PREGNANCY RECOGNITION SIGNALING, FETAL-PLACENTAL DEVELOPMENT AND PRENATAL FETAL PROGRAMMING

Fuller W. Bazer
Texas A&M University1, College Station, Texas 77843-2471 USA and
WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-921, Korea

Greg A. Johnson
Texas A&M University, College Station, Texas 77843-2471, USA

Gwonhwa Song
WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-921, Korea

Guoyao Wu
Texas A&M University, College Station, Texas 77843-2471, USA

Keywords: Estrous cycle, menstrual cycle, pregnancy, implantation, placentation, embryo, fetus, conceptus, growth and development, pregnancy recognition signaling, hormone receptors, gene expression, luteolytic and luteotrophic hormones, corpus luteum, primate, rodent, ruminant, pig, horse, rabbit, cat, dog, hormone servomechanism, uterus, intra-uterine growth restriction, fetal programming, adult onset of disease

Contents

1. Introduction
2. Gene expression for uterine proteins and nutrient transporters
2.1. Temporal and spatial changes in steroid hormone receptors in the uterus during the peri-implantation period of pregnancy
3. Pregnancy recognition
3.1. Luteotrophic versus antiluteolytic signaling for pregnancy recognition
3.2. Corpus luteum formation and regression
3.3. Pregnancy recognition signals for maintenance of corpora lutea
3.3.1. Primates
3.3.2. Rodents
3.3.3. Ruminants
3.3.4. Pigs
3.3.5. Horse
3.3.6. Cat
3.3.7. Dog
3.3.8. Rabbit
4. Implantation
4.1. Strategies for implantation
4.1.1. Primates
4.1.2. Rodents
4.1.3. Ruminants
4.1.4. Pigs
5. Servomechanisms regulating uterine gland morphogenesis and secretory function
6. Fetal growth and development
6.1. Sheep
6.2. Pigs
7. Intra-uterine growth restriction
8. Adult onset of diseases and fetal programming
9. Summary and Conclusions

Summary

Reproduction is a highly complex biological process requiring a dialogue between the developing conceptus (embryo-fetus and associated placental membranes) and maternal uterus which must be established during the peri-implantation period for pregnancy recognition signaling and regulation of gene expression by uterine epithelial and stromal cells. The uterus provides a microenvironment in which molecules secreted by uterine epithelia or transported into the uterine lumen represent histotroph or the secretome required for growth and development of the conceptus and receptivity of the uterus to implantation by the conceptus. Pregnancy recognition signaling as related to sustaining the functional lifespan of the corpora lutea (CL) which produce progesterone; the hormone of pregnancy essential for uterine functions that support implantation and placentation required for successful outcomes of pregnancy. It is within the peri-implantation period that most embryonic deaths occur in mammals due to deficiencies attributed to uterine functions or failure of the conceptus to develop appropriately, signal pregnancy recognition and/or undergo implantation and placentation. The endocrine status of the pregnant female and her nutritional status are critical for successful establishment and maintenance of pregnancy. The challenge is to understand the complexity of key mechanisms that are characteristic of successful reproduction and to use that knowledge to enhance fertility and reproductive health of animals including nonhuman primates. It is important to translate knowledge gained from studies of animals to address issues of fertility and reproductive health in humans.

1. Introduction

Reproduction in mammals is a highly complex and variable biological process among species. This is apparent when one reflects on the natural evolution of multiple strategies involving the hypothalamic-pituitary-gonadal-uterine axis employed to achieve pregnancy recognition signaling, implantation and placentation, fetal growth and development and parturition for successful outcomes of pregnancy. Regardless of species, a dialogue between the conceptus (embryo-fetus and associated placental membranes) and maternal uterus is established during the peri-implantation period for pregnancy recognition and maintenance of pregnancy. In response to pregnancy recognition signaling and secretions from the conceptus, the uterus expresses a multitude of genes in a cell-specific and temporal manner that encode for secretions by uterine luminal (LE), superficial glandular (sGE) and glandular (GE) epithelia, stromal cells (SC) and resident immune cells and for nutrient transporters to create within the uterine lumen a complex mixture of molecules in the uterine lumen called histotroph (see Figures 1, 2 and 3). Histotroph, primarily from uterine sGE and GE includes
nutrient transport proteins, ions, mitogens, cytokines, lymphokines, enzymes, hormones, growth factors, proteases and protease inhibitors, amino acids, glucose, fructose, vitamins and other substances. In the absence of uterine glands pregnancy fails early in the peri-implantation period of pregnancy. Within the appropriate uterine environment, mammalian conceptuses must signal pregnancy recognition to sustain the functional lifespan of corpora lutea for production of progesterone, the hormone of pregnancy. Progesterone is essential for implantation and placentation, both of which are critical events for successful pregnancy. However, it is within the peri-implantation period that most embryonic deaths occur due to deficiencies attributed to uterine functions or to failure of the conceptus to develop appropriately, signal pregnancy recognition and/or undergo implantation and placentation. If these events are successful, there remains variation in placental and fetal development that leads to intra-uterine growth insufficiency, undesirable aspects of fetal programming and failure of the conceptus to realize a successful induction of parturition. This entry focuses on the challenges, past and present, in understanding key mechanisms that ensure successful reproduction in a few species of animals. The goal is to provide an appreciation of current knowledge and gaps in knowledge related to our desire to enhance fertility and reproductive health or, alternatively, to establish acceptable methods for control of fertility.

Figure 1. [A] Oocytes fertilized in the oviduct enter the uterus at the morula stage, advance developmentally after hatching from the zona pellucida to spherical blastocysts that then transition to large spherical, tubular and filamentous conceptuses (embryo and its extra-embryonic membranes) with interferon tau (IFNT), the pregnancy recognition signal, being secreted from mononuclear trophectoderm cells between Days 10 and 21 of pregnancy. [B] The endometrial epithelia cease expressing receptors for progesterone (PGR) due to autoregulation by progesterone while IFNT silences expression of
receptors for estradiol (ESR1) and oxytocin receptors (OXTR) to abrogate development of the mechanism for oxytocin-mediated pulsatile release of prostaglandin F2α (PGF) which would otherwise cause regression of the corpus luteum and cessation of their secretion of progesterone. The endometrial stromal fibroblasts express PGR and secrete progestamedins, particularly fibroblast growth factor 10 that regulates uterine epithelia cell function. With down-regulation of PGR in uterine epithelia the uterine luminal (LE) and superficial glandular (sGE) epithelia express genes that are either induced by progesterone (P4) or induced by P4 and further stimulated by IFNT. Further, IFNT induces expression of interferon regulatory factor 2 (IRF2) in uterine LE and sGE to silence expression of classical interferon stimulated genes and allow expression of a unique set of genes that promote conceptus growth and development. The endometrial glandular epithelial cells (GE) and stromal fibroblasts do not express IRF2 and, therefore, express classical interferon stimulated proteins. Collectively, molecules secreted by uterine epithelia or transported into the uterine lumen by uterine epithelia form histotroph required for conceptus development. C. The ovine conceptus undergoes the adhesion cascade for implantation.

Conceptus-Maternal Interactions

PREGNANCY RECOGNITION SIGNALING

Antiluteolytic
IFNT
Estradiol/PRL

Luteotrophic
CGB
PRL
Lactogenic Hormones

Figure 2. Conceptus-maternal interactions include pregnancy recognition signaling to ensure prolonged maintenance of the corpus luteum (CL) for production of progesterone that acts on the uterus to maintain a secretory progestational endometrium to secrete and transport nutrients into the uterine lumen to support growth and development of the blastocyst. Interferon tau (IFNT), the pregnancy recognition hormone in ruminants silences expression of estrogen receptor alpha (ESR1) and, in turn, oxytocin receptor (OXTR) to prevent development of the luteolytic mechanism that requires oxytocin (OXT) from the corpus luteum (CL) and posterior pituitary to induce luteolytic pulses of prostaglandin F2α (PGF2α). In pigs, estradiol-17β (E2) secreted by conceptuses between Days 11 and 15 of pregnancy acts, along with prolactin (PRL), to cause PGF2α to change its direction of secretion from endocrine (into the uterine blood vessels) to...
exocrine (into the uterine lumen) which protects the CL from luteolytic PGF$_{2 \alpha}$. Thus, IFNT and E2 are antiluteolytic pregnancy recognition signals. Blastocysts of primates secrete chorionic gonadotrophin beta (CGB) that acts directly on the CL to ensure its maintenance and secretion of progesterone. In rodents, mating induces diurnal release of prolactin for 12 days that is required for formation of CL and their secretion of progesterone until other lactogenic hormones are produced by the placental and uterine decidual cells from about Day 10 of pregnancy.

The Uterine Microenvironment Includes Histotroph Critical to Growth and Development of the Conceptus

Figure 3. The uterine microenvironment of histotroph includes a variety of molecules that are secreted and or transported into the uterine lumen in response to hormonally regulated genes that are expressed in a temporal and cell-specific manner. Components of histotroph stimulate growth and development of the conceptus during the peri-implantation period and, indeed, for the duration of pregnancy in species with epitheliochorial and syndesmochorial placentas such as the pig and sheep, respectively.

2. Gene Expression for Uterine Proteins and Nutrient Transporters

Uterine receptivity to peri-implantation conceptus (embryo/fetus and associated extraembryonic membranes) development varies among species, and involves coordinate changes in expression of genes associated with attachment of trophectoderm. The trophectoderm is the outer layer of the blastocyst which gives rise to the chorion of the placenta. The trophectoderm/chorion secretes key hormones for establishment and maintenance of pregnancy and it also transports nutrients from the maternal system to the conceptus to allow normal development. The trophectoderm/chorion attaches to the
uterine LE and sGE for implantation. During the peri-implantation period, the coordinate changes in gene expression affect the uterine LE, as well as mid- to deep uterine GE, as well as modification of the phenotype of uterine stromal cells. Further, there is silencing of receptors for progesterone (PGR) and estrogen (ESR1) in uterine epithelia, suppression of genes for immune recognition of trophectoderm, alterations in membrane permeability to enhance conceptus-maternal exchange of growth factors and nutrients, angiogenesis and vasculogenesis, increased vascularity of the endometrium for increased uterine blood flow, and enhanced signaling for pregnancy recognition. Each of the changes in expression of epithelial and stromal genes in response to progesterone (P4), estrogens (E2), glucocorticoids (GCs), prostaglandins (PGs) and interferons (IFNs) affects biological functions including uterine receptivity to implantation and conceptus development through actions on the trophoblast and/or endometrium in mammals.

Bibliography


Barker D.J.P. (2007). The origins of the developmental origins theory. Journal of Internal Medicine 261, 412-417, 2007. [This paper describes the concepts of adult onset of disease due to environmental effects resulting in genomic reprogramming in the fetus.]


Prostaglandins 14, 397-401. [This paper describes the theory for the mechanism for pregnancy recognition in pigs.]


©Encyclopedia of Life Support Systems (EOLSS)


Dindot S.V., Person R., Strivens M., Garcia R., Beaudet A.L. (2009). Epigenetic profiling at mouse imprinted gene clusters reveals novel epigenetic and genetic features at differentially methylated regions. *Genome Research* 19, 1374-1383. [This paper describes epigenetic effects on imprinted genes which are those preferentially expressed by contributions from the paternal genome in the placenta and maternal genome in the embryo.]


Fazleabas A.T., Kim J.J., Strakova Z. (2004). Implantation: embryonic signals and the modulation of the uterine environment – a review. *Placenta* 25, S26-S31. [This paper details key events in the implantation process for humans and nonhuman primates, as well as effects of key cell signaling molecules from the conceptus involved in this process.]

Feinberg A.P. (2008). Epigenetics at the epicenter of modern medicine. *Journal of the American Medical Association* 299, 1345-1350. [This paper details the importance of understanding epigenetic events that affect health of humans and animals.]


Gertler A., Djiane J. (2002). Mechanism of ruminant placental lactogen action: Molecular and in vivo studies. *Molecular Genetics Metabolism* 75, 189–201. [This paper details biological actions of placental lactogen following binding to receptors.]

Gootwine E., Spencer T.E., Bazer F.W. (2007). Litter-size-dependent intrauterine growth restriction in sheep. Animal. 1:547-64. [This paper provides considerable insight into factors affecting growth and development of the fetus, particularly when there are multiple fetuses in the pregnant uterus of the ewe.]


Grillo M.A., Lanza A., Colombatto S. (2008). Transport of amino acids through the placenta and their role. Amino Acids 34, 517-523. [This paper details mechanisms for transport across the placenta to be utilized by the conceptus.]


Ka H., Jaeger L.A., Johnson G.A., Spencer T.E., Bazer F.W. (2001). Keratinocyte growth factor expression is up-regulated by estrogen in porcine uterine endometrium and it functions in trophoblast cell proliferation and differentiation. Endocrinology 142, 2303-2310. [This paper describes the discovery that fibroblast growth factor 7 (keratinocyte growth factor) is secreted by uterine luminal epithelium of pigs and that it can act via receptors on uterine epithelia and conceptus trophoblast to affect proliferation and differentiation of those cells.]

©Encyclopedia of Life Support Systems (EOLSS)


Lefèvre F., Guillemot M., D'Andrea S., Battegay S., La Bonnardière C. (1998). Interferon-delta: the first member of a novel type I interferon family. *Biochimie* 80, 779-788. [This paper describes the discovery of interferon delta that is now considered to be unique to pig and horse conceptuses.]


Martin P.M., Sutherland A.E., Winkle L.J.V. (2003). Amino acid transport regulates blastocyst implantation. *Biology of Reproduction* 69, 1101-1108. [This is an important paper in that it reports that arginine and leucine are required for outgrowth of the trophodermot of mouse blastocysts.]


Platanias L.C. (2005). Mechanisms of type-I- and type-II-interferon-mediated signaling. *Nature Reviews in Immunology* 5, 375-386. [This is a classic paper that first identifies the various cell signaling pathways that can be induced by Type I interferons.]


Rooney K., Ozanne S.E. (2011). Maternal over-nutrition and offspring obesity predisposition: targets for preventative interventions. *International Journal of Obstetrics* 35, 883-890. [This paper describes consequences of over-nutrition of the mother on predisposition of offspring to metabolic syndrome, as well as interventions that may prevent or ameliorate adverse outcomes of pregnancy.]


Satterfield M.C., Bazer F.W., Spencer T.E, Wu G. (2010). Sildenafil citrate treatment enhances amino acid availability in the conceptus and fetal growth in an ovine model of intrauterine growth restriction. *Journal of Nutrition* 140, 251-258. [This paper describes the use of “Viagra” to increase uterine blood flow in ewes to enhance growth and development of their fetuses.]


Simmons R. (2011). Epigenetics and maternal nutrition: nature v. nurture. *Proceedings of Nutrition Society* 70, 73-81. [This paper provides insight into links between over- and under-nutrition, epigenetic events leading to reprogramming of the genome and adult onset of disease.]


stromal cells of the primate uterus that then regulate function of conceptus trophectoderm and uterine epithelium.

So M., Kim Y., Choi C.K., Ka H. (2008). Analysis of lysophosphatidic acid (LPA receptor and LPA-induced endometrial prostaglandin-endoperoxide synthase 2 expression in the porcine uterus. *Endocrinology* 149, 6166-6175. [This paper describes the role of lysophosphatidic acid in migration of blastocysts prior to their elongation in pigs in order to allow each as much uterine surface area as possible to establish its placenta.]

Soares M. J. (2004). The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. *Reproductive Biology and Endocrinology* 2, 51. [This is an excellent review of the roles of lactogenic hormones in the establishment and maintenance of pregnancy.]


Spencer T.E., Johnson G.A., Burghardt R.C., Bazer F.W. (2004). Progesterone and placental hormone actions on the uterus: Insights from domestic animals. *Biological Reproduction* 71, 2-10. [This review provides insight into the servomechanism whereby effects of placental lactogen and growth hormone effects are dependent on prior exposure of the uterine epithelial cells to interferon tau.]

Spencer T.E., Johnson G.A., Bazer F.W., Burghardt R.C., Palmarini M. (2007). Pregnancy recognition and conceptus implantation in domestic ruminants: Roles of progesterone, interferons and endogenous retroviruses. *Reproduction Fertility and Development* 19, 65-78. [This paper reviews pregnancy recognition signaling in ruminants, but also reviews the abundant expression of endogenous retrovirus in uterine epithelia and conceptus trophectoderm that is important in successful establishment and maintenance of pregnancy in ewes.]


Thamotharan M., Garg M., Oak S., Rogers L.M., Pan A., Sangiorgi F., Lee P.N., Devaskar S.U. (2007). Transgenerational inheritance of the insulin resistant phenotypes in embryo-transferred intrauterine growth-restricted adult female rat offspring. *Am J Physiol Endocrinol Metab* 292: E1270-E1279. [This paper provides insight into links between over- and under-nutrition, epigenetic events leading to reprogramming of the genome that present as metabolic disease for multiple generations.]


newborn pigs. *Journal of Nutrition* 138, 60-66. [This paper provides insight into differences in the proteomes of selected fetal tissues of fetuses suffering from intra-uterine growth restriction.]


Williams P.J., Kurlak L.O., Perkins A.C., Budge H., Stephenson T., Keisler D., Symonds M.E., Gardner D.S. (2007). Hypertension and impaired renal function accompany juvenile obesity: the effect of prenatal diet. *Kidney International* 72, 279-289. [This paper provides insight into links between over- and under-nutrition, epigenetic events leading to reprogramming of the genome and adult onset of disease, as well as hypertension.]


**Biographical Sketches**

**Fuller Warren Bazer**, Ph.D., is Regents Fellow, Distinguished Professor and O.D. Butler Chair in the Department of Animal Science at Texas A&M University, College Station, Texas. He received the B.S. in Biology from Centenary College of Louisiana, the M.S. in Animal Science from Louisiana State University, and the Ph.D. in Animal Science (Reproductive Biology) from North Carolina State University. His research in reproductive biology focuses on uterine biology and pregnancy, particularly mechanisms of action of pregnancy recognition signals from the conceptus to the maternal uterus, including interferon tau and estrogen from ruminant and pig conceptuses, respectively. His studies are conducted at the whole animal level, as well as the molecular and cellular levels. Dr. Bazer also studies the roles of uterine secretions as transport proteins, regulatory molecules, growth factors and enzymes and endocrine regulation of their secretion, as well as the role of select nutrients such as arginine, leucine, glutamine, glucose and fructose on development of the conceptus. The endocrinology of pregnancy, especially the roles of lactogenic and growth hormones in fetal-placental development and uterine functions are also studied in his laboratory. The mechanism(s) of action and potential therapeutic value of conceptus interferons and uterine-derived hematopoietic growth factors are areas of research with both pigs and sheep as models for human disease. Dr. Bazer is author or co-author of more than 445 refereed journal articles.

©Encyclopedia of Life Support Systems (EOLSS)
Guoyao Wu, Ph.D., is Professor of Nutrition, University Faculty Fellow, and AgriLife Research Senior Faculty Fellow in the Department of Animal Science at Texas A&M University. He received the B.S. in Animal Science from South China Agricultural University, the M.S. in Animal Nutrition from Beijing Agricultural University, and M.S. and Ph.D. degrees in Animal Biochemistry from the University of Alberta, Canada. He also obtained postdoctoral training in Biochemistry and Nutrition at McGill University Medical School and the Memorial University of Newfoundland Medical School in Canada. His research interests include biochemistry, nutrition and physiology of amino acids in animals at molecular, cellular, and whole body levels. Specific research projects include: (1) functions of amino acids in gene expression and cell signaling; (2) mechanisms that regulate intracellular synthesis and catabolism of proteins and amino acids; (3) hormonal and nutritional regulation of metabolic pathways and fuel homeostasis; (4) biology and pathobiology of nitric oxide and polyamines; (5) key roles of amino acids in preventing diabetes and obesity as well as associated vascular complications; (6) essential roles of amino acids in survival and growth of embryos, fetuses, and neonates; and (7) dietary requirements of proteins and amino acids in the life cycle. Dr. Wu is author or co-author of more than 330 refereed journal articles.

Gregory A. Johnson, Ph.D., is an Associate Professor in the Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences at Texas A&M University. He obtained the B.S. in Zoology, the M.S in Microbiology, and the Ph.D. in Animal Science from the University of Wyoming. He received postdoctoral training in Reproductive Biology at Texas A&M University. Dr. Johnson’s research utilizes pigs and sheep to investigate the molecular, cellular and physiological interactions between the conceptus (embryo/fetus and its extraembryonic membranes) and the uterus during the processes of pregnancy recognition, implantation and placental development. His work has focused on placental interferons and the genes they stimulate in the uterus, as well as interactions between the extracellular matrix protein secreted phosphoprotein 1 (also known as osteopontin), and integrins during implantation and placentation. He is author or co-author of over 85 refereed journal articles.

Gwonhwa Song, Ph.D. is an Assistant Professor in the World Class University Biomodulation Major in the Department of Agricultural Biotechnology, Seoul National University, Republic of Korea. He earned the M.S. in Molecular Animal Genetics from Seoul National University, the Ph.D. in Reproductive Biology, and post-doctoral training in the Department of Animal Science and Center for Animal Biotechnology, Texas A&M University. Dr. Song has discovered important genes related to development and differentiation of avian oviduct in response to estrogen. He also studies mechanisms in the oviduct regulated by reproductive hormones and relationships between hormone-related genes and cell signaling pathways. Dr. Song conducts research with laying hen as an animal model system to study mechanisms responsible for ovarian cancer, genomic variation and gene expression associated with development, differentiation, and metastasis of cancer cells. Another area of research is on mechanisms responsible for morphological and developmental of the avian reproductive tract that account for sex differentiation and asymmetric development. His research also includes uterine biology and pregnancy, especially implantation-related gene expression and associated regulatory mechanism induced by pregnancy recognition signals, such as interferon tau and estrogen, from conceptuses of ruminants and pig, respectively. Dr. Song has been author or co-author of more than 36 refereed journal articles.