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The Pregnant Sheep as a Model for Human Pregnancy

James S. Barry[1] and **Russell V. Anthony**[1,2,*]

1*Perinatal Research Facility, Department of Pediatrics, University of Colorado Health Sciences Center, Aurora, CO 80045-0508, USA*

2*Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1683, USA*

Abstract

Successful outcome of human pregnancy not only impacts the quality of infant life and well-being, but considerable evidence now suggests that what happens during fetal development may well impact health and well-being into adulthood. Consequently, a thorough understanding of the developmental events that occur between conception and delivery is needed. For obvious ethical reasons, many of the questions remaining about the progression of human pregnancy can not be answered directly, necessitating the use of appropriate animal models. A variety of animal models exist for the study of both normal and compromised pregnancies, including laboratory rodents, non-human primates and domestic ruminants. While all of these animal models have merit, most suffer from the inability to repetitively sample from both the maternal and fetal side of the placenta, limiting their usefulness in the study of placental or fetal physiology under non-stressed *in vivo* conditions. No animal model truly recapitulates human pregnancy, yet the pregnant sheep has been used extensively to investigate maternal-fetal interactions. This is due in part to the ability to surgically place and maintain catheters in both the maternal and fetal vasculature, allowing repeated sampling from non-anesthetized pregnancies. Considerable insight has been gained on placental oxygen and nutrient transfer and utilization from use of pregnant sheep. These findings were often confirmed in human pregnancies once appropriate technologies became available. The purpose of this review is to provide an overview of human and sheep pregnancy, with emphasis placed on placental development and function as an organ of nutrient transfer.

Keywords

Sheep; Human; Placenta; Fetus; Fetal growth restriction

1. Introduction

The establishment, maintenance and the successful outcome of pregnancy in the birth of a live, healthy offspring is the ultimate goal of the reproductive system. However, it has been estimated [1] that the likelihood of a woman conceiving during a given menstrual cycle is only 30%, and only 50 to 60% of these conceptions are expected to survive to 20 weeks of gestation [2]. Of the pregnancies lost, 75% can be attributed to implantation failure [2]. Furthermore, in the United States during 2004, 12.5% of live births were delivered premature [3] with 8.1% of live infants weighing less than 2500 g [3]. This represents an increase in the incidence of low

*Corresponding author. E-mail address: Russ.Anthony@ColoState.edu.

birthweight of 11% since 1994. Worldwide, the incidence of low birth weight is estimated at 15% [4]. The definition of low birthweight in this context refers to live births weighing less than 2500 g, but is also defined as infants with ≤2 standard deviations of weight for the gestational age. By contrast, intrauterine growth restriction (IUGR) or fetal growth restriction (FGR) refer to infants that failed to reach their genetic growth potential *in utero* as determined by prenatal screening, many of which have low birthweight, but not all do. Approximately 30% of low birthweight infants result from clinically defined IUGR [5]. Low birthweight, IUGR and premature delivery are often interrelated, and the compilation of these adverse pregnancy outcomes result in increased infant mortality and morbidity [6,7]. Furthermore, epidemiological studies provide evidence that adverse pregnancy outcomes predispose individuals to coronary heart disease, diabetes, hypertension and stroke later in life [8]. Consequently, an adverse conclusion of human pregnancy is a significant health issue worldwide.

While our knowledge of human fetal growth and development has increased significantly during the past 50 years [9], in conjunction with improved prenatal diagnosis and care, there are many questions regarding human pregnancy, especially complicated pregnancies, that have yet to be answered. For both ethical and practical reasons, many aspects of human pregnancy can not be adequately investigated. Consequently, the use of animal models has played an integral part of our current understanding of both normal and complicated pregnancies [9]. A variety of animal species, spanning from laboratory rodents to domestic ruminants, have been used to investigate various aspects of normal and complicated pregnancies. All of the animal models used have merit, but most suffer from the inability to repetitively sample from both the maternal and fetal side of the placenta, limiting their usefulness in the measurement of placental or fetal physiology under steady-state conditions. While no animal truly recapitulates human pregnancy, the pregnant sheep has been used extensively over the past 40 years to investigate maternal-fetal interactions, in part due to the ability to surgically place and maintain catheters in both the maternal and fetal vasculature [10,11], allowing repetitive sampling from non-anesthetized pregnant ewes. Since these early studies [10,11], considerable insight on placental oxygen and nutrient utilization and transfer has been obtained using pregnant sheep, often confirmed in human pregnancies once appropriate technologies became available [9].

The purpose of this review is to provide an overview of human and sheep pregnancy, with emphasis being placed on placental development and function as an organ of nutrient transfer to the fetus. Comparisons will be made between the two species, and where possible discussion of functional changes associated with FGR pregnancies will be provided. It is our aim to provide an appreciation for the utility of pregnant sheep as an investigative model of human pregnancy.

2. Placental Development

Placental development begins quite early in human pregnancy, continues throughout gestation, and is closely tied to vascular development within the placenta, as increasing blood flow is required to meet the needs of the growing fetus. At the time of implantation, lacunae develop within the syncytiotrophoblast layer, coalescing to form the intervillous space that eventually fills with maternal blood. Between days 8 and 13 post coitus (p.c.) syncytiotrophoblast trabeculae separate the lacunae, and on about day 13 p.c., the trabeculae are invaded by proliferating cytotrophoblasts, giving rise to the primary villi concomitant with maternal blood cells entering the lacunae. Primary villi are rapidly transformed into secondary villi due to primary chorionic plate-derived mesenchyme cell invasion. The first fetal capillaries are present within the mesenchyme between day 18 and 20 p.c., marking the development of the first tertiary villi [12]. As this process progresses, extravillous cytotrophoblasts migrate and invade the spiral arteries within the decidua and myometrium, thereby remodeling the spiral

artery walls [13,14]. Immature intermediate villi are derived from tertiary villi, and intermediate villi give rise to stem villi. As blood flow and pressure increases within the primitive vessels, further vessel development occurs, and mature intermediate villi are produced in conjunction with increasing endothelial cell proliferation [15]. When vessel length within the intermediate villi exceeds the villi length, terminal villi are formed. Vessels within the terminal villi lie adjacent to the intervillous space, such that placental and maternal blood are separated by only a thin layer of syncytiotropoblast, providing a thin diffusion barrier and efficient transfer of nutrients to the fetus.

Anatomically, many view the sheep and human placenta to be quite divergent, which they are, based on the classification of Grosser [16]. However, there are important similarities in function and functional structure between human and sheep placentae. In humans the conceptus truly invades into the uterine lining, whereas in sheep there are specialized regions of well vascularized, but non-glandular endometrium, know as caruncles that reside within the uterus [17]. It is the association of the developing chorionic epithelium with the raised surface of the caruncle that provides the foundation for placental development in sheep. Following expansion of the allantois from the hind-gut at day 15 p.c., allowing fusion with the chorion, the vascularized allantois provides placental vascularization in the areas of the caruncular projections [17]. Interdigitation of fetal and maternal tissues occurs early in the fourth week of pregnancy [18], in areas of chorion-caruncle apposition. As pregnancy progresses, the caruncles continue to grow and develop deeply branched crypts into which the fetal villi project, elongate and branch forming an apposing network of fetal villi within the maternal crypts [19]. The sheep placenta has numerous discrete attachment sites comprised of the fetal cotyledon and maternal caruncle, rather than the large discoid placenta that occurs in humans, yet the human placenta is structurally divided into cotyledons as well. Regardless, the villous tree of the sheep cotyledon is structurally similar to that of the human placenta, as both can be divided into stem, intermediate and terminal villi [20], and the fetal vessels within the villi are comprised of stem arteries and veins, intermediate arterioles and venules, and terminal capillaries in both species. By no means is the sheep placenta a perfect model for the human, but the similarity in fetal placenta vascular structure coupled with the relative maturity of the fetus at birth, and the ability to repetitively obtain both maternal and fetal blood samples from singleton pregnancies [10.11], allows the sheep to serve as a useful model of placental vascular development and placental nutrient exchange.

2.1 Placental development in compromised pregnancies

As indicated in the introduction, fetal growth restriction (FGR) is a common problem in human pregnancy, with an incidence of 8-10% [21], leading to increased infant mortality and morbidity. The most common etiology of FGR results from functional placental insufficiency, i.e., a failure to deliver sufficient nutrients and oxygen to the growing fetus, resulting in asymmetric growth restriction where the trunk of the infant is proportionately smaller than the head. Placental structural abnormalities that have been associated with FGR pregnancies include reductions in placental villous number, diameter and surface area, along with reductions in villous arterial number, diameter and degree of branching [22-27]. However, not all FGR presentations are the same, and neither are the placental abnormalities associated with FGR pregnancy. Severe, early-onset FGR is associated with altered umbilical artery Doppler waveforms indicative of increased placental vascular resistance, and placentae from these pregnancies exhibit long straight terminal villi with a simpler vascular network and fewer interconnections [28]. On the other hand, placental villi that are more tortuous with more interconnections are associated with altered placental development during late gestation, and these pregnancies often do not exhibit altered Doppler waveforms indicative of increased placental vascular resistance, i.e. preserved diastolic flow in the umbilical arteries [28].

The altered placental villi morphology is likely driven by altered placental vascular development, as development of the placental vascular network begins early, with an initial phase of vasculogenesis, followed by branching and then non-branching angiogenesis [29]. Vascular endothelial growth factor (VEGF) promotes both vasculogenesis and angiogenesis within the placenta [30] through interactions with the receptors VEGF-R1 and –R2, whereas the angiopoietins (Ang 1 and Ang 2) likely only promote angiogenesis [31]. Both Ang 1 and Ang 2 interact with equal affinity with the receptor Tie 2 [32], and while Ang 1 induces maturation and stabilization of the developing vasculature [33], Ang 2 promotes vessel destabilization required for additional sprout formation and branching angiogenesis [32]. Within the human placenta, Ang 2 expression is greatest during the first trimester, and wanes as gestation progresses [34,35]. Greater expression of Ang 2 during the first trimester, with Ang 1 and VEGF expression increasing from early to late gestation [35], fits with the concept that branching angiogenesis occurs during early placental development, followed by non-branching angiogenesis [29]. The altered placental morphology (i.e., long straight terminal villi) associated early-onset severe FGR may result from increased non-branching angiogenesis, as both VEGF and Ang 2 are reduced in term placenta from this type of pregnancy [36,37], consistent with reduced branching angiogenesis during late gestation.

Unfortunately, the term human placenta does not provide adequate information on the progression of placental development in compromised pregnancies, which is required for development of clinical interventions. Consequently, appropriate animal models are needed to fill in the gaps, until which time the resulting data are confirmed in the human. Our current understanding of placental development and function in placental insufficiency-induced FGR is primarily derived from animal studies, with the pregnant sheep and rodent used most commonly. As mentioned earlier, the sheep allows for measurement of fetal and placental physiology and metabolism during FGR pregnancies, which is not feasible in pregnant rodents. Fetal growth restriction in sheep can be induced by a variety of methods, including maternal nutrient restriction or excess, placental embolization, restriction of placental attachment sites (carunclectomy), restriction of uterine blood flow, and steroid administration [38]. We utilize the natural phenomenon of high ambient temperature-induced FGR in sheep [39] to study placental development and function. Exposure of pregnant ewes to hyperthermic conditions for approximately 80 days (days 40 to120 p.c.) results in a fetus whose placenta is also growth restricted [40,41]. Similar results are obtained when exposure is for only 55 days (days 37 to 93 p.c.), with significant reductions in fetal and placental weights [42,43]. Fetal growth in these pregnancies is asymmetric in nature, as evidenced by greater biparietal diameter/abdominal circumference ratios [42]. Furthermore, current evidence indicates that development of FGR in chronically hyperthermic ewes occurs as a consequence of reduction in placental growth in early gestation [41,42,44], making this a model to examine impaired placental development, leading to FGR, comparable to early-onset severe FGR in humans.

Using these FGR pregnancies, we have determined that the placental vascular architecture is modified by day 90 p.c. [39], and that near-term uterine and umbilical blood flows are reduced, while umbilical artery systolic/diastolic ratio, pulsatility index and resistance index are all increased [45.46]. It should be noted that while absolute (ml/min) uterine and umbilical blood flows are reduced, relative (ml/min/kg of conceptus or fetus) umbilical blood flow is also reduced whereas relative uterine blood flow is actually increased [45]. These data infer that perfusion of the pregnant uterus during late gestation is not diminished, rather the problem exists within the placental vasculature. Doppler velocimetry measures of the umbilical artery support the concept of increased placental vascular resistance [46], similar to what has been reported for early-onset severe FGR in humans [25,26,28]. Furthermore, similar as to what has been reported for term human FGR pregnancies [36,37], fetal cotyledon VEGF, VEGF-R1 Ang 2, and Tie 2 expression is reduced in our near-term sheep FGR pregnancies [45,47]. By contrast, at day 55 p.c. in our sheep FGR pregnancies, VEGF, Ang 1, Ang 2 and Tie 2 expression

are all increased within the fetal cotyledon [47,48], indicative of accelerated angiogenesis within these placentae. These data, obtained during early placental development in our FGR pregnancies are not available for human FGR pregnancies, and suggest that accelerated vasculogenesis/angiogenesis during early placental development may set the stage for the abnormal placental vasculature structure, increased placental vascular resistance and impaired placental function described for human and sheep FGR pregnancies.

3. Placental transport of oxygen and nutrients

The placenta is not simply a quiescent transporter of fetal nutrients and hormones, but is a metabolically active organ that utilizes nutrients and oxygen at rates similar to the fetus for its own growth. There is a complex balance between placental utilization of substrates and oxygen for its own growth and the transfer of these nutrients to the fetus, allowing for fetal growth. The nutrient transfer capacity of the placenta, which is dependent on adequate placental development and growth, plays a critical role in fetal growth trajectory allowing for fetal growth to reach its potential in both humans and animals [49,50]. In humans and other mammals, fetal growth is dependent on the placental supply of oxygen and nutrients which are provided through two main processes, simple diffusion and transporter-mediated transfer, respectively [51]. The major fetal nutrients are glucose (and its metabolic byproduct, lactate) and amino acids [52,53]. Transport capacity for these nutrients varies as a function of placental size, vascular and structural formation, blood flow, and transporter abundance [54]. Additionally, fetal nutrient, oxygen, and hormone supply depends on placental substrate utilization and hormone metabolism. With advancing gestation, both placental growth and fetal growth increase dramatically and, in general, placental size is directly correlated with fetal growth in both humans and sheep [53-55]. At mid-gestation, the placenta consumes a majority of the substrate taken up by the gravid uterus, but as gestation advances placental substrate consumption decreases and more substrate is transferred to the fetus [56].

Much of our current knowledge of placental-fetal interactions, placental metabolism, and placental transfer function and capacity has been gleaned from investigations using large animal models, most commonly, the sheep. *In vivo* studies of placental metabolism have involved the application of the Fick Principle to experimental conditions in the chronically catheterized fetal sheep and ewe [57]. In order to apply the Fick Principle to measure placental metabolism, arterial-venous substrate and oxygen concentration differences are measured concurrently in the uterine and umbilical circulation (see Figure 1) [58]. This enables measurement of the metabolism of the gravid uterus and fetus simultaneously. Utero-placental metabolic determinations are the subtractive differences between the metabolic measurements of the gravid uterus and fetus.

Our understanding of human placental-fetal physiology is much less robust than for the pregnant sheep given the ethical constraints associated with the study of the human fetus. It has been repeatedly shown that sheep pregnancy is an invaluable model of placental physiology and much of the data and conclusions from the studies of the pregnant sheep are applicable to human placental physiology. In the following sections, we will highlight current knowledge of oxygen, glucose, and amino acid placental metabolism in the sheep and contrast the findings to the present understanding of human placental metabolism.

3.1 Placental oxygen transfer and metabolism

Oxygen diffusion into the human and sheep fetus is dependent on the rate of umbilical and uterine blood flow, fetal and maternal blood oxygen carrying capacity and hemoglobin oxygen binding affinity, placental surface area, placental permeability, and the quantity of oxygen consumed by the placenta [59]. Blood flow is the major determinant of fetal oxygen delivery. In sheep and humans, uterine blood flow increases by three fold (from 0.4 to 1.2 L/min) and

two and a half fold (from 0.3 to 0.8 L/min), respectively, throughout the later half of gestation [60,61]. Oxygen diffusion across the placenta is minimal during early pregnancy. Gas exchange occurs across the various placental cellular layers between the uterine circulation and fetal umbilical circulation only after maturation of their respective vascular beds, allowing adequate approximation of the two circulations. As a result, through much of the first trimester, oxygen levels within human placental tissue are quite low with a PO_2 <10 mmHg at 8 weeks of gestation, but by the end of the first trimester, placental PO_2 is increased to 60 mmHg on average [62,63]. However, it is not known what umbilical PO_2 is at this stage of human gestation. These data illustrate the fetus develops in a hypoxic environment during the first trimester, and that maturation of the utero-placental vasculature and villous structures at the beginning of the second trimester, provides measurable gas transfer to the fetus. Even in pregnant sheep there is a paucity of information on efficiency of oxygen transfer to the umbilical circulation during the first third of gestation. We recently obtained fetal blood gas data from day 55 p.c. sheep pregnancies and found umbilical vein PO_2 values of ≈17 mmHg with no difference between control and FGR pregnancies (Dohnal, Barry and Anthony unpublished results) at this stage of gestation, which is lower than what is observed later in gestation (see below).

Our present understanding of fetal-placental respiratory function comes from study of the late gestation sheep. As the placenta is a highly metabolically active organ, it accounts for a large fraction of the total oxygen consumed by the gravid uterus. Bell et al. [56] revealed that, during mid-gestation, placental oxygen consumption accounts for 80% of the total oxygen taken up by the uterus. Battaglia and Meschia, pioneers in the investigation of placental and fetal physiology, were among the first to report placental oxygen consumption rates during late gestation in sheep. Their studies demonstrated that utero-placental oxygen consumption rates (0.98 mmol/min) were nearly equivalent to fetal oxygen consumption rates (1.18 mmol/min) and thereby, accounted for nearly half of the total uterine oxygen uptake (2.16 mmol/min) [64]. Nearly 90% of placental oxygen consumption is accounted for by the oxidative phosphorylation of glucose in sheep [57].

Until recently, comparative data from human pregnancies were unavailable, but with advances in technology and the advent of Doppler ultrasonography, inferences of fetal and placental oxygen consumption rates have been made near term. Human umbilical blood flow is in the range of 70-111 ml/min/kg [65-67], and based upon umbilical arterial-venous oxygen differences determined from cord blood sampling at delivery, it has been calculated that human fetal oxygen consumption is 0.9 to 1.2 mmol/min, similar to that found in the sheep fetus [9]. Bonds et al. [68], using indirect calorimetric methodologies, showed that 40% of total gravid uterus oxygen uptake can be accounted for by the placental uptake of oxygen in women undergoing cesarean sections at term. Additionally, when comparing near term human and sheep placental oxygen consumption on a per kilogram basis, they are similar. Human placental oxygen consumption has been reported as 37 ml/kg/min and for the sheep as 34 ml/kg/min [57,68]. Therefore, the rates of human fetal and placental oxygen consumption are quite similar to those found in the sheep.

During late gestation, fetal oxygen consumption remains constant even under conditions that result in a large deficit of maternal oxygenation and utero-placental oxygen delivery. In the 1960's, Campbell et al. [69] determined that the PO_2 gradient across the placenta remains constant despite decreases in maternal oxygenation and uterine blood flow. The constant oxygen gradient between maternal and fetal circulation sustains fetal oxygen delivery at normal rates. However, it has been demonstrated in multiple sheep studies that there is a critical level of fetal oxygen delivery below which fetal oxygen consumption decreases. The fetal arterial oxygen concentration of 0.6-0.5 mmol/L appears to be a critical value at which fetal oxygen consumption becomes reduced in hypoxic fetal sheep, induced by reductions in uterine blood

flow, fetal or maternal blood oxygen carrying capacity, or fetal hemoglobin oxygen affinity [70-73]. Such studies have not been undertaken in human pregnancy. However, cordocentesis data from the near term human fetus demonstrates a similar umbilical vein-artery oxygen difference (Figure 2). These data demonstrate that placental oxygen consumption, transfer, and fetal oxygen consumption rates are similar between the human and sheep.

Fetal hypoxemia is commonly found in both human and sheep FGR [38,74]. Doppler ultrasound investigation of human FGR pregnancies exhibit increased impedance to umbilical arterial blood flows [75,76] and uterine blood flows [77,78]. Additionally, it has been shown during *in vivo* human study that the limitation in fetal oxygen delivery during FGR results from decreased placental transfer of oxygen as blood leaving the conceptus through the uterine veins has a higher PO_2 than control pregnancies and concomitantly, there is a lower uterine oxygen extraction rate [79]. In our sheep FGR pregnancies, there are reductions in both umbilical venous PO_2 (Figure 3) and relative umbilical blood flows (ml/min/kg fetus or ml/min/100 g placenta) [45,80,81]. Doppler velocimetry measures of umbilical arterial circulation illustrate increased placental vascular impedance during late gestation in these FGR pregnancies [45, 46]. Additionally, uterine venous oxygen content and PO_2 (Figure 4) are higher in FGR pregnancies, similar to the results found in human IUGR pregnancies (Figure 5) [79]. Fetal hypoxemia is also a consequence of decreased fetal oxygen transfer through the placenta in the FGR sheep fetus as uterine-umbilical PO_2 differences are increased by 65 percent compared to controls [81]. Through these human and sheep studies, it is clear that FGR, as a result of placental insufficiency, causes significant fetal hypoxemia as a result of restricted placental oxygen transfer and uterine-umbilical blood flows. However, it should be noted that not all growth restricted pregnancies exhibit significant fetal hypoxemia. Pardi et al. [74] subdivided human FGR based on fetal heart rate (FHR) and umbilical artery Doppler pulsatility index (PI), and demonstrated that FGR pregnancies with normal FHR and PI were not hypoxemic, but that the majority of fetuses with abnormal FHR and PI were hypoxemic. We have observed a similar scenario in sheep, in that moderately growth restricted pregnancies did not exhibit the significant reduction in umbilical blood flow and fetal oxygenation observed in the severe FGR pregnancies [80].

3.2 Placental glucose transfer and metabolism

Glucose is the primary substrate for the human and sheep fetus and placenta. Normally, the fetus has a limited ability for glucose production [64,82]. Therefore, fetal glucose supply is dependent on the supply from the maternal circulation. Placental glucose uptake and transfer to the fetus occurs down a concentration gradient from the maternal circulation to the fetal circulation mediated by sodium-independent transport proteins on maternal-facing microvillous and fetal-facing basal membranes of the trophoblasts [83]. Glucose entry into the fetal circulation in depends on three steps: 1) uptake from the maternal circulation by transporters found in the maternal facing microvillous membrane of the trophoblast, 2) transport across the cytoplasm of the trophoblast, 3) transport across the fetal facing basal membrane of the trophoblast into the fetal circulation. At present, only glucose transporter proteins 1 (Glut-1) and 3 (Glut-3) have been found in human and sheep placental tissue in locations that would allow for such glucose transport [83,84].

Glut-1, the most prominent placental isoform, is found in vascular endothelial cells [85], and in both the basal [86] and microvillous membranes [87,88] of human syncytiotrophoblasts. In humans, a higher protein concentration of Glut-1 has been found in the maternal-facing microvillus membranes compared with basal membrane concentrations [89] which may allow for a higher glucose uptake capacity into the syncytiotrophoblast from the maternal circulation. It is also possible that the rate-limiting step of glucose transport is from the intracellular syncytiotrophoblast glucose pool into the fetal circulation as a result of the lower Glut-1

concentrations in the basal or fetal facing membrane in human placentas. The microvillus membrane has a much larger surface area compared with the overall surface area of the fetal facing basal membrane [90]. In the human placenta across gestation (starting early second trimester through mid third trimester), Glut-1 concentrations are constant in the microvillus membrane, but in the basal membrane they double in concentration allowing for increased placental glucose transport closer to term [91]. In sheep, Glut-1 is localized to the base of the syncytial layer of the placenta [83], derived from the fusion of maternal epithelial cells and chorionic binucleate cells, as well as the baso-lateral surface of the trophoblast layer. Glut-1 was not found on the interdigitated microvilli of the apical trophoblast surface and syncytial layer, indicating that a different glucose transporter is responsible for transfer from the fetomaternal syncytium to the fetal-derived trophoblast layer.

In the human, Glut-3 has been localized to the vascular endothelium, not in the trophoblast despite the presence of Glut-3 mRNA being present in the trophoblast as well as other placental cell types [84]. In comparison, Glut-3 has been found on the microvillar junction between the syncytium and trophoblast layer in sheep [83]. Interestingly, Glut-3 has only been found in one other tissue, the brain. The brain, like the fetus, also depends on a constant supply of glucose to maintain energy production. It may be that Glut-3 is present in the sheep placenta to maintain glucose supply to the fetus even under very low concentrations of glucose in maternal circulation.

Human and sheep placenta differ in that expression of Glut-1 and Glut-3 is developmentally regulated in sheep. As gestation advances, the concentration of Glut-1 decreases and Glut-3 increases in the sheep [92]. When comparing glucose transporter concentrations and location within human and ovine placenta, there are differences that have been clearly identified, but the known functional significance of these differences is limited given the ethical restraints of human investigation. Recent human studies, using the technique of *in vitro* dual cotyledon perfusion to determine placental glucose transport have shed light on the significance of the polarized location and concentration of the two important glucose transporter isoforms within the human placenta [93]. These studies demonstrated a differential placental uptake of glucose from maternal and fetal vasculature after simultaneous infusion with physiologic solutions of equivalent glucose concentration. Glucose uptake from maternal circulation was twice that taken up from fetal circulation. This functional data is supported by previous reports showing a six fold increase in microvillus surface area compared with the fetal facing basal membrane [90,94] and a three fold increase in Glut-1 density on the apical membranes [88]. The human placental perfusion studies demonstrate that the maternal to fetal glucose gradient is a major determinant of fetal blood glucose concentrations and may be based on polarized glucose transporter concentration differences in the trophoblast. Data from *in vivo* sheep studies by Hay et al. have also shown that the maternal to fetal glucose gradient is the major driving force for fetal glucose concentration [95]. It has also been determined in both sheep and humans that the transplacental glucose gradient is increased near term as a result of decreasing fetal glucose concentrations [96,97]. From mid-gestation to term, fetal glucose demand increases 14 fold, while placental glucose transfer capacity only increases by 5-6 fold. To increase placental to fetal glucose transfer to meet this increased fetal glucose demand, the transplacental glucose gradient increases as a result of decreased fetal glucose concentrations [97].

The placenta is not simply a passive organ involved in the transfer of glucose to the fetus, but it is a highly metabolic organ that has a significant nutrient requirement to maintain function and increase growth as gestation advances. In the near term human fetus, *in vivo* and *in vitro* studies have shown that as much as 60-80% of glucose taken up by the placenta is not transferred to the fetus, but is instead consumed by the placenta [93,98]. During sheep gestation, placental consumption of glucose also contributes significantly to the maternal-fetal glucose

concentration gradient as 80% of utero-placental glucose uptake is also consumed by the placenta [97].

Fetal hypoglycemia is common during both human and sheep FGR [38,80,99-101]. With human FGR, placental expression of the predominant glucose transporter isoform, Glut-1, is unchanged in the syncytiotrophoblast [102], and that glucose uptake by isolated syncytiotrophoblasts is not different between FGR and controls. In sheep FGR, fetal glucose uptake is lower on an absolute basis, but it is not different from control if determinations are made based on fetal weight [103]. On an absolute basis, placental glucose transport is reduced by 65%, but on a relative weight basis it is only slightly less than controls [41]. However, using our model of sheep FGR and glucose clamp methodologies, Thureen et al. [41] demonstrated that the FGR placenta did not have the equivalent glucose transport capacity when compared to control pregnancies. By contrast, using a different sheep FGR model, Wallace et al. [104] did not find a weight-specific deficit in glucose transport capacity. This may suggest that the severity of FGR may impact the degree and mechanism of fetal hypoglycemia, similar to the impact on oxygen transport [80].

3.3 Placental amino acid transfer and metabolism

The fetal supply of amino acids depends on their active transport across the trophoblast and also on rates of placental consumption and interconversion of amino acids [105]. In general, fetal concentrations of amino acids are higher than those in the maternal circulation [106-108]. Study of placental amino acid metabolism has proven to be quite complex. It is implausible to devote extreme detail on this subject matter given the complexity of placental amino acid metabolism. Please refer to recent excellent reviews of this subject matter for more details [53,105,109,110].

The transfer of amino acids is much more complex than that of glucose or oxygen as multiple amino acid transporter systems have been identified. In addition, many amino acids can competitively inhibit transfer of one another making proper *in vitro* and *in vivo* study necessary to fully understand placental amino acid metabolism. Fetal amino acid transport is dependent on the specific transporters present on the maternal and fetal facing membranes of the syncytiotrophoblasts, the polarity and density of these transport systems, and on the surface area of the placenta [109]. During the second half of gestation, increasing placental surface area does not match the increase in fetal size, therefore increasing amino acid transporter abundance and capacity is necessary for appropriate fetal growth. In addition, *in vivo* human and sheep studies have shown that many amino acids transferred to the fetus are produced in the placenta through interconversion of metabolically related amino acids [111,112].

The development of techniques using isotope tracers has allowed the study of the bidirectional flow of amino acids and has improved the understanding of placental transport and metabolism of amino acids [107,112-115]. In the human placenta, the fetal endothelial and syncytiotrophoblast layers are the only two cell layers separating maternal blood from fetal blood. The endothelial layer does not restrict amino acid transport as it does not form a complete barrier at endothelial cell junctions [116]. Therefore, the syncytiotrophoblast layer regulates fetal and placental amino acid transport and metabolism.

Amino acid transport study has been performed *in vitro* on microvillous and basal membrane fractions in humans. Currently, fifteen amino acid transport systems have been identified and are broadly grouped into neutral, cationic, and anionic transport systems [109]. Overall, there is a net transfer of amino acids from the maternal to fetal circulation during human pregnancy. This does not hold true for all amino acids as there is no net placental transfer of glutamate or aspartate to the human fetus [117]. As in humans, there is no net fetal uptake of aspartate or

glutamate during sheep gestation [113]. In the sheep, there is also transfer of serine from the fetus to the placenta [118].

During human FGR, fetal amino acid concentrations are lower than those found in control pregnancies [119,120]. The decrease in amino acid concentrations within fetal blood results from decreased placental transport. In humans, *in vitro* studies have shown an inverse relationship between birth weight and maternal facing microvillous amino acid transport activity [121]. Furthermore, it has been shown that with more severe human fetal growth restriction, defined by an increased umbilical arterial pulsatility index, amino acid transport activity decreases [122]. *In vivo* studies of human FGR observed a reduced transport of the essential amino acids, leucine and phenylalanine [123]. Additional *in vitro* studies using microvillous fractions of human FGR placentas have shown a reduced transport of leucine, lysine, and taurine [124,125].

In sheep FGR, there are variable changes in the concentrations of fetal amino acids. In our laboratory we have found phenotypic differences between animals with moderate and severe fetal growth restriction [80]. During moderate FGR, concentrations of amino acids in fetal circulation are lower than in control pregnancies, but during severe FGR, amino acid concentrations tend to be higher than in controls. It is likely that these discrepancies are a result of an altered catabolic state within the fetus. In moderate FGR, there is a less severe reduction in fetal glucose and oxygen delivery [80], thus the fetus likely continues under a state of anabolism and growth, albeit at a reduced rate. In contrast, with severe FGR there are greater reductions in oxygen and glucose delivery to the fetus (see above), which restricts growth coinciding with a catabolic metabolism. With catabolism, protein is broken down and likely utilized as a fuel, therefore increasing fetal arterial amino acid concentrations. In general, human and sheep FGR exhibit reduced placental amino acid transport, but the impact on fetal amino acid concentrations may well be due to the severity of growth restriction.

4. Conclusions

In this review, we have provided an overview as to why the study of pregnancy and pregnancy outcome is important, reviewed placental development and function, and have compared human and sheep pregnancies. From the comparisons presented here, we recognize that sheep are not a perfect model for human pregnancy, but it has many commonalities with the human, especially from the standpoint of placental development, metabolic function and nutrient transport. Obviously, many of the questions yet unanswered about the progression of human pregnancy, especially those compromised by fetal growth restriction, can not be investigated in the human for ethical reasons. If for no other reason, the pregnant sheep has been an invaluable model for the study of placental and fetal physiology due to our ability to repetitively sample, under steady-state conditions, from both the maternal and fetal circulations. We hope that the information provided here provides an appreciation for using sheep as a model for human pregnancy.

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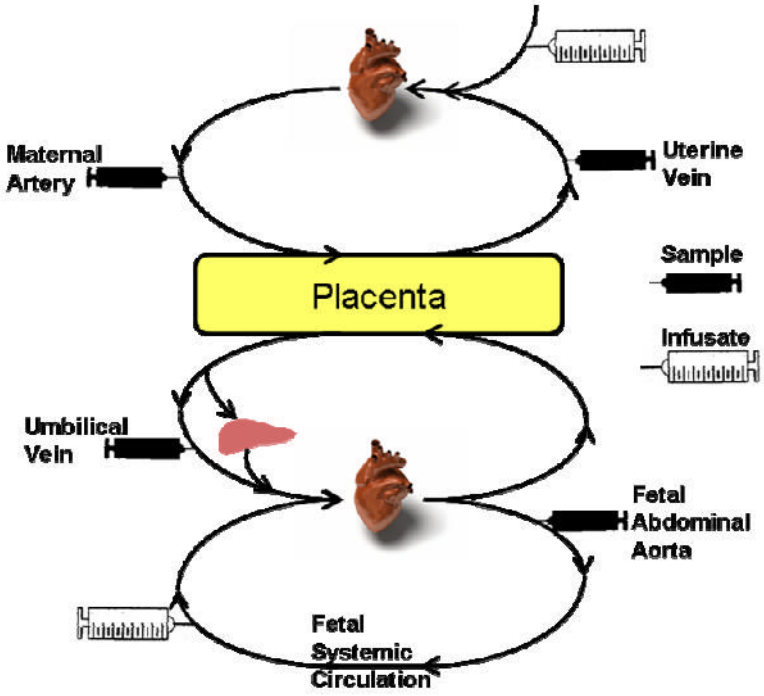

Figure 1.
Schematic representation of pregnant sheep *in vivo* preparation used to measure the metabolism of the gravid uterus and fetus simultaneously. Infusate can be into either the maternal or fetal circulation, and blood samples can be drawn from either circulation simultaneously.

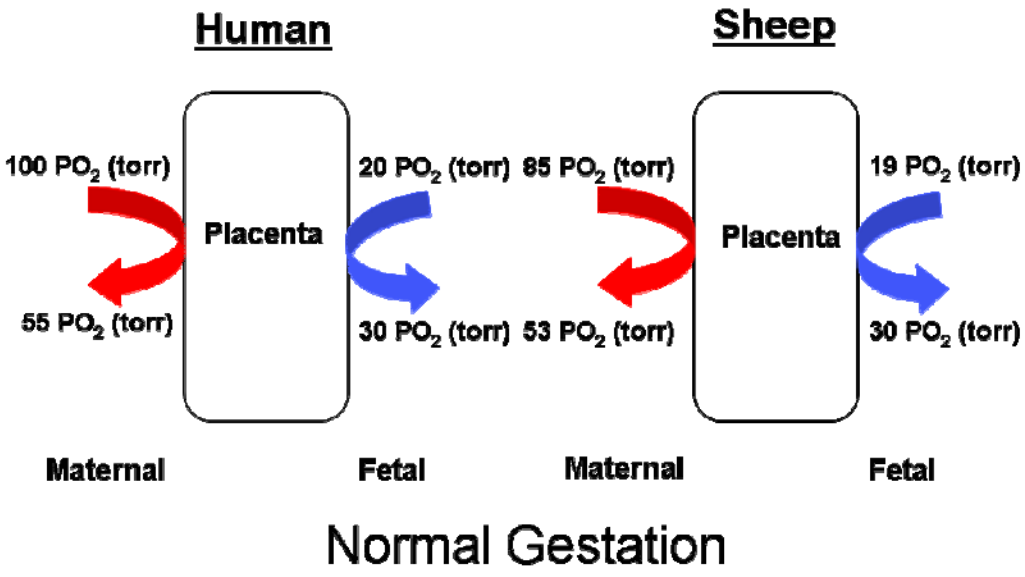

Figure 2.
Schematic representation of maternal artery, uterine vein, umbilical vein and fetal arterial oxygen tensions during late gestation in human and sheep pregnancies.

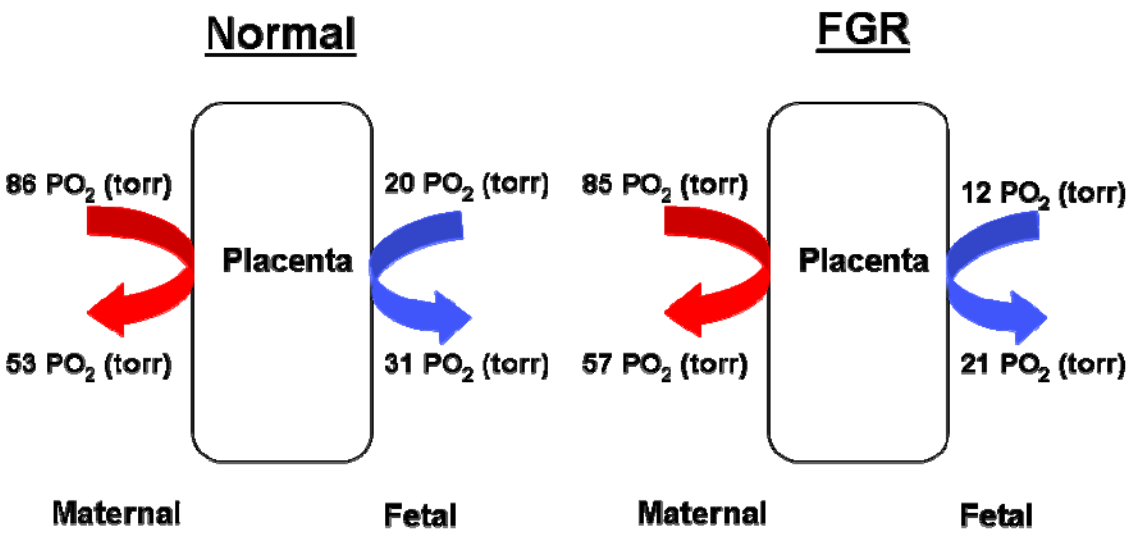

Figure 3.
Schematic representation of maternal artery, uterine vein, umbilical vein and fetal arterial oxygen tensions in late gestation normal and severe FGR sheep pregnancies. Note the reductions in fetal oxygenation and the elevated PO_2 in the uterine vein, indicative of reduced placental oxygen extraction.

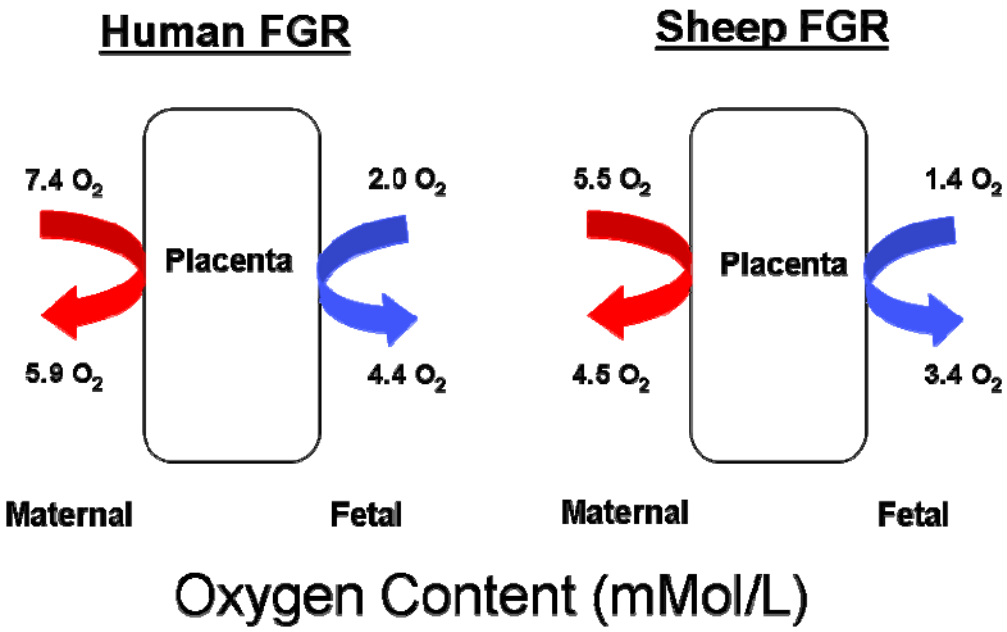

Figure 4.
Schematic representation of maternal and fetal oxygen content (mMol/L) values for late gestation human and sheep growth restricted pregnancies.