly die in early pregnancy, moreover, SphK deficiency results in reduced proliferation of undifferentiated endometrial stromal cells and massive rupture of the decidual vessels, ultimately leading to massive uterine bleeding and high early embryonic lethality (Table 1) [43]. SphK deficiency (*Sphk1*$^{-/-}$ or *Sphk2*$^{+/-}$) in mice does not affect the levels of PGs or COX2 expression [43]. However, some studies suggest that S1P induces COX2 expression in pre-decidual stromal cells, indicating associations between sphingolipid metabolism and PGs signaling in decidual tissues [44]. Furthermore, neutrophil extracellular traps have been implicated as a potential mechanism in pathogenesis of Sphk-mediated pregnancy loss [45].

Recently, a bidirectional homeostatic crosstalk was demonstrated between sphingolipids and glycerophospholipids, and that dysregulation contributes to lipotoxicity and induces metabolic stress [46]. S1P and dihydro-S1P can be degraded by S1P lyase to produce fatty aldehydes and phosphoethanolamine, which are used in the biosynthesis of PE, and PE deficiency may also be the cause of early pregnancy failure in mice with *Sphk* gene silencing [43]. Sphingolipid metabolism plays an important regulatory role on uterine decidualization in early pregnancy. However, multiple sphingolipid metabolic enzymes with opposite effects are upregulated in mouse decidual tissues [43]. Therefore, a consistent and exact mechanism should be identified to fully understand the modulation of sphingolipid metabolism in uterine receptivity.

Steroid hormones resourced from cholesterols

The steroid hormones (including progesterone and estradiol) contribute to the maintenance of pregnancy, especially during early pregnancy in mammals [6,7]. Recent studies demonstrate that progesterone-induced blocking factor (PIBF) deficiency alters immune response (including impaired T cell activation, disordered Th1 differentiation, and increased NK activity) and leads to embryo implantation failure in mice [47]. Notably, elevating circulating progesterone level during gestation is an effective method to maintain pregnancy and reduce early embryonic loss. Luteal

phase defect (LPD) is a complex endocrine disease in women related to recurrent embryo implantation failure or miscarriage. Although the etiology of LPD is still not clear, women with LPD usually have low serum progesterone and insufficient endometrial growth. Therefore, progesterone supplementation is an important therapy for women with LPD and can reduce the risk of miscarriage by enhancing uterine receptivity [48]. Moreover, progesterone is clarified to alter the lipid metabolic pathways, including inositol, phospholipid, glycerolipid, and primary bile acid in bovine uterine fluid during the period of elongation [49]. Recently, progesterone and estradiol are found to induce human trophoblast tubulogenesis associated with the LPA/LPA3 pathway [50]. Moreover, Pla2g10 is revealed to be induced by progesterone receptors and exclusively localized in the apical region of mouse uterine luminal epithelium to contribute to the uterine receptivity [51], while the endocannabinoid system and PGE2 levels in the human uterus are clarified to be modulated by estrogen [52]. Therefore, there may be a plausible mechanism by which steroid hormones effectively affect the phospholipid metabolism and endocannabinoid system for uterine receptivity modulation in mammals, but further studies are needed to validate these hypotheses. Moreover, intensive studies are needed to validate the physiological effects and mechanisms of crosstalk among cholesterol, phospholipids, and endocannabinoid on early pregnancy efficiency.

The dynamics of steroid hormones synthesis at early pregnancy in humans and mice and its regulatory roles on uterine receptivity and embryo implantation have been described in detail [6,7]. Cholesterols act as the precursors for steroid hormone synthesis, and the cell membrane receptors for cholesterols have critical functions in ovarian steroidogenesis [53]. Here, we integrate the resources of cellular cholesterols and the downstream processes of steroid hormone synthesis (steroidogenesis), as well as the major regulatory roles of luteinizing hormone in luteal cells during early pregnancy (Box 3). Certain efforts should be made in future studies to fully understand the crosstalk between gonadal cholesterol metabolism and steroidogenesis, and the central nervous system regulatory roles of maternal lipid metabolism signaling on the hypothalamic–pituitary–gonadal axis (**reproductive axis**).

Maternal lipid metabolism signals regulate the reproductive axis through the central nervous system

The adipose tissue hormones and metabolic signals could be transferred to reproductive-related neurons to exert their reproductive regulatory functions. Notably, leptin is reported to not only regulate energy metabolism (such as appetite and thermogenesis-related reactions), but also

Box 3. Synthesis of steroid hormones from cholesterol

The synthesis of steroid hormones in luteal cells are shown in Figure I. Several sources of cholesterol are used in steroid hormones synthesis in luteal cells at early pregnancy. (i) *De novo* synthesis of cholesterol from acetate in the endoplasmic reticulum (ER). The rate-limiting step of *de novo* synthesis of cholesterol is the synthesis of mevalonate from acetyl-CoA catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. (ii) Intracellular stored cholesterol released from lipid droplets by hormone sensitive lipase E (HSL/LIPE). (iii) Dietary cholesterol reaches the gonadal cells through blood circulation in the form of lipoprotein cholesterol. Cholesterol used for steroidogenesis mostly comes from circulating lipoprotein cholesterol. The high-density lipoprotein cholesterol (HDL-C) can be transported into cells by membrane scavenger receptor B1 (SR-B1), whereas LDL-C can enter cells through its receptor (LDLR)-mediated endocytosis. The cholesteryl ester that enters cells through LDLR-mediated endocytosis is hydrolyzed by lysosomal acid lipase (LAL) to release cholesterol.

Intracellular free cholesterol can be re-esterified by acyl-CoA: acyltransferase (ACAT) and stored in lipid droplets, or reaches the mitochondrial outer membrane using the START-domain protein (StarD1-15) or other cholesterol transport proteins. START protein is closely related to steroidogenic acute regulatory (StAR) protein, and binds cholesterol after being induced by sterol regulatory element-binding protein (SREBP). StAR is an acute regulatory protein in promoting steroidogenesis, which is responsible for transporting cholesterol from the mitochondrial outer membrane to inner membrane, which is the rate-limiting step of steroidogenesis. The mitochondrial inner membrane cholesterol is converted to pregnenolone by the cholesterol side-chain cleavage enzyme CYP11A1, then converted to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD) and orderly converted to androgen or estrogen by CYP17A1 or CYP19A1. Luteinizing hormone (LH) can increase the cAMP levels in gonadal cells, which on one hand can stimulate HSL but inhibit ACAT, thereby increasing the intracellular free cholesterol level; on the other hand, it can stimulate HMG-CoA reductase activity and increase LDL-C uptake, thereby increasing the cholesterol available for steroidogenesis. Future studies will be necessary to understand the metabolic hypothalamic signaling, which regulates the hypothalamic–pituitary–gonadal axis to influence ovarian steroidogenesis.

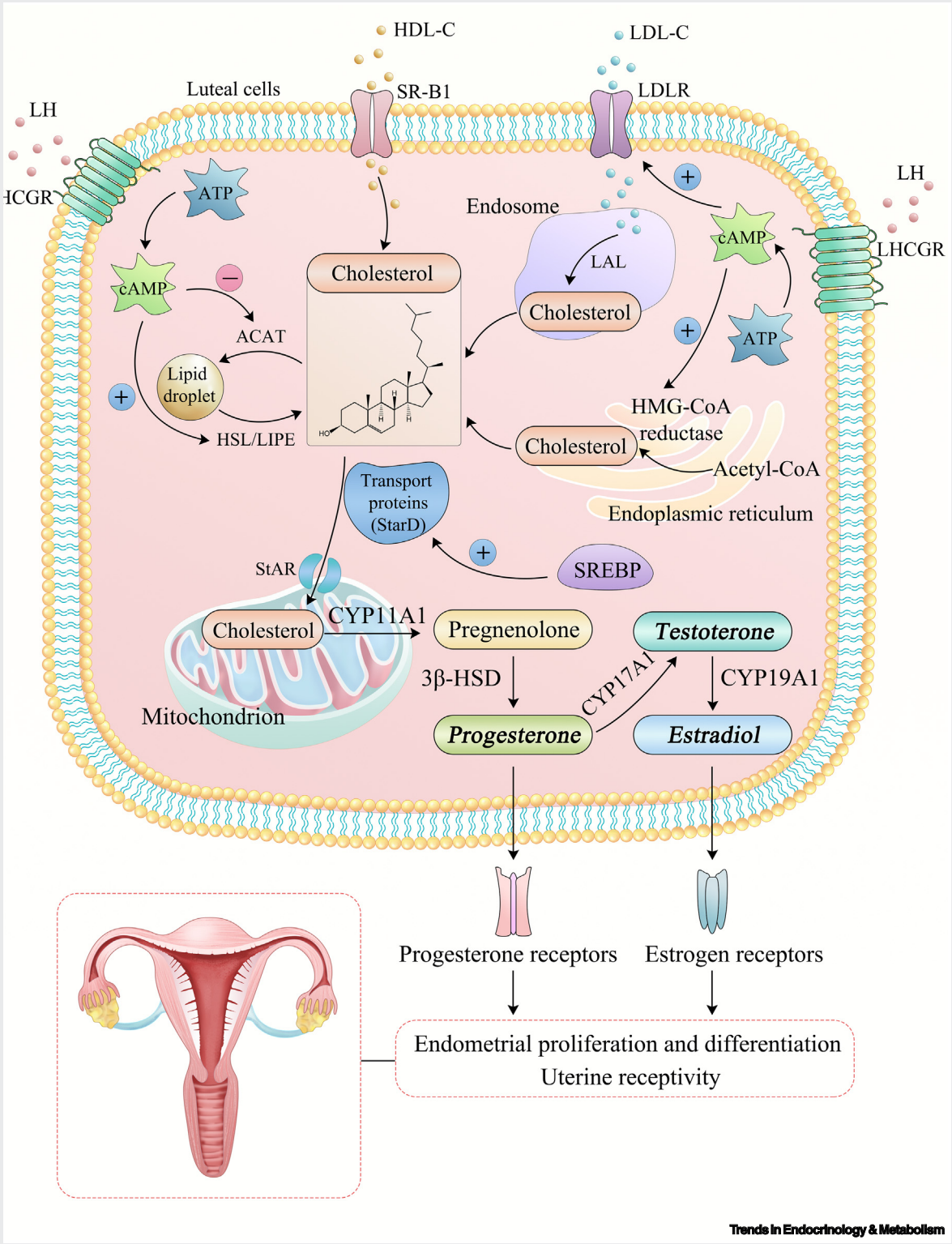

Figure I. Synthesis of steroid hormones in luteal cells.

intermediate mammalian reproductive capacity [54]. Blockage or mutation of leptin signaling causes reproductive dysfunction, similarly, insufficient leptin (e.g., as a result of anorexia or malnutrition) is usually associated with delayed gonad development and reduced reproductive capacity [55]. Notably, not only does leptin deficiency interfere with the reproductive axis, but too much leptin (e.g., due to morbid obesity) also affects the reproductive axis [4,56]. Although the neuron-specific silencing of leptin receptor blocks estrus and induces infertility in female mice, selective silencing of leptin receptor in hypothalamic gonadotropin-releasing hormone (GnRH) neurons does not affect the estrus and fertility of female mice, indicating an indirect effect of leptin on GnRH neurons [57]. Recently, the neuropeptide α-melanocyte stimulating hormone (α-MSH), synthesized by proopiomelanocortin (POMC) neurons, is reported to mediate the leptin signal and then regulate the synthesis of kisspeptin to control puberty onset in rats [58].

Considering that GnRH neurons are gatekeepers in the reproductive axis, novel central neuroendocrine pathways are extensively investigated, including kisspeptin, neurokinin B, and the products of POMC and nerve peptide Y (NPY) neurons that modulate GnRH synthesis and release by nutritional and metabolic factors, such as leptin, ghrelin, and insulin [59]. Recent progresses are primarily focused on elucidating the central regulatory roles of kisspeptin and its receptor GPR54 (also named Kiss1R) in the reproductive axis, particularly through their regulation of metabolic signaling [60,61]. Evidence shows that leptin deficiency due to malnutrition or lack of energy is associated with the inhibition of Kiss1 expression, whereas leptin supplementation activates Kiss1 expression [62,63]. In addition, both negative energy balance and excess energy supply both decrease hypothalamic Kiss1 expression and delay gonad development [64,65]. Recent studies show that the metabolic signals modulating Kiss1 activity are regulated by the mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) signaling in the hypothalamus in mice [64,66]. Importantly, the upstream regulators of mTOR and AMPK signaling in Kiss1 neurons deserve further study, which may include mTOR signaling regulated by protein metabolism and AMPK signaling regulated by adiponectin and leptin. Moreover, the peripheral actions of kisspeptin in oocytes, trophoblast, and uterus can also indicate actions of kisspeptin in embryo development, trophoblast attachment, and implantation [67].

Lipid droplets and fatty acids in oocytes and embryos regulate early embryonic developmental potential

The dynamics of lipid droplets and fatty acids metabolism

Intracellular fatty acids are esterified to triglycerides, phospholipids, and cholesteryl esters and stored in lipid droplets, which are essential for pre-implantation embryonic development and protect against lipotoxicity induced by fatty acids [1]. Importantly, the lipidomic patterns of endometrial fluid between implantative and nonimplantative IVF cycles mostly reveal alteration of glycerophospholipids and fatty acids, suggesting that phospholipids and fatty acids are critical to uterine receptivity during the embryo implantation window [2]. The dynamics of lipid droplets and fatty acids in oocytes and embryos are significantly affected by lipids from the diets and the serum albumin in cultured medium [68,69]. Notably, addition of carnitine (transporter of fatty acids from the cytoplasm into mitochondria) could effectively mitigate the difference in blastocyst development under different commercial serum albumin supplementation [70]. In humans, L-carnitine is a promising culture media supplement to reverse the dysfunction of mitochondria induced by maternal aging and stress [71]. Thus, optimizing utilization of L-carnitine in the culture medium for early embryos could effectively improve the metabolism of fatty acids and promote embryo viability via antioxidation and metabolic-regulating effects [72,73]. As regards long chain acyl-CoA dehydrogenase (ACADL), the rate-limiting enzyme of fatty acid β-oxidation, studies show that the blastocyst formation rate of ACADL-deficient embryos is reduced compared with wild-type embryos in mice (Table 1) [74].

Recent studies reveal that PPARs, which are nuclear receptors of lipid ligands that control lipid metabolism and cell proliferation and differentiation, regulate blastocyst viability [3,4,37]. In ruminants, the transcription level of genes related to uptake, metabolism, and *de novo* biosynthesis of fatty acids and prostaglandins in the trophectoderm during elongation are markedly increased, substantially with an activation of PPARγ binding lipid ligands to coordinate energy metabolism and cell fate [3]. Moreover, recent work suggests that PPARδ stimulation has no effect on blastocyst formation but improves blastocyst hatching by activating fatty acid β-oxidation in pigs [4]. However, both blastocyst formation and hatching are impaired in PPARδ$^{(-/-)}$ embryos in mice [37], and it has been identified that PPARδ contributes to embryonic cell proliferation and mediates the stimulation effects of PGI2 on embryo development and blastocyst hatching [37]. Therefore, certain lipid ligands including both prostaglandins and fatty acids targeting embryonic PPARs to accurately determine embryo survival should be considered in future studies.

Fatty acid composition

The reliable biological markers and molecular mechanisms regulated by lipid mediators of maternal and embryonic origin are needed to predict the uterine and embryonic development competence. Here, the effects of fatty acids on embryo development are shown in Table 2. In pigs, palmitic acid affects cellular proliferation, apoptosis, and endoplasmic reticulum stress through ceramide accumulation in granulosa cells, and affects epigenetic markers of histone H3K9 demethylation and H4K12 acetylation in oocytes, which induced deterioration of oocyte viability and reduced the blastocyst formation [75]. In addition, NEFAs during *in vitro* maturation of bovine oocytes leads to abnormal lipid content and decreases cell proliferation and viability, but oleic acid can compensate for the adverse effects of NEFAs and improve the blastocyst formation [76]. Moreover, NEFA exposure of bovine oocytes leads to disorders of mitochondrial metabolism and developmental epigenetic programming in the subsequent blastocyst [9]. However, the effects of polyunsaturated fatty acids on early embryo survival show varying results among different species and doses [77]. For example, N-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) supplementation with a lower dose (1 μM) during *in vitro* maturation of bovine oocytes can improve the cleavage rate and blastocyst formation without affecting lipid metabolism, whereas a higher dose (100 μM) of DHA diminishes the cleavage rate and embryo developmental competence associated with perturbation of lipid metabolism and steroidogenesis in granulosa cells [77]. Evidence in mice suggest that the medium-chain fatty acid octanoate, which could enter mitochondria being β-oxidized without a transporter, is a potential alternative energy source for pre-implantation embryos [78]. Moreover, recent studies indicate that butyrate can impede the meiosis of mouse oocytes, but promote the embryo development potential through increasing transcription levels of HDAC1, Sox2, and Pou5f1 [79]. However, the molecular mechanisms of butyrate regulating expression of vital developmental genes of embryos still need further investigations.

Emerging evidence suggests potential effects of dietary fatty acids on improving fertility through prostaglandin synthesis and steroidogenesis [80–82]. In humans, dietary docosapentaenoic acid (22:5n-3) is associated with increased serum progesterone and reduced risk of anovulation [80]. In bovines, dietary conjugated linoleic acid supplementation from 21 days *prepartum* to day 42 *postpartum* increases plasma progesterone concentration but decreases the transcription of genes (NAPE-PLD and CB2) involved in the uterine endocannabinoid system [81]. In rats, dietary short- and medium-chain fatty acids (butyrate, hexanoate, and caprylate) are identified to increase the serum levels of arachidonic acid and progesterone, and improve embryo survival in early pregnancy [82]. Recent studies reveal that butyrate drives the acetylation of histone H3K9 to activate steroidogenesis through stimulating PPARγ and PPARγ co-activator 1α (PGC1α) pathways in human ovarian granulosa cells [83]. Studies are needed to investigate the regulatory

Table 2. Effects of fatty acids on early embryo development, steroidogenesis, and embryo implantation

Fatty acids	Species	Effects	Refs
Stearic acid (SA)	Bovine	Oocyte maturation under elevated SA concentration compromised early embryo quality, viability and metabolism, and dose-dependently resulted an inhibitory effect on fat storage in lipid droplets	[76]
Palmitic acid (PA)	Bovine	Oocyte maturation under elevated PA concentration compromised early embryo quality, viability and metabolism, and dose-dependently resulted an inhibitory effect on fat storage in lipid droplets	[75,76]
Oleic acid (OA)	Bovine	Oocyte maturation under elevated OA concentration has no effect on developmental competence nor on total blastocyst cell number, but elevated the blastocyst apoptotic cell index; OA caused an increase of lipid storage in lipid droplets and an improvement of oocyte developmental competence	[76]
Non-esterified fatty acids (NEFA)	Bovine	High level of NEFA-exposed oocytes and embryos displayed a lower competence to develop into blastocysts and resulted in blastocysts with altered DNA methylation and transcriptomic fingerprints related to lipid and carbohydrate metabolism, cell death, immune response and metabolic disorders, compared with basal level of NEFA-exposed counterparts	[9,10]
Docosahexaenoic acid (DHA)	Cattle	A low physiological dose of DHA (1 μM) during *in vitro* maturation of oocytes improves blastocyst formation rate after *in vitro* fertilization, whereas a higher dose of DHA (100 μM) decreases the cleavage rate and the >4-cells embryo rate after parthenogenetic activation	[77]
Docosapentaenoic acid	Human	Dietary docosapentaenoic acid is associated with increasing serum progesterone and reducing risk of anovulation	[80]
Conjugated linoleic acid	Bovine	Dietary conjugated linoleic acid supplementation alters the expression of genes involved in the endocannabinoid system in the bovine endometrium and increases plasma progesterone concentrations	[81]
Octanoate	Mouse, Rats	Octanoate provides an efficient alternative energy source for mouse pre-implantation embryo; octanoate increases the number of implanted embryos but reduces pregnancy rate in rats	[78,82]
Butyrate	Rats, Mouse	Maternal butyrate increases the number of implanted embryos and improves ovarian progesterone synthesis in rats; butyrate interrupts the maturation of mouse oocytes but improves early embryo competence *in vitro*	[79,82]
Hexanoate	Rats	Maternal hexanoate increases the number of implanted embryos but reduces pregnancy rate	[82]

effects and validate the mechanisms of dietary fatty acids supplementation on sex hormones synthesis in early pregnancy.

Regulation of embryonic epigenetic modification by lipid metabolism and fatty acids

Growing evidence indicates that the effects of lipid metabolism on embryo development potential could be attributed to its regulation of embryo epigenetic modification [84–86]. For example, diet-induced obesity and maternal lipid metabolism disorders can affect the epigenetic reprogramming of oocytes and embryos, and cause metabolic syndrome in offspring in mice [84]. At present, researchers generally consider that the oocyte maturation and early embryo development phages constitute the most sensitive window of epigenetic reprogramming [1,9]. Recent studies in bovines show that exposure to high levels of NEFA during oocyte maturation and early embryo development alter the DNA methylation pattern of genes related to cell fate, immunity, and metabolism in cumulus–oocyte complexes and blastocysts [9,10]. Moreover, the abnormal DNA methylation patterns of genes related to caspase activation, p53-induced apoptosis, Ras signal transduction, and Wnt signal transduction induced by NEFA exposure are consistent with the observed phenotype of increasing apoptotic cells in blastocysts [9,10].

Whole-genome reprogramming regulated by histone modifications are pivotal in embryo development and later growth, including histone methylation and demethylation, acetylation and deacetylation, and other modifications of specific chromatin regions [87]. The fate-determining mechanisms are demonstrated to center on several key metabolism-regulating signals, such as AMPK/mTOR signaling pathways and α-ketoglutarate, *S*-adenosyl methionine, and acetyl-CoA intermediate metabolites [88]. However, the metabolite pools arise from plural sources and participate in several downstream pathways, such as acetyl-CoA originated from both fatty acid β-oxidation and glycolysis of pyruvate, and influenced both the tricarboxylic acid (TCA) cycle and acetylation of histones. Current studies have revealed the importance of pyruvate and the nucleus localization of TCA enzymes on mouse **embryonic genome activation** [89]. Notably, interesting studies suggest that acetyl-CoA derived from fatty acid β-oxidation is the predominant contributor to the global acetyl-CoA pool in mice [90]. Notably, octanoate is verified to activate the retinoic acid receptor (RAR)/retinoid X receptor (RXR) and become the major source of acetyl-CoA to reprogram cellular metabolism in mouse hepatocytes [90]. Moreover, acetylcarnitine formed in mitochondria can be a source of nucleus acetyl carbons for histone acetylation via transport out of mitochondria by carnitine acyl-carnitine translocase and conversion to acetyl-CoA via nuclear carnitine acetyltransferase [90]. However, whether this model applies in oocytes and early embryos and the regulating enzymes and metabolic signaling underlying lipids and acetyl-CoA still need to be determined. Emerging evidence shows that maternal circulating lipids are associated with fetal developmental epigenetic reprogramming [14,85,86], therefore, further studies are required to investigate the underlying mechanisms of maternal lipid metabolism dynamics on offspring epigenome modification.

Concluding remarks and future perspectives

Maternal lipid metabolism before and during early pregnancy have profound impacts on oocyte quality and embryo survival, as well as the ovarian and uterine micro-environments, which determines the subsequent growth and development trajectories of offspring. The connection between the fetus and the uterus becomes increasingly close starting from the embryo implantation stage. The developmental defects of early embryos during embryo implantation stage have serious consequences for late pregnancy and directly determine the pregnancy outcomes. There is a huge amount of biomarker information provided by omics approaches, which highlights the potential of genetic and metabolic interventions to improve endometrial receptivity, embryo viability, and embryo implantation to advance our knowledge of embryo implantation. However, there are still more challenges to link and integrate data from all omics to reduce early embryonic loss and improve pregnancy outcomes when elucidating the implantation mechanisms [91].

Lipid metabolism not only serves as an energy supply through the TCA cycle of acetyl-CoA resourced from fatty acid β-oxidation, but also plays crucial biological functions in the synthesis of LPA, PGs, cannabinoids, and steroid hormones, which contribute to embryo implantation by regulating early embryo development and uterine receptivity during early pregnancy. Moreover, lipid metabolism and fatty acids in oocytes and embryos also influence embryonic genome activation and subsequent blastocyst viability through modulating epigenetic modifications. Great advances in our understanding of lipid metabolism in early embryo development and uterine receptivity have been made; the next steps are to understand upstream regulators and downstream impacts that are directly hard-wired into early pregnancy success. In addition, although the interactions between lipid metabolism, neuroendocrine, energy consumption, and epigenetic reprograming are being confirmed, more future challenges (see Outstanding questions) will also be needed to reveal the comprehensive crosstalk and mechanisms of lipid metabolism from maternal and embryonic origin. Moreover, there are tremendous opportunities to understand lipid

Outstanding questions

Does an exogenous supply of arachidonic acid reduce embryo loss and improve embryo implantation in early pregnancy by acting as the precursor of PG synthesis?

In sphingolipid metabolism, does the key metabolite, S1P or PE, regulate uterine receptivity in early pregnancy? Which sphingolipid metabolic enzyme plays the leading role in regulating uterine receptivity?

What are the effects and mechanisms of lipid metabolism on the expression patterns of leptin or adiponectin in modulating hypothalamic Kiss1 signaling and regulating GnRH synthesis?

Do unsaturated fatty acids and short- and medium-chain fatty acids impact early embryonic genome activation? If so, how? Do the histone inhibitor butyrate and fatty acid β-oxidation product acetyl-CoA play biological roles in early embryonic genome activation?

How can we identify the ideal lipid pattern and determine balanced fatty acid profiles for oocytes and embryos through maternal intervention to improve early embryonic potential?

metabolism in the physiological context of early embryo development, and in the pathological context of malnutrition or obesity, and ultimately to balance the dietary lipids and fatty acids profile for improvement of early embryo survival and final pregnancy outcomes.

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Declaration of interests

The authors declare no conflicts of interest.

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