



Phosphate, Calcium, and Vitamin D: Key Regulators of Fetal and Placental Development in Mammals

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Abstract

Normal calcium and bone homeostasis in the adult is virtually fully explained by the interactions of several key regulatory hormones, including parathyroid hormone, 1,25 dihydroxy vitamin D₃, fibroblast growth factor-23, calcitonin, and sex steroids (estradiol and testosterone). In utero, bone and mineral metabolism is regulated differently from the adult. During development, it is the placenta and not the fetal kidneys, intestines, or skeleton that is the primary source of minerals for the fetus. The placenta is able to meet the almost inexhaustible needs of the fetus for minerals by actively driving the transport of calcium and phosphorus from the maternal circulation to the growing fetus.

These fundamentally important minerals are maintained in the fetal circulation at higher concentrations than those in maternal blood. Maintenance of these inordinately higher fetal levels is necessary for the developing skeleton to accrue sufficient minerals by term. Importantly, in livestock species, prenatal mineralization of the skeleton is crucial for the high levels of offspring activity soon after birth. Calcium is required for mineralization, as well as a plethora of other physiological functions. Placental calcium and phosphate transport are regulated by several mechanisms that are discussed in this review. It is clear that phosphate and calcium metabolism is intimately interrelated and, therefore, placental transport of these minerals cannot be considered in isolation.

Keywords

Phosphate · Calcium · Vitamin D · Placenta · Endometrium · Pregnancy

List of Abbreviations

1,25(OH) ₂ D ₃	1,25 Dihydroxyvitamin D ₃
ADAM	A disintegrin and metalloprotease domain
cAMP	Cyclic adenosine monophosphate
CTB	Cytotrophoblasts
CYPs	Cytochrome P450 mixed-function oxidases

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E2	Estradiol
ECM	Extracellular Matrix
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
GE	Glandular epithelium
IFNT	Interferon tau
KL	Klotho
LE	Luminal epithelium
P21	Cyclin-dependent kinase inhibitor
P4	Progesterone
P53	Tumor protein 53
PGR	Progesterone receptor
PKC	Protein kinase C
PMCA	Plasma membrane Ca^{2+} ATPase
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone-related protein
S100	S100 calcium binding proteins
sFRP4	Secreted frizzled-related protein 4
sGE	Superficial glandular epithelium
SLC	Solute carrier
SLC20	Solute carrier family 20
SLC34	Solute carrier family 34
SPP1	Secreted phosphoprotein 1
STB	Syncytiotrophoblast
STC	Stanniocalcin
TRPV	Transient receptor potential cation channel
VDR	Vitamin D receptor
WNT	Wingless-related integration site

5.1 Introduction

The strength and durability of the adult vertebrate skeleton come not from the construction of a monolithic static structure but from building a dynamic and renewable biologically active scaffold (Suva et al. 2005). This process requires an enormous supply of minerals, principally calcium and phosphate, along with the meticulous regulation of mineral storage, availability, and utility by a plethora of hormones acting in concert on kidney, intestine, and bone. Indeed, normal adult calcium and bone homeostasis can be almost

fully explained by the interactions of several regulatory hormones, including parathyroid hormone (PTH), PTH-related protein (PTHrP), 1,25 dihydroxyvitamin D3 (1,25(OH)₂D₃), fibroblast growth factor-23 (FGF23), calcitonin, and sex steroids (estradiol, progesterone, and testosterone).

During normal fetal development minerals such as calcium, phosphate and magnesium are maintained at significantly higher concentrations in utero to achieve adequate bone accretion (Taylor-Miller and Allgrove 2021). The availability of these critical minerals is an integral component of fetal development that facilitates the safe neonatal transition to post-natal life in all mammalian species (Taylor-Miller and Allgrove 2021). It is perhaps not surprising that many of the same mineral transport regulators active throughout adult life are associated with mineral transport across the placenta to the developing fetus; a concept that will be discussed throughout this review.

With regard to mineral ion action, inorganic phosphate (Pi) is critical for a wide variety of metabolic pathways including synthesis of DNA and RNA, formation of phospholipids and membranes, the generation of ATP/GTP/UTP, cellular signaling (phosphorylation and dephosphorylation), the buffering of pH in intracellular fluids and urine, as well as in the extracellular matrix (ECM) of bone and teeth in the forms of hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$) (Voelkl et al. 2021) and glucosamine-phosphate-derived glycoproteins (Wu 2021).

During embryonic development after the early chondrocyte anlage pattern for the endochondral skeleton is laid down, rapid bone formation and mineralization of the developing fetal skeleton creates a significant demand for minerals. In this scenario, kidneys, intestines, and skeleton (active in adults to control the extent of mineral ion metabolism) do not supply minerals to the normal fetus. Rather, the placenta meets the extensive fetal need for minerals by actively transporting calcium, phosphorus, and magnesium from the maternal circulation (Kovacs 2014). The placenta provides these essential minerals even when confronted with their

reduced concentrations in the maternal circulation. Indeed, the fetus develops in the face of a significant hypercalcemia yet maintains even higher mineral concentrations than in the mother or a normal adult (Kovacs 2014). These significantly elevated levels are required for the skeleton to form and mineralize normally (Care 1991). In this review, the regulatory mechanisms responsible for the transport of minerals across the placenta to the developing fetus are described in relation to the well-characterized mechanisms in play in the adult.

5.2 Roles of Phosphate, Calcium, and Vitamin D Postnatally in Bone and Kidney

5.2.1 Phosphate

Several factors and endocrine hormones modulate homeostasis of renal calcium and phosphate. Among those, (1,25(OH)₂D₃), PTH, and FGF23 have gained the most attention as very important regulators of phosphate homeostasis (Suva and Friedman 2020). These three hormones regulate each other, thereby forming a classic regulatory endocrine loop (Kovacs 2014).

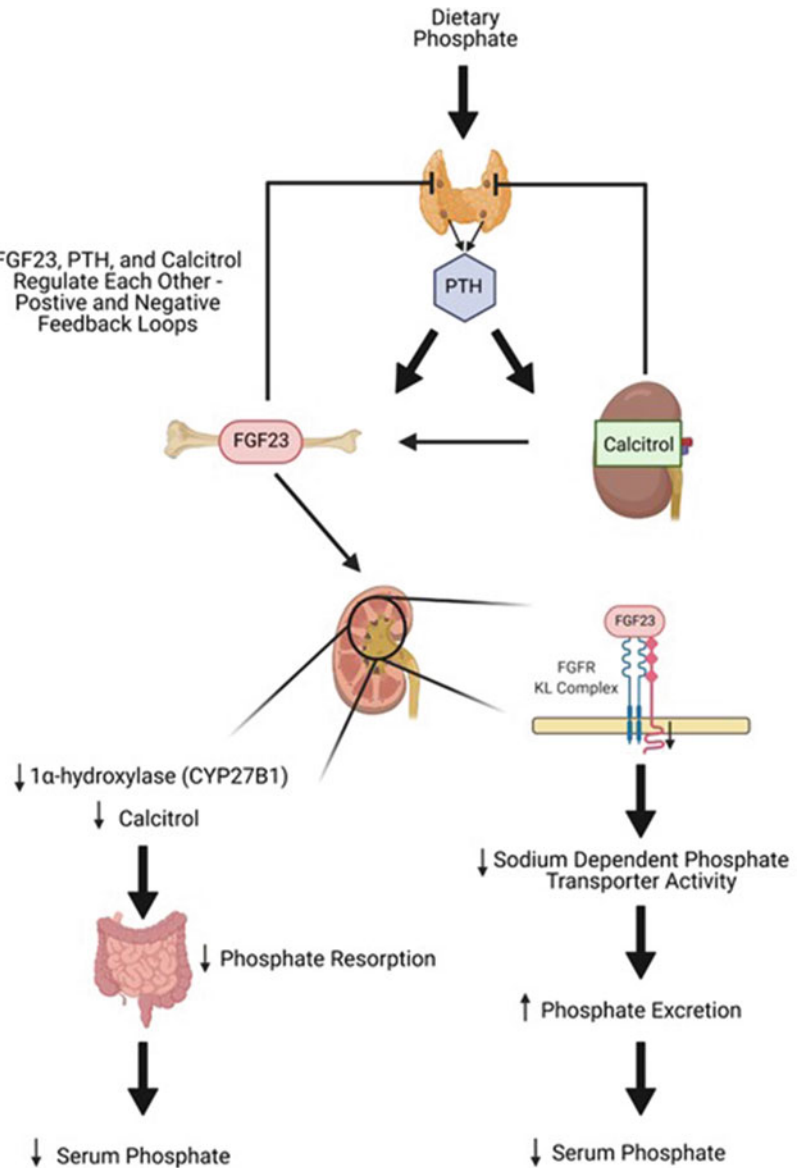
An increase in dietary phosphate intake or plasma phosphate levels stimulates PTH secretion, which enhances bone-derived FGF23 synthesis and release as well as the synthesis of 1,25(OH)₂D₃ by kidneys (Fig. 5.1). Similarly, modest decreases in serum calcium also produce profound and rapid increases in PTH secretion that enhance bone-derived FGF23 formation and release, as well as the synthesis of 1,25(OH)₂D₃ by the kidney. The release of FGF23 suppresses PTH and 1,25(OH)₂D₃ levels (PTH inhibits 1 α hydroxylase action in the kidney), whereas 1,25(OH)₂D₃ stimulates FGF23 release and inhibits PTH synthesis and secretion. In addition to these positive and negative feedback loops, all three hormones are regulated by a plethora of other factors.

Blood in the vascular circulation and extracellular fluid are responsible for the transport of phosphate to and from the organs involved in phosphate metabolism. It is the skeleton that provides the largest reservoir of phosphate, and it is also the source of a major hormone regulating phosphate transport, namely FGF23 (Dias et al. 2006; Peacock 2021). Bone mineral phosphate is exchangeable with extracellular fluid phosphate, but this flux does not substantially contribute to extracellular fluid phosphate homeostasis (Dias et al. 2006; Peacock 2021). Fundamentally, two transport pathways for phosphate exist in bone. One involves the transport of extracellular fluid phosphate into and out of bone whereas the second is the transport of phosphate within bone, directly from the bone-forming osteoblast as a component of the mineralizing apatite crystal (Peacock 2021). Daily net transport of phosphate from extracellular fluid to new bone formation and back to the extracellular fluid by bone resorption is less than that managed by the kidney and the gut (Peacock 2021).

It is the kidney that handles the major fraction of daily phosphate transport, as well as being the primary source for the generation of the active form of vitamin D (1,25(OH)₂D₃), through the action of the PTH-regulated 1 α hydroxylase (Li et al. 2020). 1,25(OH)₂D₃ is a critically important hormone for the regulation of phosphate transport. The process of glomerular filtration transports more than 5,000 mg phosphate every 24 h to the proximal renal tubule that reabsorbs and transfers the majority (>80%) back to extracellular fluid. As such, the kidney is a primary organ responsible for controlling the circulating concentrations of phosphate (Peacock 2018).

Given the fundamental role of the kidney, the impact of the gut on the supply of phosphate to the circulation is also important to consider. The amount of dietary phosphate is inadequate for herbivorous mammals without receiving supplementation (Wu 2018) and humans consuming little or no animal products. It is possible that

Fig. 5.1 PTH, FGF23, and 1,25(OH)₂D₃ are essential regulators of phosphate homeostasis postnatally. An increase in dietary phosphate intake or plasma phosphate levels stimulates PTH secretion, which enhances bone-derived FGF23 formation and release as well as the synthesis of 1,25(OH)₂D₃ by kidneys. The release of FGF23 suppresses PTH and 1,25(OH)₂D₃ levels (PTH inhibits 1 α hydroxylase action in the kidney), whereas 1,25(OH)₂D₃ stimulates FGF23 release and inhibits PTH synthesis and secretion. In addition to these positive and negative feedback loops, all three hormones are regulated by a plethora of other factors.



dietary phosphate is surplus over the daily requirements for phosphate in carnivores. Paracellular diffusion is an important mechanism for phosphate uptake in humans and other animals, but the mechanistic detail(s) and its relative magnitude remain to be fully elucidated. The absorption of phosphate occurs by both paracellular diffusion and active, saturable, transcellular mechanisms that act at low phosphate intakes (Peacock 2021). In addition, the small intestine is

an important site for phosphate transport, and it is directly responsible for the bulk of dietary phosphate absorption (Hernando and Wagner 2018). In contrast to animal products, the bioavailability of phosphate in plant-sourced foods is relatively low (Wu 2018). The efficiency of absorption is estimated at around 80% with some 20% of the absorbed phosphate returning to the lumen of the gut from the extracellular fluid as endogenous secretions.

With this in mind, it is clear that phosphate transport is both passively and actively performed and regulated by a number of transport mechanisms distributed across many tissues, but with a particularly important focus on the kidney, gut and bone (Peacock 2021).

5.2.2 Calcium

Similar to phosphate, the minute to minute regulation of serum calcium is also intimately and elegantly controlled by the interplay between calcium and phosphate as well as the level of the major calcium regulating hormones in the adult, PTH and 1,25(OH)₂D₃ (Kovacs 2014). Calcium is involved in many physiological and biochemical processes as it is an essential element for cardiac function, the structural integrity of bone, muscle contraction, and it also acts as a second messenger in cell signaling and a cofactor for many enzymes in a multitude of biochemical pathways. Serum calcium can be measured in venous blood samples, with normal physiologic levels ranging from 8.8 to 10.4 mg/dL for total calcium, and 4.7–5.2 mg/dL for free calcium (Kavsak 2017). Total calcium values should be corrected for current concentrations of albumin as their interaction can affect reported levels.

A consistent finding throughout fetal development is the high demand for calcium. Indeed, concentrations of calcium in fetal serum are significantly greater than the simultaneous concentrations of calcium in maternal serum in many species (Kovacs 2014). In fetal mice, free calcium is 0.25–0.50 mM greater than maternal values (Kovacs et al. 1996), and the values can be even higher in relation to maternal levels in a wide variety of species including pigs (Care et al. 1986), lambs (Delivoria-Papadopoulos et al. 1967), foals (Garel 1972), and perinatal primates (Fleischman et al. 1975). These important measurements indicate that the fetus is indeed hypercalcemic relative to both the maternal and normal adult serum calcium values. Interestingly, an explanation continues to elude us regarding how early in gestation do concentrations of

calcium in fetal serum exceed those in maternal serum.

Given the profound hypercalcemia of the fetus, an obvious question is why? What purpose is served by the fetus maintaining a high concentration of calcium in its blood? The conservation of this fetal hypercalcemia across mammals suggests some fundamentally important physiological function. Several rationales exist including the obvious need for high fetal calcium to ensure normal skeletal mineralization (especially in large animals) and the possibility that elevated blood calcium in utero provides a survival advantage after birth. Whatever the physiological reason, the details of why are unclear, especially since moderate fetal hypocalcemia does not appear to impair survival to the end of gestation as shown in murine studies (Kovacs et al. 2001a; Suzuki et al. 2008).

During endochondral bone formation in the fetus, the regulation of fetal mineral homeostasis is of utmost importance. In addition to the requirement for calcium, phosphorus also plays a key role. Since the mineralization of osteoid requires the incorporation of phosphate prior to calcium binding (Zhang et al. 2011), dietary phosphorus is an important determinant of bone mineralization during fetal development. In sum, concentrations of calcium, ionized calcium, and phosphorus in serum are significantly higher in fetuses than in the mother and can generally be maintained despite abnormal concentrations in the maternal circulation (Kovacs 2014).

As discussed previously, and throughout this review, the transport of both phosphate and calcium is regulated by a complex hormonal axis comprised of FGF23, PTH, and 1,25(OH)₂D₃. Perhaps most importantly, the metabolism of phosphate and calcium does not occur in isolation from each other. They closely interact at transport mechanisms in extracellular fluid, gut, bone, and kidney. Changes in the extracellular fluid concentrations of phosphate and calcium independently regulate secretion of the hormones, FGF23, PTH, and 1,25(OH)₂D₃ that maintain phosphate and calcium homeostasis. The regulation of phosphate and calcium

metabolism is intimately inter-related and neither should be considered in isolation.

5.3 Animal Models for the Study of Placental Mineral Transport (see Enders and Carter 2004; Carter 2007; Barry and Anthony 2008; Grigsby 2016)

The placenta is an underappreciated organ, which is critical for the exchange of minerals, gases, amino acids, sugars, and proteins, while producing regulatory molecules such as cytokines, growth factors, and hormones that are crucial for the development and growth of the conceptus (Enders and Blankenship 1999). Given the ethical concerns and the significant limitations associated with performing human placental research, comparative physiology is essential to improve understanding of the mechanisms governing placental nutrient and mineral transport, and conceptus development and growth. As there are often confounding factors in human pregnancy studies, animal models can provide valuable data from a controlled experimental design to allow further investigation into the importance of mineral transport in pregnancy. Despite the striking similarities that exist between mammalian species during pregnancy, placentation varies substantially, which can lead to differences in mineral and nutrient transport to the fetus (Enders and Blankenship 1999; Enders and Carter 2004; Montiel et al. 2013).

There are many published reports on placental transport of minerals with rodents, sheep, and pigs commonly used as animal models. Rodents are an extensively utilized as an inexpensive animal model for studies of conceptus growth and development. The gestation period for rats, mice, and hamsters is 20–22 days, allowing generation of data from large numbers of animals in a timely manner. Rodents undergo invasive implantation and have hemochorial placentae. While there are several similarities between human and rodent placentae in structure and function, there are several considerations that must be made when interpreting results from

studies with rodents (Wu 2022). Considering the significant interspecies differences in bone structure, composition, and density (Aeressens et al. 1998) and physiological maturity at birth, it is unsurprising that there are significant variations in the timings of ossification and skeletal mineralization. Compared with humans, rodents are relatively immature at birth, and their small size makes both surgical and non-surgical procedures challenging (Swanson and David 2015). In contrast to humans, rodents are a litter-bearing species, with individual fetoplacental units allowing for direct comparison among samples from different fetuses within the same uterus, without the confounding factors of maternal genotype, nutrition, or husbandry practices. However, as they are a litter bearing species, there are much greater demands being placed upon the mother to ensure that adequate mineral transfer occurs to allow appropriate fetal growth and development. Data suggest that the fetal mineral demand is much greater in rodents than in other commonly utilized animal models, with approximately 80% of the calcium in maternal blood transported to the fetuses in late gestation in rats, compared with 5–10% of calcium in maternal blood of women (Comar 1956; Widowson 1962). This makes rodents highly susceptible to secondary hyperparathyroidism and hypocalcemia compared with other species. While this allows for unique opportunities to study the effects of these conditions on fetal growth and development, it does raise the question of how comparable placental mineral transport is in rodents compared with other species.

In contrast to rodents, sheep are much larger in size, making them easier to handle and perform experimental manipulations. Importantly, the sheep fetus has a similar physiological maturity at birth to human infants. Furthermore, like humans, sheep usually have singleton or twin pregnancies. Sheep have a much longer gestation length than rodents, which allows investigations to focus on comparable stages of gestation to those for human pregnancies. While the sheep placenta is viewed by some as being anatomically distinct from the human placenta, there are many important similarities. The sheep

have a multi-cotyledonary placenta, consisting of placentomes composed of maternal caruncular tissue and fetal cotyledonary tissue. Large variations in cotyledonary numbers exist and it is known that breed, litter size, and parity can influence cotyledonary number and size (Dwyer et al. 2005). Despite the gross anatomical differences, the human placenta also has cotyledonary structures, making them more similar in structure to those for sheep than may be appreciated by some scientists. Additionally, while the fetal vascular trees of sheep and human placentae differ in size, the architecture of the stem, intermediate, and terminal villi is comparable (Leiser et al. 1997).

Similar to rodents, pigs are a litter-bearing species with individual feto-placental units, so comparisons can be made among different fetuses within the same uterus, with minimal confounding effects. Pigs are much larger in size than rodents, making them easier to handle and perform experimental manipulations. Furthermore, piglets have a similar physiological maturity at birth to human infants and a longer gestation length than rodents, which allows investigations to occur at desired, comparable stages of gestation to human pregnancies. Pigs have a true epitheliochorial placenta, wherein the uterine luminal epithelium (LE) remains intact throughout pregnancy and the trophoctoderm directly attaches to the LE. This type of conceptus attachment is much less invasive than for other types of placental types; therefore, extensive remodeling must occur to maximize placental surface area available for nutrient, mineral, and gas exchange.

It is important to consider these species-specific differences when designing an experiment to investigate placental transport of minerals and nutrients and when extrapolating the findings from studies of one species to another species. While each animal model has its own merits, it is important to note that no animal model fully recapitulates human pregnancy.

5.4 Maternal Mineral Adaptations

Calcium and phosphorous are two of the most abundant minerals in the body, with approximately 99 and 85% of this calcium and phosphorous, respectively, stored in bone in the form of hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$) (Mitchell et al. 1945; Penido and Alon 2012). In addition to their critical roles in the skeletal and renal systems outlined earlier, calcium and phosphate are regulators of many processes including cellular proliferation, protein synthesis, and cellular metabolism (Chin et al. 1987; Santella 1998; Brostrom and Brostrom 2003; Jeon 2008; Glancy and Balaban 2012; Penido and Alon 2012; Kovacs 2014). During pregnancy, the mother must adapt to meet the nutritional and mineral requirements of the developing fetus, without compromising the maternal mineral reserve. Studies investigating placental transport of minerals have demonstrated that the rate of calcium and phosphate transport significantly increases in the last trimester of pregnancy to allow for fetal skeletal mineralization. In fact, it is estimated that around 80% of the minerals present in a fetus at birth are transported during the last trimester of pregnancy. In humans, this translates to approximately 20 g of phosphate and 30 g of calcium (Givens and Macy 1933; Widdowson and McCance 1965; Ziegler et al. 1976). Unsurprisingly, this is a significant burden on the mother's mineral reserve and the mechanisms regulating maternal mineral homeostasis must be altered to avoid the mother becoming hypocalcemic or hypophosphatemic.

To accommodate the increasing mineral demands, the maternal intestine must double the absorption of phosphate and calcium (Kent et al. 1991; Ritchie et al. 1998; Kovacs 2016) (Fig. 5.2). Increasing intestinal mineral absorption is a crucial gestational adaptation to provide sufficient minerals for transport across the placenta to meet the demands of the developing fetus. In humans, this begins after approximately

12 weeks of pregnancy and is maintained until parturition (Heaney and Skillman 1971; Kent et al. 1991; Cross et al. 1995). This strategy increases the maternal mineral reserve so that in the latter stages of gestation, during the period of exponential fetal growth and skeletal mineralization, there are sufficient amounts of calcium and phosphate available for the fetus. There are conflicting theories regarding what regulates this change in maternal mineral absorption. Some consider calcitriol to be the central regulator of this adaptation as it is known to increase intestinal expression of molecules involved in calcium transport including calcium-binding proteins, transient receptor potential cation channel family members, and Ca^{2+} ATPases [reviewed by (Kovacs 2016)]. While this is plausible, the increase in intestinal calcium absorption begins early in gestation, prior to the increase in maternal calcitriol. Additionally, recent evidence from research with mice indicates that calcitriol is not the key regulator of this increase in intestinal calcium absorption during pregnancy (Fudge and Kovacs 2010; Gillies et al. 2018). Evidence from animal models suggests that prolactin and placental lactogen can regulate intestinal calcium absorption; therefore, the potential role of these molecules in the regulation of increased intestinal calcium absorption during the first trimester warrants further investigation. Despite this crucial and significant increase in intestinal calcium absorption, the maternal kidneys do not alter their resorption of minerals, ultimately leading to significant increases in calcium excretion in urine.

Considering the extensive transport of calcium that must occur across the placenta to the fetus, it is unsurprising that calcium in maternal serum decreases in late gestation in many species including rats, sheep, goats, and cows [reviewed by Kovacs (2016)]. Results of several studies of women suggest that calcium in maternal serum decreases in pregnancy; however, results of other studies suggest that the abundance of free calcium in maternal serum is not affected by stage of pregnancy [reviewed by Kovacs (2016)].

5.5 Gestational Changes in Phosphate and Calcium in Fetal Fluids

Prior to the establishment of a functional placenta for transplacental exchange of gases, micronutrients (amino acids, glucose), and macromolecules (proteins), the conceptus is entirely reliant upon the secretion and transport of nutrients into the uterine lumen by the maternal uterine glandular epithelium (GE) and LE. A simple and effective manner to assess the nutrient and mineral composition within the uterine luminal environment is to flush the uterus with a buffer solution and compare the nutrient and mineral composition across different days of gestation. Data from pigs and sheep indicate that calcium in uterine flushings increases during the peri-implantation period and is more abundant in uterine flushings from pregnant animals when compared with cyclic animals during the mid-luteal phase of the estrous cycle (Gao et al. 2009; Choi et al. 2019; Stenhouse et al. 2021a). Furthermore, it has been demonstrated in mice that this increase in calcium is essential for the regulation of conceptus development and implantation, with infusion of calcium channel blockers into the mouse uterine lumen causing failure of blastocysts to implant (Banerjee et al. 2011).

As the major components of the skeleton, calcium, and phosphate must be transported across the placenta to the fetus for adequate mineralization of the skeleton (Kovacs 2014, 2016). Studies investigating placental transport of minerals have demonstrated that the rate of calcium and phosphate transport increases significantly during the last trimester of pregnancy to allow mineralization of the fetal skeletal system to occur, as noted previously. In humans, >300 mg of calcium must be transported per day to the fetus between weeks 35 and 38 of gestation. It has been suggested that pregnant ewes in late gestation must transport 1.25 g of phosphate and 2.7 g of calcium to the ovine fetus each day (Grace et al. 1986).

Fetal fluids (allantoic and amniotic fluids) are of maternal origin via active transport of water,

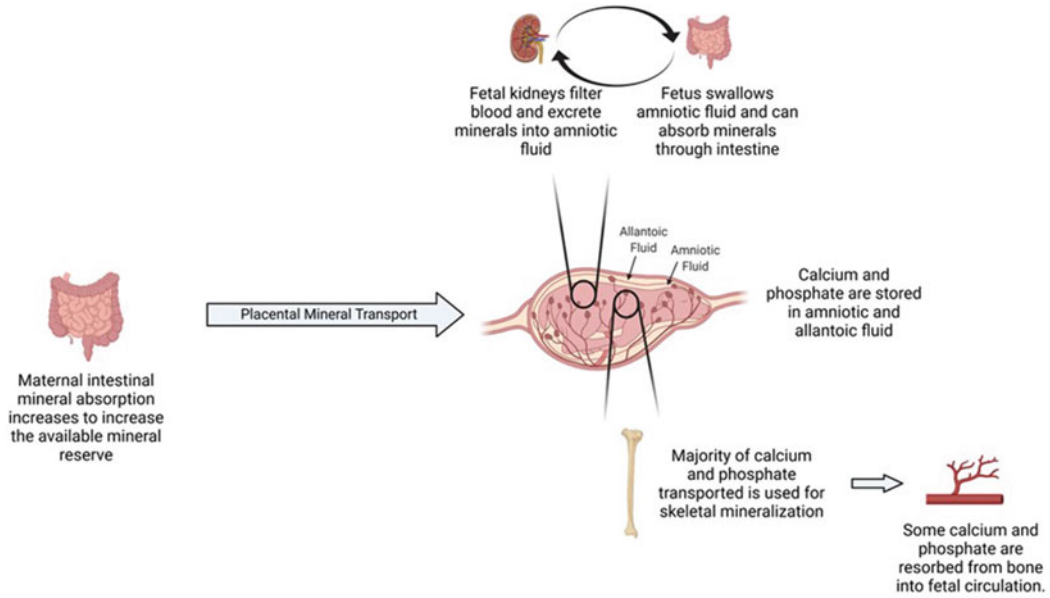


Fig. 5.2 Maternal mineral absorption increases during gestation from an increased mineral reserve for fetal skeletal mineralization. During pregnancy, the mother must adapt to meet the nutritional and mineral requirements of the developing fetus, without compromising the maternal mineral reserve. Studies investigating placental mineral transport have demonstrated that the rate of calcium and phosphate transport significantly increases in the last trimester of pregnancy to allow for fetal skeletal mineralization, with the majority of transported calcium and phosphate forming the fetal skeleton. To

accommodate the increasing mineral demands, the maternal intestine must double the absorption of phosphate and calcium. Following placental transport of these minerals, they can be stored in amniotic and allantoic fluid. The fetus can drink amniotic fluid, which is an important additional source of minerals for the fetus as the fetal intestines can absorb minerals present in amniotic fluid. The fetal kidneys then filter the blood and excrete minerals into urine, which in turn makes up much of the volume of amniotic fluid; thereby forming an intestinal-renal-amniotic fluid loop

as well as other molecules, across the placenta and into the allantoic sac for distribution to other components of the conceptus including the fetus and amniotic sac. The driving force for expansion of the allantois, and in turn the chorioallantois, is the rapid accumulation of water in the allantoic sac. In the pregnant ewe, this volume increases from about 1 ml on Day 18 to 90 ml on Day 40 and then from Day 70 (32 ml) to Day 140 (438 ml) of the 147-day period of gestation. The rapid changes in allantoic fluid volume allow expansion of the chorioallantoic membranes and ensure their intimate apposition across the maximum surface area for attachment to the maternal endometrium. Allantoic fluid is a nutrient reservoir that is rich in electrolytes, sugars, amino acids, minerals, and other nutrients, as well as

proteins. Importantly, the allantoic epithelium derives from the hindgut and functions to transport nutrients from allantoic fluid into the fetal-placental vasculature. Amniotic fluid is isosmotic to fetal serum, and its volume increases throughout gestation in sheep from 2 ml on Day 30 to over 700 ml on Day 140 of gestation. Amniotic fluid has several important roles. First, it buoys the fetus to allow it to develop symmetrically in three dimensions. Second, it prevents fetal skin from adhering to the amnion. Third, the fetus may drink up to 1 L of amniotic fluid in the last one-third of gestation to gain water and other nutrients, including proteins secreted by the lungs, salivary glands, and amniotic membranes. This act of drinking amniotic fluid is an important additional source

of minerals for the fetus as the fetal intestines can absorb minerals present in amniotic fluid.

Phosphate and calcium are both very abundant in ovine amniotic and allantoic fluids across gestation (Stenhouse et al. 2021a; b), indicative of extensive mineral transport across the placenta. Total phosphate and calcium in ovine allantoic fluid increase significantly with advancing gestational day, highlighting that in late gestation when increased placental mineral transport occurs, these minerals are stored in the allantoic fluid for the fetus to utilize for skeletal mineralization. However, total calcium and phosphate are relatively constant in ovine amniotic fluid across gestation, despite significant changes in fluid volume across pregnancy (Stenhouse et al. 2021a; b). In goats, the concentrations of phosphorous and calcium in allantoic fluid increase with advancing gestational day, whereas in amniotic fluid, the concentrations of both calcium and phosphorous decrease with advancing gestational stage (Tabatabaei 2012). Similarly, it has been suggested that the concentrations of phosphate in human amniotic fluid decrease with advancing gestational day (Fotiou et al. 2015; Correia-Branco et al. 2020). It is important to note that differences in placentation type, stage of gestation, and fetal number may all influence the abundance of these minerals in fetal fluids. Additionally, it is known that the volume of fetal fluids fluctuates significantly across gestation (Bazer et al. 2012; Dubil and Magann 2013). Therefore, care should be taken when comparing fetal fluid data presented as concentration as compared with total abundance (concentration X fluid volume).

While it is understood that mineral transport at the maternal–conceptus interface increases in late gestation, it is important to ascertain the spatiotemporal profile of molecules that regulate calcium and phosphate transport and vitamin D metabolism at the maternal–conceptus interface across species. Many studies have investigated calcium, phosphate, and vitamin D signaling in pregnancy. While these are all important signaling pathways individually, we must ascertain how these pathways work in concert at the

maternal–conceptus interface to fully understand the importance of these minerals during the course of gestation.

5.6 Impact of Maternal Calcium and Vitamin D Deficiencies on Fetal Development and Pregnancy Outcomes

Hypocalcemia in pregnancy occurs when the fetal demands for calcium exceed the availability of calcium in maternal serum. Deficiency in maternal calcium is a significant problem in pregnancy and has been associated with several adverse pregnancy outcomes including pre-eclampsia, preterm birth, and intrauterine growth restriction (Chhabra and Singh 2017; Wilson et al. 2020). Thus, calcium has an important role in the regulation of conceptus development and growth. In mothers with severe hypocalcemia, the increase in intestinal calcium absorption that occurs in a normal pregnancy is not sufficient to increase the available calcium reserve in the mother. In these instances, calcium must be resorbed from maternal bone, which can decrease maternal bone density and increase the risk of the mother developing osteoporosis during pregnancy (Kovacs and Ralston 2015). Despite these adaptations in mineral status of the mother, it is necessary to monitor mineral status and ensure that there is sufficient mineral transport across the placenta because severe calcium deficiencies impact placental transport of calcium to the fetus which, in severe cases, can lead to demineralization of the fetal skeleton and development of secondary hyperparathyroidism [reviewed by Kovacs (2014)]. Similar findings have been reported for pregnant rats fed a calcium-deficient diet that led to maternal hypocalcemia and secondary hyperparathyroidism in dams, and resorption of minerals from the fetal skeletal system and secondary hyperparathyroidism in the fetuses [reviewed by Kovacs (2014)].

In dairy cows, the periparturient period (4 weeks before to 4 weeks after calving) is often associated with the development of ‘milk fever’

(hypocalcemia). Reports vary substantially on the incidence of this condition (DeGaris and Lean 2009); however, a meta-analysis of 137 controlled trials estimated that the mean incidence of the condition is 21% (range 0–83%) (Lean et al. 2006). In this condition, calcium in maternal serum can reach < 50% of the recommended normal concentrations of calcium in serum (Mayer et al. 1969; Allen and Sansom 1986), which can have severe consequences for both the mother and fetus. Hypocalcemia in dairy cows is associated with an increased risk for many undesirable conditions including parturition recumbency, dystocia, still born calves, ketosis, displaced abomasum, and even death. Uterine involution is less efficient in cows with hypocalcemia, therefore, there is a higher incidence of uterine prolapse and retained fetal membranes in hypocalcemic cows (Risco et al. 1994). Additionally, cows with hypocalcemia are more susceptible to infections, including mastitis and metritis after calving (Ducusin et al. 2003). This condition is not restricted to dairy cows as a similar condition occurs in ewes during the periparturient period that is estimated to account for between 4 and 6% of the mortality rate in sheep (Hindson and Winter 2002). Collectively, these findings indicate that hypocalcemia in large animals during the periparturient period has significant adverse impacts on pregnancy outcomes and maternal and offspring health, which can be costly for the livestock industry.

Feeding low amounts of calcium to pregnant ewes for 2 months from Day 60 of gestation significantly decreased maternal calcium while increasing circulating concentrations of hydroxyproline, parathyroid hormone, and 1,25(OH)₂D (Lima et al. 1993). Interestingly, maternal hypocalcemia in ewes does not influence calcium, hydroxyproline, parathyroid hormone, or 1,25(OH)₂D in fetal plasma at Day 120 of gestation. Despite the lack of differences in calcium in fetal plasma, higher concentrations of phosphate in fetal plasma result in delayed ossification of the skeletal system, a lower proportion of bone compared with cartilage, lower specific gravity, and lower ash content in fetuses from hypocalcemic mothers (Lima et al. 1993).

Maternal vitamin D deficiency is also a significant problem in pregnancy and has been linked to several adverse pregnancy outcomes including pre-eclampsia, preterm birth, and intrauterine growth restriction (Bodnar et al. 2007; Ma et al. 2012; Germand et al. 2014; Achkar et al. 2015; Chen et al. 2015; Qin et al. 2016; Zhou et al. 2017; Alimohamadi et al. 2020; Wilson et al. 2020). Thus, vitamin D has an important role in the regulation of conceptus development and growth. In addition to impacts on pregnancy outcome, it has been suggested that maternal hypovitaminosis D (defined as maternal 25(OH)D] levels <20 ng/ml or <50 nmol/l) is a significant risk factor for adverse neonatal outcomes in humans (Karras et al. 2016). There are large variations in the reported incidence of maternal hypovitaminosis D, presumably due to seasonal and locational variations in maternal vitamin D status [reviewed by Karras et al. (2016)], but this problem does affect the global population. Despite the clear associations between maternal calcium status and fetal skeletal development, there are contradicting reports on the association between maternal vitamin D status and fetal skeletal development in humans [reviewed by Karras et al. (2016)]. However, there is some evidence suggesting a link between maternal vitamin D status and fetal bone development, with maternal hypovitaminosis D impacting fetal bone development from as early as 19 weeks of gestation (Mahon et al. 2010; Ioannou et al. 2012; Young et al. 2012).

5.7 Regulators of Placental Calcium and Phosphate Transport

Given the importance of phosphate, calcium, and vitamin D for many critical processes postnatally, it is unsurprising that many regulatory mechanisms exist (Fig. 5.3). In this review, we will briefly summarize some of the available evidence for the roles of several of the regulatory processes present at the maternal–conceptus interface across species affecting phosphate, calcium, and vitamin D.

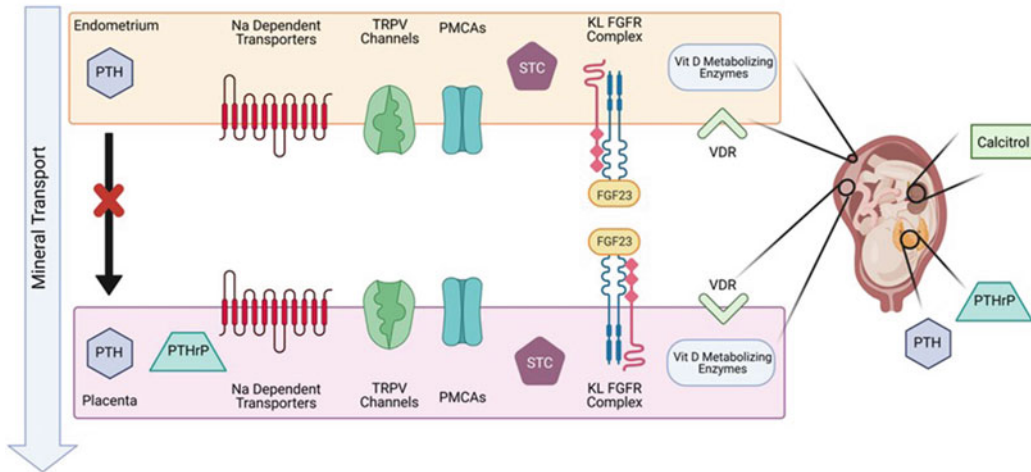


Fig. 5.3 Many signaling pathways are present at the maternal–conceptus interface across species for the regulation of phosphate, calcium, and vitamin D transport and metabolism. As the major components of the skeleton, calcium and phosphate must be transported across the placenta to the fetus, where they must be present in excess abundance compared with that in the

mother to allow adequate mineralization of the skeleton. Considering this, it is perhaps unsurprising that many pathways are expressed at the maternal–conceptus interface for the regulation of the transport of these molecules. These pathways are intimately related to one another and therefore must not be considered in isolation from one another

5.7.1 PTH and PTHrP

Parathyroid hormone (PTH), PTH-related protein (PTHrP), and the cognate G protein-coupled PTH receptor (PTHrR) play defining roles in the regulation of extracellular calcium and phosphate metabolism and in controlling skeletal growth and repair. Through a series of complex signaling mechanisms that, in many instances proceed in a tissue-specific manner, precise control of these processes is achieved and maintained.

The shared receptor (PTHrR) mediates all known biological actions of PTH and PTHrP (Suva and Friedman 2020). Full length PTH is a peptide of 84 amino acids whereas PTHrP exists as peptide products of 139, 141, or 173 amino acid residues derived from a single alternatively spliced gene distinct from the PTH gene (Suva and Friedman 2020). The fundamental role of PTH in phosphate and calcium metabolism in adults is well defined as it has been the focus of intense investigation for many years (Quarles 2008).

Within the fetal circulation, PTH is less abundant when compared with concentrations in maternal blood of several species such as lambs and rodents (Thomas et al. 1981; Collignon et al. 1996; Simmonds et al. 2010). These low values for PTH in the fetus are from sources within the fetus since intact PTH does not cross the placenta (Garel 1972; Northrop et al. 1977; Günther et al. 2000). During development, fetal parathyroid glands begin to express PTH by mid-gestation (Tucci et al. 1996). The fetal parathyroid gland appears to be the primary source since genetic ablation of the fetal parathyroids using *Hoxa3* null mice results in undetectable PTH in serum, measured using a rodent PTH immunoradiometric assay (Kovacs et al. 2001b). An additional source of PTH is likely the placenta since the PTH gene is expressed by placentae of mice. As such, the placenta may contribute a small amount of PTH to fetal circulation (Simmonds and Kovacs 2010). The normally low concentration of PTH in fetal blood is critical for the maintenance of calcium concentrations in fetal blood

since fetal mice lacking PTH or the PTHR are hypocalcemic compared with WT littermates (Kovacs et al. 1996).

Only since the discovery and cloning of PTHrP (Suva et al. 1987) have details of the movement of calcium across the placenta begun to be elucidated (Abbas et al. 1989; MacIsaac et al. 1991). Since fetal blood contains high PTH-like bioactivity, but low levels of immunoreactive PTH (Allgrove et al. 1985; Loveridge et al. 1988; Rodda et al. 1988; Bourdeau et al. 1990), the finding of elevated PTHrP in fetal blood (Khosla et al. 1990; Seki et al. 1994) explained earlier reports of PTH-like bioactivity. These results led to the overarching hypothesis that PTHrP is the primary calcium-regulating hormone in fetuses, and that the suppressed PTH levels suggested that PTH is largely unimportant until after birth. Furthermore, studies in chronically cannulated sheep fetuses demonstrated a PTHrP-specific action (Abbas et al. 1989) and investigation of PTHrP null mice fetuses compared with their WT and PTHrP \pm littermates demonstrated the importance of PTHrP in regulating mineral and bone metabolism in the fetus (Kovacs 2014).

5.7.2 Calcitriol

Postnatally, vitamin D has a central role in the regulation of mineral homeostasis (Bouillon et al. 2019). Vitamin D is acquired through the diet, in the form of fish liver and fortified dairy products, and vitamin D₃ (cholecalciferol) can be synthesized from cholesterol in the skin following exposure to ultraviolet irradiation (Schmid and Walther 2013). Cytochrome P450 mixed-function oxidases (CYPs) are the regulators of vitamin D metabolism (Jones et al. 2014). Vitamin D enters the systemic circulation bound to vitamin D binding protein and is transported to the liver, where it is hydroxylated by CYP2R1 to 25-hydroxyvitamin D (25[OH]D₃) (Bikle 2014). 25[OH]D₃ is then transported to the kidney, bone, and placenta where it is further hydroxylated to the active hormonal form 1,25-dihydroxyvitamin D (1,25[OH]₂D₃) by 1α -

hydroxylase CYP27B1 (Bikle 2014; Wu 2018). The activity of calcitriol is regulated by the catabolic activity of CYP24A1 (24-hydroxylase) (Bikle 2014), which inactivates 1,25[OH]₂D₃ via conversion to 1,24,25[OH]₃D₃. The biologically active form of vitamin D (1,25(OH)₂D₃ or calcitriol) binds to the vitamin D receptor (VDR). The VDR (also known as NR1I1 (nuclear receptor subfamily 1, group I, member 1) is a member of the nuclear receptor family of transcription factors. 1,25(OH)₂D₃ binds to the VDR, which then heterodimerizes with the retinoid-X receptor to elicit transcriptional effects. Vitamin D₂ from sunlight-dried plants is almost as effective as vitamin D₃ in most mammals, including humans, cattle, sheep, and pigs (Wu 2018).

As gestation progresses, the abundance of calcitriol in maternal blood increases significantly in many species including humans, rabbits, rodents, and pigs (Pike et al. 1979; Kubota et al. 1982; Ardawi et al. 1997; Boass et al. 1997; Kovacs et al. 2005; Kirby et al. 2013; Jang et al. 2017). This increase in maternal calcitriol occurs due to increased synthesis of calcitriol and not due to alterations in the metabolic clearance of calcitriol (Ross et al. 1989; Paulson et al. 1990). Species specific differences in the abundance of calcitriol in fetal blood versus maternal blood do exist. In rodents (Lester et al. 1978; Verhaeghe et al. 1988; Kovacs et al. 2005) and pigs (Lachenmaier-Currle et al. 1989; Lachenmaier-Currle and Harmeyer 1989), the abundance of calcitriol in fetal blood is <50% of that in maternal blood. It has been suggested that calcitriol cannot cross the placenta in rats (Noff and Edelstein 1978); therefore, the conceptus itself must be responsible for the generation of calcitriol from 25-hydroxyvitamin D that can be transported across the placenta in rats (Haddad et al. 1971). In contrast, calcitriol abundance is significantly greater in fetal blood than maternal blood in pregnant ewes (Abbas et al. 1987; Paulson et al. 1987) and calcitriol can pass between maternal and fetal blood in pregnant ewes (Devaskar et al. 1984). Furthermore, given the rapid metabolism of calcitriol in sheep fetuses, it is thought that calcitriol production is

significantly upregulated in the ovine conceptus when compared with maternal production of calcitriol (Ross et al. 1989).

While it is well established that vitamin D is an important regulator of mineral homeostasis postnatally, contradicting data from animal models exists regarding whether vitamin D is required to maintain normal concentrations of minerals in fetal serum (Kovacs 2014). Loss of 1α -hydroxylase in fetal pigs, vitamin D deficiencies in rats, and some data from VDR null mice suggest that abundances of calcium, phosphorus, and PTH in fetal serum are not regulated by calcitriol, vitamin D, or VDR [reviewed by Kovacs (2014)]. However, other data from studies of other VDR null mice lines are inconsistent with these reports [reviewed by Kovacs (2014)].

In humans, rodents, and pigs, vitamin D metabolizing enzymes and the production of vitamin D at the maternal–conceptus interface have been demonstrated (Shahbazi et al. 2011; Bergada et al. 2014; O’Brien et al. 2014; Jang et al. 2017), suggesting potentially important roles of vitamin D in the establishment and maintenance of pregnancy, and in the regulation of conceptus growth and development. The expression of *CYP27B1* mRNA and protein in placenta is positively correlated to the abundance of vitamin D in blood of humans (O’Brien et al. 2014). VDR is more abundant in the endometrium of pregnant mice compared with cyclic mice, and VDR expression increases in both the placenta and decidua with advancing days of gestation (Shahbazi et al. 2011). Additionally, the expression of *CYP2R1*, *CYP27B1*, *CYP24A1*, *GC*, and VDR varies significantly across gestation at the porcine maternal–conceptus interface (Jang et al. 2017), suggesting that vitamin D metabolism at the maternal–conceptus interface is regulated locally and varies across gestation.

Recently, we demonstrated the expression of vitamin D regulatory molecules at the ovine maternal–conceptus interface across gestation (Stenhouse et al. 2021a). VDR protein is expressed by both the trophectoderm and endoderm of the Day 17 conceptus, with more intense

staining in the endoderm. Furthermore, VDR protein is expressed by the myometrium, blood vessels, uterine LE, uterine superficial glandular epithelium (sGE), uterine caruncles, and chorioallantois throughout pregnancy. The expression of VDR protein at the ovine maternal–conceptus interface throughout pregnancy demonstrates that the synthesis of active vitamin D may have broad functional consequences locally throughout pregnancy. Molecules involved in the metabolism of vitamin D (*CYP2R1*, *CYP11A1*, *CYP24*, and *CYP27B1*, and VDR) are expressed at the mRNA level in both endometria and placentae across gestation in sheep (Stenhouse et al. 2021a). Interestingly, *CYP2R1* and *CYP27B1* mRNAs have stable expression in ovine endometria and placenta across days of gestation (Stenhouse et al. 2021a). Since *CYP2R1* and *CYP27B1* are essential for the production of calcitriol, stable expression across gestation suggests an essential role for vitamin D throughout pregnancy across the maternal–conceptus interface. The expression of *CYP24*, VDR, and *CYP11A1* mRNAs at the ovine maternal–conceptus interface varies depending upon the gestational day investigated, with high expression in early pregnancy that decreases with advancing days of gestation (Stenhouse et al. 2021a). The activity of calcitriol is regulated by the catabolic activity of *CYP24A1* (24-hydroxylase) that inactivates $1,25\text{[OH]}_2\text{D}_3$ via conversion to $1,24,25\text{[OH]}_2\text{D}_3$ (Bikle 2014). The high expression of *CYP24* mRNA in ovine endometria and placenta in early pregnancy compared with mid- and late-gestation could be indicative of high requirements for these tissues to catabolize calcitriol during implantation and placentation, which may not be required as extensively in mid- to late-gestation when the placenta is established and readily transporting minerals and nutrients to the fetus. Similar findings have been reported for porcine endometria, with high expression of *CYP24* mRNA on Days 12 and 15 of gestation (Jang et al. 2017). Thus, it could be postulated that a localized negative feedback mechanism is present to regulate the abundance of calcitriol at the maternal–conceptus interface. Further

investigations into the spatiotemporal expression and enzymatic activity of these enzymes would provide valuable insights into the importance of vitamin D in the establishment and maintenance of pregnancy in mammals.

Using porcine endometrial explant cultures, it has been demonstrated that calcitriol regulates the expression of several genes with essential roles in the establishment and maintenance of pregnancy including fibroblast growth factor 7 (FGF7), and secreted phosphoprotein 1 (SPP1) (Jang et al. 2017). In addition to the essential roles of vitamin D in the regulation of phosphate and calcium homeostasis, there are several ‘non-classical’ extra-skeletal functions of vitamin D. Vitamin D is a known regulator of both the adaptive and innate immune systems (Adams and Hewison 2008), targeting multiple immune cell types including T- and B-lymphocytes, monocytes, macrophages, and dendritic cells [reviewed by Baeke et al. (2010)]. Importantly, vitamin D modulates cytokine production by T-lymphocytes and antigen-presenting cells, and enhances anti-microbial activity of macrophages and monocytes, [reviewed by Baeke et al. (2010)]. During pregnancy, the uterine immune system must be tightly regulated to ensure that required anti-microbial protection occurs while ensuring that the conceptus is not rejected by the maternal immune system (Hunt 2006). Therefore, an immunomodulatory role of vitamin D at the maternal–conceptus interface has been suggested [reviewed by Tamblyn et al. (2015)]. Metabolism of vitamin D stimulates both anti-bacterial and anti-inflammatory responses by human decidual and trophoblast cells (Evans et al. 2006; Díaz et al. 2009; Liu et al. 2009). Additionally, trophoblast-derived vitamin D has been suggested to be a regulator of placental inflammation in mice (Liu et al. 2011). Given the importance of immune modulation during pregnancy, and the described spatiotemporal expression profile of vitamin D regulatory molecules at the maternal–conceptus interface in multiple species, it could be hypothesized that vitamin D not only modulates phosphate and calcium transport but plays an important role in

immunomodulation at the maternal–conceptus interface during implantation and placentation, which warrants further investigation.

5.7.3 Phosphatonins

Fibroblast growth factor 23 (FGF23), secreted frizzled-related protein 4 (sFRP4), matrix extracellular phosphoglycoprotein, and fibroblast growth factor 7 (FGF7) are members of the phosphatonin family of circulating molecules with suggested roles in the maintenance of phosphate homeostasis (Berndt and Kumar 2007; Shaikh et al. 2008). Of the phosphatonins, FGF23 has been the most extensively investigated and is considered a significant regulator of phosphate transport through its interactions with Klotho (KL). KL acts as a coreceptor with members of the fibroblast growth factor receptor family (FGFR), and FGF23 binds to the FGFR-KL complex. Binding to this complex can regulate WNT (wingless-related integration site), PKC (protein kinase C), cAMP (cyclic adenosine monophosphate), p53 (tumor protein 53), and p21 (cyclin-dependent kinase inhibitor) signaling cascades to maintain phosphate homeostasis (Wang and Sun 2009). The metalloproteinases ADAM10 and ADAM17 can cleave KL from the plasma membrane to form a secreted form of KL (Chen et al. 2007). KL can mediate phosphate absorption by the intestine, phosphate excretion in urine, and phosphate distribution in bone in both an FGF23-dependent and -independent manner. Furthermore, the expression of type II and type III sodium-dependent phosphate transporters is tightly regulated via KL and FGF23 (Bon et al. 2018; Hu et al. 2019). In addition, other FGF family members such as FGF19 and FGF21 can utilize the KL-FGFR complex to enhance their cell signaling (Dolegowska et al. 2019). In addition to the role of KL signaling in the regulation of phosphate homeostasis, KL likely has essential roles in the regulation of calcium (Nabeshima and Imura 2008), acting as a glucuronidase to activate transient receptor potential cation channel 5 (TRPV5) (Chang et al.

2005). Furthermore, KL is a mediator of calcitriol synthesis (Yoshida et al. 2002; Haussler et al. 2013).

In mice, the fetal heart, liver, and somites express FGF23 from embryonic day 12.5 (Sitara et al. 2004). Despite low expression of *FGF23* mRNA by the murine placenta, several genes involved in FGF23-KL signaling are expressed including KL, SLC34A1, SLC34A2, SLC34A3, CYP27B1, CYP24A1, FGFR1, FGFR2, FGFR3, and FGFR4 (Ma et al. 2014). Transgenic mouse lines have provided interesting insights into the role of FGF23 in the regulation of placental phosphate transport. While FGF23 null mice are fertile, they have a growth restriction phenotype accompanied by shorter longevity (Sitara et al. 2004). Interestingly, these studies revealed that intact FGF23 cannot cross the murine placenta despite fetal levels of intact FGF23 being comparable to intact FGF23 levels in wild-type pregnant mice (Ma et al. 2014).

A role of KL in the maintenance of calcium and phosphate transport in pigs has also been suggested (Choi et al. 2014a). *KL* mRNA is expressed in both the porcine endometria and chorioallantois and analysis of porcine endometrial *KL* mRNA expression demonstrated that *KL* mRNA decreased between Days 12–15 of the estrous cycle. Interestingly, porcine endometrial *KL* mRNA has a biphasic expression profile, with the highest expression observed on Days 12 and 90 of gestation. A role of KL-FGF23 signaling in the regulation of phosphate transport during pregnancy in ewes has also been suggested (Stenhouse et al. 2021b). FGF23 and KL proteins are localized to both the endoderm and trophoderm of Day 17 ovine conceptuses, and both were abundantly expressed in the myometrium, uterine LE, sGE, and GE, and stratum compactum stroma, as well as blood vessels throughout gestation. FGF23 protein is expressed by the mononuclear and binucleate cells at the interface of the chorioallantois and caruncular tissue, and in areas undergoing syncytialization within the placentome. As pregnancy progresses, FGF23 protein is expressed in greater abundance in caruncular tissue than cotyledonary tissue of placentomes. KL protein is expressed in

caruncular tissue, the chorioallantois and blood vessels throughout gestation. There is expression of FGF23-KL signaling molecules (FGF23, FGFR1-4, KL, KLB, ADAM10, and ADAM17) at the mRNA level at the ovine maternal–conceptus interface throughout gestation. The spatiotemporal expression profiles suggest potential roles of KL-FGF23 signaling in the regulation of conceptus implantation in sheep, which warrants further investigation.

KL is very abundant in human cord blood (Ohata et al. 2011) and it is expressed by the syncytiotrophoblast (STB) cells of the chorionic and basal plates of the human placenta (Iñiguez et al. 2019). Data from studies with humans suggest that KL regulates placental transport of phosphate and fetal growth, with alterations in the expression of KL, ADAM17, and FGFR1 associated with pre-eclampsia and small for gestational age term placentae (Miranda et al. 2014; Loichinger et al. 2016; Iñiguez et al. 2019).

In addition to the role of KL in the regulation of pathways associated with cellular proliferation and apoptosis, KL can regulate angiogenesis, nitric oxide production by endothelial cells, and protect against endothelial dysfunction, all of which are important in the establishment and maintenance of pregnancy (Saito et al. 1998; Fukino et al. 2002; Yamamoto et al. 2005; Kusaba et al. 2010). It is also known that vascular smooth muscle cells have high expression of KL and that knockdown of KL expression in human vascular cells revealed that they are a KL-dependent target tissue for FGF23 (Lim et al. 2012). Extensive angiogenesis must occur in the uterus and placentae to allow adequate nutrient transport from the mother to the fetus. Future studies to ascertain whether KL-FGF signaling also acts as an essential regulator of angiogenesis at the ovine maternal–conceptus interface should be performed.

Despite extensive research into the role of FGF23 in the regulation of phosphate transport, few studies have investigated the mechanisms whereby the phosphatonin FGF7 regulates phosphate transport. FGF7 inhibits phosphate uptake in tumor cells (Carpenter et al. 2005), and

its expression may be regulated by vitamin D in breast cancer cells (Lyakhovich et al. 2000). FGF7 may also have an essential role in the female reproductive tract as it is classified as a progestamedin. FGF7 is expressed by uterine stromal cells in response to progesterone (P4) and acts in a paracrine manner via FGFR_{2IIIb} to regulate the function of uterine epithelial cells and conceptus trophoctoderm (Chen et al. 2000). Ovine *FGF7* mRNA localizes to the tunica muscularis of blood vessels in the endometrium and myometrium (Chen et al. 2000). Given the essential roles of the progestamedins in the establishment and maintenance of pregnancy, and the limited studies that have implicated FGF7 in phosphate transport, future studies should determine whether progestamedins act as regulators of phosphate and calcium transport during pregnancy.

5.7.4 Calcium-Binding Proteins

The S100 family of proteins are cytosolic calcium-binding proteins that have central roles in the regulation of many cellular processes including cellular proliferation, differentiation, migration, metabolism, apoptosis, protein phosphorylation, and the maintenance of calcium homeostasis (Hermann et al. 2012).

Calbindin-D9K, also known as S100G, is a vitamin D-regulated calcium binding protein expressed by epithelial cells of the intestine for the modulation of calcium transport (Darwish and DeLuca 1992). While S100G is a known regulator of calcium absorption in the kidney and intestine, and multiple studies provide evidence for a critical role of this protein in the establishment of pregnancy and regulation of conceptus growth and development. In the mouse uterus, *S100G* mRNA is localized to uterine LE and GE when the endometrium is receptive to implantation of the blastocyst (Nie et al. 2000). Furthermore, *S100G* mRNA expression is down-regulated at the implantation site in mice (Nie et al. 2000). Similarly, S100G protein is localized to the uterine stroma and myometrium in the rat, with decreased uterine expression during the

period of blastocyst implantation (An et al. 2003). In contrast to findings from rodents, S100G protein is expressed only by uterine LE and GE of the non-pregnant bovine endometrium, and not by myometrial or uterine stromal cells (Inpanbutr et al. 1994). S100G mRNA and protein expression are greatest during the luteal phase of the estrous cycle, suggesting that S100G may be important in the regulation of uterine gland secretions during this phase of the estrous cycle in cattle. Furthermore, S100G is highly expressed by the porcine uterus on Day 12 of pregnancy, and its expression may be regulated by P4 (Choi et al. 2009, 2012). In sheep, *S100G* mRNA is expressed in endometria across gestation (Stenhouse et al. 2021a), with high expression at Day 30 of gestation, which could indicate a vital role for S100G in placental development and function. Both the cytotrophoblast (CTB) and STB cells of human term placentae express S100G mRNA, with greater expression by STB than CTB cells (Belkacemi et al. 2004), highlighting a role of S100G in the mediation of calcium flux at the maternal–conceptus interface. S100G is expressed by the yolk sac and placenta in multiple species (reviewed by Choi and Jeung (2008)), and it is hypothesized that this protein has a critical role in regulation of the accumulation of excess calcium required in late gestation for mineralization of the fetal skeletal system.

While S100G is expressed at the conceptus–maternal interface in multiple species, with differing spatiotemporal expression profiles across species, the functional significance of S100G in pregnancy remains poorly understood. It is proposed that other calcium regulatory molecules may undergo compensatory increases in the kidney and intestines of S100G null mice, such as TRPV6 and plasma membrane Ca²⁺-ATPase 1 (PMCA1) (reviewed by (Choi and Jeung 2008)). S100G null mice are fertile but it could be hypothesized that in the absence of S100G, other calcium regulatory molecules undergo compensatory increases in expression at the maternal–conceptus interface to ensure that calcium transport is appropriately regulated.

Additional S100 family members, while not as extensively investigated, have been suggested

to play critical roles in pregnancy. S100A8 is associated with early recurrent pregnancy loss in humans (Nair et al. 2013) and S100A8 null mice (Passey et al. 1999; Baker et al. 2011). S100A7, S100A8, S100A9, and S100A12 have been suggested to have a role in the establishment of pregnancy in pigs (Zeng et al. 2018, 2019). Similarly, roles for S100A9 and S100A12 in the establishment of pregnancy in sheep have been suggested because of high endometrial expression early in gestation (Days 9, 12, and 17) (Stenhouse et al. 2021a). Interestingly, the spatiotemporal profile of S100A9 protein in the ovine uterus varies significantly across gestation. S100A9 protein is localized to uterine LE, sGE, and GE on Days 9, 12, and 17 of gestation, suggesting a pivotal role of this molecule in the regulation of calcium trafficking at the maternal–conceptus interface during the peri-implantation period of pregnancy. In contrast, S100A9 protein is only detected in uterine GE on Days 30 and 90 of gestation, and uterine LE at Day 125 of gestation. This striking change in the localization of this protein across gestation in uteri of sheep suggests alterations in the functional importance of S100A9 during gestation that warrants further investigation. S100A11 may have a role in the regulation of endometrial receptivity to implantation of blastocysts and immunotolerance through regulation of epidermal growth factor-stimulated adhesion through its roles in regulating intracellular calcium uptake and release (Liu et al. 2012; Poeter et al. 2013). Knockdown of S100A11 in mice reduced the expression of genes associated with uterine receptivity to implantation and reduced rates of implantation of blastocysts (Liu et al. 2012). Additionally, S100A11 has been detected in uterine flushings from ewes on Days 14 and 16 of pregnancy, suggesting a potential role of S100A11 during the peri-implantation period of pregnancy (Romero et al. 2017). The results from studies with these animal models translate directly to those from humans in which S100A11 is a factor associated with pregnancy failure, and low endometrial expression of S100A11 is associated with adverse immune responses (Liu et al. 2012).

While S100 family members are known to regulate calcium flux, many of the S100 proteins are also mediators of inflammation (Donato et al. 2013; Xia et al. 2018). Available data suggest that the S100 family of calcium-binding proteins plays a critical role in early pregnancy when the uterine immune environment must be tightly regulated to ensure that anti-microbial protection occurs and the conceptus is not rejected by the maternal immune system (Hunt 2006). It could be speculated that in addition to ensuring that sufficient calcium is available for the rapidly dividing and differentiating conceptus tissues in early gestation, members of the S100 family play an important role in the establishment of immunotolerance at the maternal–conceptus interface.

5.7.5 Transient Receptor Potential Cation Channel (TRPV) Family

The TRPV family members, TRPV5 and TRPV6, have a critical role in the regulation of calcium transport by epithelial cells in mammals (Islam 2011). The TRPV channels, such as TRPV5 and TRPV6, have important functions in the regulation of calcium transport at the maternal–conceptus interface to regulate calcium transport in a manner similar to that for transport of calcium by intestinal epithelia. It is speculated that TRPV channels are important for the transfer of calcium in maternal blood to cells on the maternal side of the maternal–conceptus interface, and that Ca^{2+} -ATPases are present on the basement membranes of cells on the fetal side of the maternal–conceptus interface that allow that calcium to enter the fetal circulation.

TRPV6 mRNA and protein are expressed by the murine yolk sac (Suzuki et al. 2008) with expression of TRPV6 increasing 14-fold in the placenta of mice during the last 4 days of gestation (Suzuki et al. 2008) to ensure that required calcium is available for mineralization of the fetal skeletal system. TRPV6 null mice have a 40% reduction in placental calcium transport,

accompanied by severe hypocalcemia and a 50% decrease in weight of fetal skeletal ash, indicating that placental TRPV6 is critical for the regulation of placental transport of calcium (Suzuki et al. 2008). TRPV6 is highly expressed by the porcine uterus on Day 12 of pregnancy (Choi et al. 2009, 2012), and a role for TRPV channels in early pregnancy has also been suggested for sheep (Stenhouse et al. 2021a). Day 17 ovine conceptus tissue expresses *TRPV5* mRNA, and there is also high expression of *TRPV6* mRNA in Day 17 endometria and Day 30 placentae. Interestingly, endometria and placentae supplying twin fetuses have greater expression of *TRPV6* mRNA compared with endometria from ewes with singleton pregnancies at Day 125 of gestation. Thus, TRPV6 may be critical in regulating the increase in calcium transport required to meet the increased mineral demands in twin pregnancies.

5.7.6 Sodium-Dependent Phosphate Transporters

Solute carrier family members (SLCs) have central roles in the regulation of solute transport across the plasma membrane, which is critical for the regulation of intracellular contents of metabolites and ions, cellular volume, and the removal of waste products from cells (Pizzagalli et al. 2020). Many SLC transporter family members exist, allowing for passive facilitative transport or secondary active transport of molecules such as sugars, amino acids, polyamines, vitamins, nucleotides, metals, and inorganic and organic ions (Pizzagalli et al. 2020). The SLC34 and SLC20 families of type II and type III sodium-dependent phosphate transporters play critical roles in bone, kidney, choroid plexus, and vascular physiology and pathophysiology (Lederer 2014; Segawa et al. 2015).

Interestingly, evidence suggests that members of the SLC34 and SLC20 families of type II and type III sodium-dependent phosphate transporters have a critical role in the regulation of growth and development of mammalian conceptuses. In vitro placental perfusion studies have provided interesting insights into the

mechanisms of mineral transport in many species including humans, guinea pigs, rats, mice, and sheep [reviewed by Kovacs (2014)]. Manipulation of Na^+ concentrations in these models demonstrated that placental phosphate transport is sodium-dependent (Husain and Mughal 1992; Stulc and Stulcova 1996). Given that sodium-dependent phosphate transporters are regulators of post-natal phosphate homeostasis, it could be hypothesized that they are regulators of phosphate transport at the maternal–fetal interface. SLC20A1 is expressed by STB cells of the human placenta (Yang et al. 2014) and defects in vascular remodeling of the yolk sac in SLC20A1-deficient mice is embryonic lethal (Wallingford and Giachelli 2014). Similarly, knockout of the type II sodium-dependent transporter SLC34A2 is embryonic lethal in mice (Shibasaki et al. 2009). SLC20A2 is localized to intravillous connective tissues in placentae of humans (Yang et al. 2014). A role for sodium-dependent phosphate transporters in the development of preeclampsia is based on evidence that a reduction in placental expression of SLC20A1 and SLC20A2 is associated with development of preeclampsia and, in late gestation, there is a significant increase in expression of SLC20A2 in preeclamptic pregnancies. SLC20A2 deficient mice are fertile, but some 25% of the mice have pregnancy complications including abnormal placental vascular remodeling, fetal growth restriction, and placental calcification and about 50% of the offspring of those mice do not survive until weaning (Wallingford et al. 2016).

A role for sodium-dependent phosphate transporters SLC20A1 and SLC20A2 in the regulation of phosphate transport at the maternal–conceptus interface in sheep has also been suggested (Stenhouse et al. 2021b). Both *SLC20A1* and *SLC20A2* mRNAs are expressed by ovine placentae and endometria throughout gestation. Interestingly, expression of *SLC20A1* mRNA decreased in ovine endometria at Day 17 of gestation, corresponding to the period of conceptus attachment to uterine LE. There was a stable expression of *SLC20A2* mRNA across gestation in both ovine endometria and placentae,

highlighting a potentially crucial role for this transporter in the regulation of placental phosphate transport across gestation.

5.7.7 Plasma Membrane Ca^{2+} -ATPases

Plasma membrane Ca^{2+} -ATPases (PMCA) are Ca^{2+} extrusion pumps that function to maintain intracellular concentrations of calcium within a tightly regulated range. There are four main isoforms of PMCA (PMCA1-4) expressed in adult tissues. PMCA expression doubles during the last week of gestation in rodents (Tuan and Bigioni 1990; Glazier et al. 1992). Similarly, PMCA 1-4 is expressed by the STB of human placentae and ATP-dependent Ca^{2+} transport increases linearly during the third trimester of pregnancy (Strid and Powell 2000). A critical role for Ca^{2+} -ATPase activity in the regulation of placental transport of calcium was suggested based on results obtained following inhibition of Ca^{2+} -ATPase activity in the rat placentae (Care 1991; Strid and Powell 2000). Homozygous PMCA1 knockout mice have an embryonic lethal phenotype prior to implantation of the blastocyst (Okunade et al. 2004; Prasad et al. 2007). In contrast, PMCA2 and PMCA4 null mice have mild phenotypes although further analyses should be performed to investigate effects on the development of the fetal skeletal system and on placental development and structure (Okunade et al. 2004; Prasad et al. 2007).

The *PMCA1-4* mRNAs are expressed by the uterine endometrium of pigs during the estrous cycle and throughout gestation, in a pregnancy status and stage of pregnancy-specific manner (Choi et al. 2014b), suggesting a role of Ca^{2+} -ATPases in the regulation of calcium ion abundance in uterine endometria. A role for plasma membrane Ca^{2+} -ATPases in early pregnancy in sheep has also been suggested (Stenhouse et al. 2021a). Day 17 ovine conceptus tissue expresses *PMCA4* mRNA which, in combination with high expression of *PMCA3* and *PMCA4* mRNA in endometria from Day 17 of pregnancy, and high expression of *PMCA4* mRNA in placentae on

Day 30 of pregnancy, suggests a role in the regulation of implantation and placental development. Collectively, these findings suggest a role for *PMCA*s in the regulation of calcium at the maternal-conceptus interface in pregnancy that warrants further investigation, particularly during the critical periods of implantation and placentation.

5.7.8 Stanniocalcin

The homodimeric phosphoglycoprotein stanniocalcin (STC) is a central regulator of calcium levels in fish (Wagner et al. 1986; Lu et al. 1994; Wagner 1994; Wagner and Jaworski 1994). STC decreases the influx of calcium from the aquatic environment through the gills and promotes absorption of phosphate in the kidney, while chelating excess calcium, and inhibiting calcium uptake in the intestine. STC1 and STC2 are mammalian orthologs of STC. STC1 has relatively high amino acid sequence identity (approximately 50%) to fish STC and is expressed in many tissues including brain, lung, and heart. While it is known that mammalian STC1 is a regulator of calcium and phosphate transport in the kidney and intestine, in part through the regulation of sodium-phosphate co-transporters, the role of STC2 remains poorly understood.

In addition to the role of STCs in the kidney and intestine, STC may have an important role in female reproduction. During pregnancy, the abundance of *STC1* mRNA in the murine ovary increases substantially and STC1 protein is present in maternal blood during pregnancy despite not normally being found in blood (Deol et al. 2000). The localization of *STC1* mRNA in the mouse uterus varies substantially depending upon pregnancy status and stage. In cyclic mice, the uterine LE expresses *STC1* mRNA (Stasko et al. 2001). Following implantation, *STC1* mRNA localizes to the mesometrial stromal cells bordering the uterine lumen until Days 6.5-8.5 when expression is in the mesometrial lateral sinusoids, after which time expression decreases. STC1 protein, however, is expressed by the uterine epithelia, stromal cells and decidual cells,

as well as trophoblast giant cells. Similarly, STC1 and STC2 may have roles in the regulation of implantation and decidualization in the rat (Xiao et al. 2006). *STC1* and *STC2* mRNA expressions are induced in stromal cells at implantation sites on Day 6 of pregnancy, with additional expression in the uterine LE. Furthermore, STC2 protein is immunolocalized to uterine GE from Day 2 of gestation, with expression peaking on Day 6. STC1 mRNA and protein are expressed by decidualized cells from Days 7–9 of gestation. Interestingly, while *STC1* mRNA is expressed by the whole decidua from Days 7–9 of gestation, *STC2* mRNA is only expressed in the decidua in close proximity to the implantation site on Days 7 and 8, with weak staining throughout the entire decidua by Day 9.

A role for STC has also been suggested for the female reproductive tract of large animals. In the pig, *STC1* mRNA is localized to uterine LE between Days 12 and 15 of the estrous cycle and, in pregnant pigs, it increases between Days 15 and 20 of gestation, suggesting that its expression may be regulated by P4 (Song et al. 2009). After Day 20, expression of *STC1* mRNA in the uterus decreases to minimal expression on Day 25 and no expression at Day 30, suggesting that STC1 may be important for conceptus attachment and implantation. Expression of *STC1* mRNA by the endometrial glands of the ovine uterus is evident from Day 18 of pregnancy and increased further to Day 80 of gestation (Song et al. 2006). STC1 protein is localized predominantly to the apical surface of uterine GE after Day 16 of pregnancy and in the placental areolae that form opposite the opening of uterine glands to transport histotroph across the placenta and into the fetal-placental blood. Ovine endometrial stroma and glands have low expression of *STC2* mRNA in both cyclic and early pregnant uteri (Song et al. 2006). The abundance of *STC2* mRNA significantly increases between Days 20 and 40 of gestation, with high expression in both placentomes and endometria until Day 120 of gestation. In late gestation, abundant expression of *STC2* mRNA is present in uterine LE and GE, trophoctoderm, and uterine caruncles.

In addition to demonstrating that STC1 and STC2 mRNAs and proteins are expressed by ovine uterine and placental tissues, Song et al. established that STC1 protein is present in ovine and porcine uterine luminal fluid (Song et al. 2006, 2009) and ovine allantoic fluid (Song et al. 2006). Thus, STC1 can be secreted by endometrial glands and enter allantoic fluid and the fetal circulation. Further investigations are required to ascertain the role of secreted STC1 in the pregnant uterus, but it may act as a regulator of conceptus growth and development.

Given the spatiotemporal profiles of STC1 expression across gestation, its expression may be hormonally regulated in the female reproductive tract. While interferon tau (IFNT; signal for maternal recognition of pregnancy in ruminants) does not appear to be a regulator of STC1 expression in the ovine uterus, treatment of ewes with P4 does induce expression of *STC1* mRNA in the ovine endometria (Song et al. 2006). Intrauterine infusions of ovine placental lactogen increased the abundance of STC1 in progesterinized ewes; demonstrating an important role of P4 and placental lactogen in the regulation of calcium transport by STC1 by the ovine uterus (Song et al. 2006). Furthermore, the expression of *STC1* mRNA by uterine LE in pigs is stimulated by P4, and this expression is enhanced by estrogen (Song et al. 2009). Given the pattern of expression of STC1 in the uterine LE of pigs, with complete downregulation occurring after conceptus attachment and implantation, the downregulation in expression may be in response to the progesterinized uterus being exposed to estrogen, making STC1 a useful marker of implantation in pigs. Furthermore, in that study, treatment of ewes with estradiol (E2) increased the expression of progesterone receptor (PGR) by uterine GE and this was associated with decreased expression of STC1, which could indicate that downregulation of PGR is critical for the expression of STC1 and other genes expressed by uterine GE.

Collectively, the spatiotemporal expression patterns suggest an important role for the calcium and phosphate regulator STC in the female

reproductive tract. Despite differences in expression profiles for STC1 among species, it is evident that STC1 plays a role in both the establishment and maintenance of pregnancy in species with differing types of placentation to influence growth and development of the conceptus. The intriguing finding that STC1 and STC2 have opposing expression profiles in the ovine uterus and placentomes indicate that they may work together to regulate calcium and phosphate transport at the ovine maternal–conceptus interface in different ways. The concept warrants further investigation to improve our understanding of these molecules.

5.7.9 Sex Steroids

Sex steroids, specifically estradiol and testosterone, are critical for the regulation of postnatal skeletal development and bone turnover throughout adulthood, and therefore are considered calcitropic hormones. Placental-derived hCG and the fetal pituitary gonadotropins can stimulate sex steroid production by developing fetal ovaries and testes. Additionally, the fetal adrenals synthesize low amounts of sex steroids. During pregnancy, concentrations of both estradiol (Habert and Picon 1984) and progesterone (Ward and Weisz 1984) are similar between male and female fetuses in rats. In contrast, concentrations of testosterone in fetuses are at least four times greater for male than female fetuses in rats (Habert and Picon 1984). In addition, concentrations of testosterone are equal to (Houtsmuller et al. 1995) or higher than those in maternal blood in rats [reviewed in Kovacs (2014)]. Similar findings have been reported for sheep, with higher concentrations of testosterone in plasma of male than female fetuses. Interestingly, there is a dramatic decrease in concentrations of testosterone in fetal plasma immediately prior to parturition, and concentrations of testosterone remain greater than those in maternal plasma. Concentrations of progesterone are less in male and female sheep fetuses compared with much higher concentrations in maternal plasma until just prior to parturition when there is

a dramatic decline in progesterone in maternal plasma (Strott et al. 1974). Concentrations of estradiol in fetal plasma are slightly greater than those in maternal plasma throughout most of pregnancy (Strott et al. 1974). Thus, circulating levels of sex steroids in fetal lambs exceed maternal concentrations at all gestational time points investigated [reviewed in Kovacs (2014)]. Finally, the testes in human fetuses produce testosterone in response to gonadotropins and hCG, whereas negligible amounts of testosterone are produced by the ovaries or adrenals in female fetuses (Reyes et al. 1973). Concentrations of estradiol in human fetuses are quite low and similar between males and females, and much lower than those in maternal plasma at term [reviewed in Kovacs (2014)].

The levels of sex steroids in the placenta can be quite high during pregnancy, with concentrations of testosterone, estradiol, or both in umbilical cord blood being equal to or greater than those in maternal plasma [reviewed in Kovacs (2014)]. This appears to result from aromatase enzymatic activity in the placenta converting maternal androgens into estrogens. In the third trimester of pregnancy in humans, the placenta is the source of nearly all of the estradiol, estrone, and estriol present in maternal blood (Menon and Sperling 1998).

Although estrogen and testosterone are considered calcitropic hormones because of their effects on development of the post-natal skeleton, evidence that they play a role in mineral transport and homeostasis during pregnancy is less clear. Studies of calcium and phosphate in serum of murine fetuses that lack sex steroids, or their receptors have not been performed, apart from describing them as being normal at birth. This lack of evidence for mineralization deficiencies suggests that development of fetal bone and mineral homeostasis in fetuses is less dependent upon sex steroids (Kovacs 2015). In the rat, it has been suggested that fluctuations in concentrations of Ca^{2+} may result from activities of different calcium transporters in uteri that respond differentially to progesterone and estradiol during the estrous cycle (Kim et al. 2006; Yang et al. 2011). Additionally, in the pig, it has been

suggested that estrogen increases the release and or transport of calcium into the uterine lumen during the peri-implantation period of gestation (Geisert et al. 1982).

Estrogen is a known regulator of calcium transport in the intestine (Arjmandi et al. 1993) and kidney (Bronner 1989). Estrogen stimulates expression of renal CYP27B1 that increases calcitriol secretion by the kidneys in multiple animal models and humans (Reddy et al. 1983; Kovacs 2016). Estradiol also reduces serum FGF23 and increases PTH in blood, which also may contribute to increase in calcitriol (Saki et al. 2020), although the requirement for estrogen to stimulate the 2–3-fold increase in calcitriol in maternal blood during pregnancy has not been tested directly (Ryan and Kovacs 2021).

In addition to the indirect regulation of calcitriol by estrogen via CYP27B1 expression and decreases in FGF23 and PTH, estrogen may also indirectly regulate calcium transport during pregnancy via regulation of TRPV6, S100G, and vitamin D metabolism. Estrogen is a key regulator of expression of TRPV6 during pregnancy (Lee and Jeung 2007), and TRPV6 is critical for the regulation of placental transport of calcium (Suzuki et al. 2008). TRPV6 is highly expressed in the porcine uterus on Day 12 of pregnancy (Choi et al. 2009, 2012) and in uteri of pregnant sheep (Stenhouse et al. 2021a). Thus, taken together, current evidence supports an indirect role for sex steroids in the regulation of mineral homeostasis during pregnancy, primarily via upregulation of expression of calcitriol.

5.8 Summary and Conclusions

Appropriate transport of nutrients and minerals at the maternal–conceptus interface is essential not only for the establishment and maintenance of pregnancy but also the appropriate regulation of conceptus development and growth. Despite our understanding that phosphate, calcium, and vitamin D are essential for conceptus development and mineralization of the fetal skeletal system, little is known regarding mechanisms of

transport and metabolism of these molecules at the maternal–conceptus interface. Current research defining the spatiotemporal expression of the multiple pathways for calcium and phosphate transport and vitamin D metabolism at the maternal–conceptus interface across gestation and species is vital to improving understanding of these processes. Furthermore, understanding how IFNT, E2, P4, and nutrients regulate phosphate and calcium transporters, and enzymes involved in vitamin D metabolism will further understanding of the roles of these molecules in the nutrition and physiology of reproduction under normal and pathological conditions.

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