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

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Contents

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.

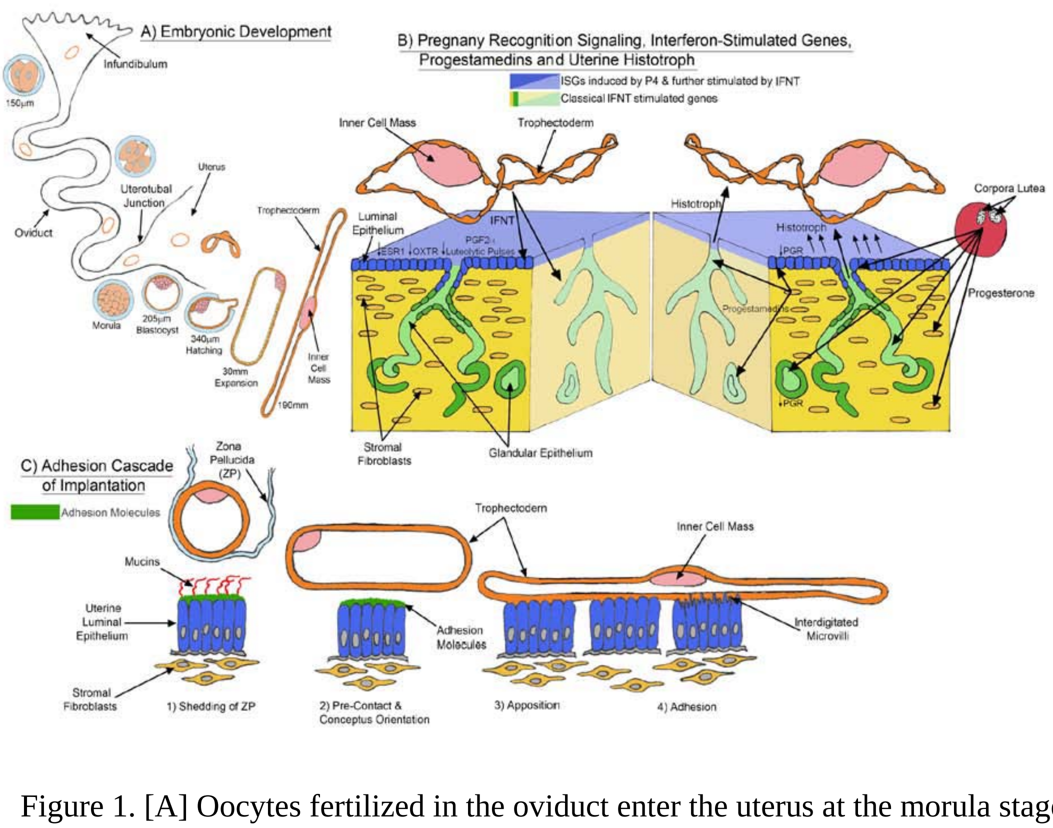

Figure 1. [A] Oocytes fertilized in the oviduct enter the uterus at the morula stage, advance developmentally after hatching from the zona pellucid to spherical blastocysts that then transition to large spherical, tubular and filamentous conceptuses (embryo and its extra-embryonic membranes) with interferon 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 $F_{2\alpha}$ (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.

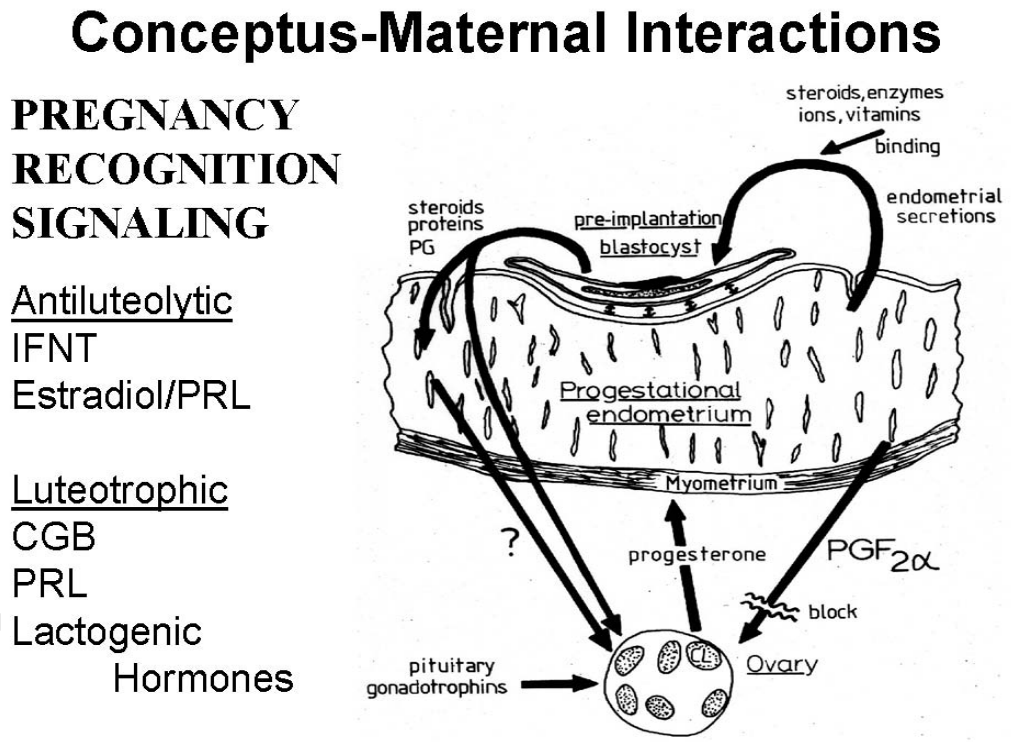

Figure 2. Conceptus-maternal interactions include pregnancy recognition signaling to ensure prolonged maintenance of the corpus luteum (CL) for production of progesterone that acts on the uterus to maintain a secretory progestational endometrium to secrete and transport nutrients into the uterine lumen 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 $F_{2\alpha}$ ($PGF_{2\alpha}$). In pigs, estradiol-17β (E2) secreted by conceptuses between Days 11 and 15 of pregnancy acts, along with prolactin (PRL), to cause $PGF_{2\alpha}$ 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.

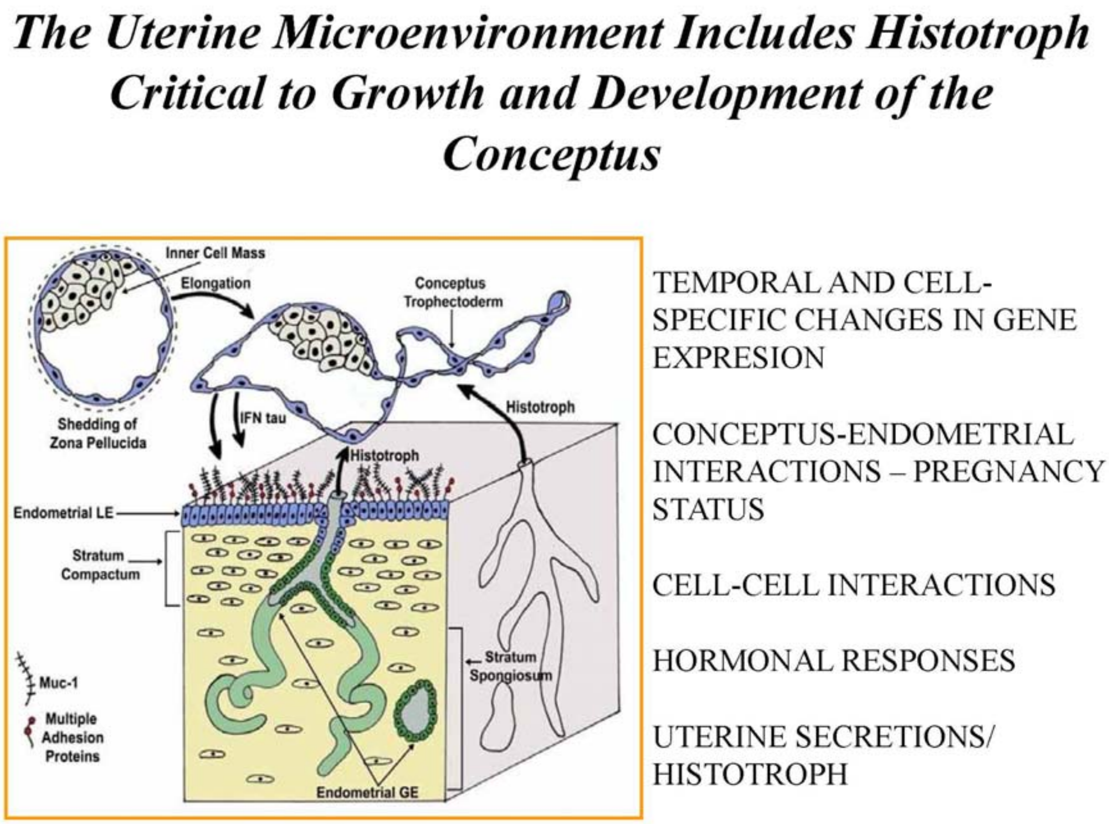

Figure 3. The uterine microenvironment of histotroph includes a variety of molecules that are secreted and or transported into the uterine lumen in response to hormonally regulated genes that are expressed in a temporal and cell-specific manner. Components of histotroph stimulate growth and development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

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Bibliography

Aboagye-Mathiesen G., Toth F.D., Zdravkovic M., Ebbesen P. (1996). Functional characteristics of human trophoblast interferons. *American Journal of Reproductive Immunology* 35, 309-317. [This paper describes Type I interferons expressed by human placental tissues.]

Afshar Y., Stanculescu A., Miele L., Fazleabas A.T. (2007). The role of chorionic gonadotropin and Notch1 in implantation. *Journal of Assisted Reproduction and Genetics* 24, 296–302. [This paper describes events associated with implantation in primates.]

Ashkar A.A., Croy B.A. (1999). Interferon-γ contributes to the normalcy of murine pregnancy. *Biology of Reproduction* 61,493-502. [This paper describes a role for interferon gamma in pregnancy in rodents.]

Ashkar A.A., Di Santo J.P., Croy B.A. (2000). Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *Journal of Experimental Medicine* 192, 259-270. [This paper describes a role for interferon gamma in pregnancy in rodents.]

Bany B.M., Cross J.C. (2006). Post-implantation mouse conceptuses produce paracrine signals that regulate the uterine endometrium undergoing decidualization. *Developmental Biology* 294, 445-456. [This paper describes a role for interferons during implantation and decidualization of the uterus in rodents.]

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.]

Bazer F.W., First N.L. (1983). Pregnancy and parturition. *Journal of Animal Science* 57(Suppl. 2), 425-460. [This paper describes embryonic and fetal development in domestic animal species, as well as mechanisms for induction of parturition.]

Bazer F.W., Thatcher W.W. (1977). Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F_{2a} by the uterine endometrium.

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

Bazer F.W., Burghardt R.C., Johnson G.A., Spencer T.E., Wu G. (2008). Interferons and progesterone for establishment and maintenance of pregnancy: Interactions among novel cell signaling pathways. *Reproductive Biology* 8, 179-211. [This paper describes the role of interferons in pregnancy recognition signaling and alternative cell signaling pathways for Type I interferons in the pregnant uterus.]

Bazer F.W., Spencer, T.E. Johnson, G.A., Burghardt R.C., Wu G. (2009) Comparative aspects of implantation. *Reproduction* 138, 195-209. [This paper describes mechanisms for implantation in primates, domestic animals and rodents.]

Bazer F.W., Wu G., Spencer T.E., Johnson G.A., Burghardt R.C., Bayless K. (2010) Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. *Molecular Human Reproduction* 16, 135-152. [This paper describes mechanisms for implantation and establishment of pregnancy in mammals.]

Bazer F.W., Spencer T.E., Thatcher W.W. (2011) Growth and development of the ovine conceptus. *Journal of Animal Science* Aug 12. [Epub ahead of print]. [This paper describes the pattern of growth of the placenta and fetus, as well as changes in fetal fluid volumes throughout gestation in ewes.]

Bertram C., Khan O., Ohri S., Phillips D.I., Matthews S.G., Hanson M.A. (2008). Transgenerational effects of prenatal nutrient restriction on cardiovascular and hypothalamic-pituitary-adrenal function. Transgenerational effects of prenatal nutrient restriction on cardiovascular and hypothalamic-pituitary-adrenal function. *Journal of Physiology* 586, 2217-2229. [This paper describes trangenerational effects of environmental insults that result in reprogramming of the genome during fetal life.]

Brown J.L. (2006). Comparative endocrinology of domestic and nondomestic felids. *Theriogenology* 66, 25-36. [This paper describes details of the physiology and endocrinology of pregnancy in cats.]

Burghardt R.C., Bowen J.A., Newton G.R., Bazer F.W. (1997). Extracellular matrix and the implantation cascade in pigs. *Journal of Reproduction and Fertility* Suppl 52, 151-164. [This paper describes roles for extracellular matrix molecules in implantation in pigs.]

Burghardt R.C., Johnson G.A., Jaeger L.A., Ka H., Garlow J.E., Spencer T.E., Bazer F.W. (2002). Integrins and extracellular matrix proteins at the maternal-fetal interface in domestic animals. *Cells Tissues Organs* 172, 202-217. [This paper describes roles for extracellular matrix molecules in implantation and in placentation in pigs.]

Burton G.J., Watson A.L., Hempstock J., Skepper J.N., Jauniaux E. (2002). Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. *Journal of Clinical Endocrinology and Metabolism* 87, 2954-2959. [This paper documents that secretions from uterine glands are essential for successful outcomes of pregnancy in women.]

Bygren L.O., Kaati G., Edvinsson S. (2001). Longevity determined by paternal ancestors' nutrition during their slow growth period. *Acta Biotheor*etica 49, 53–59. [This paper describes the consequences of under-nutrition on growth and development of human offspring and their predisposition to onset of metabolic disease as adults.]

Carson D.D., Bagchi I., Dey S.K., Enders A.C., Fazleabas A.T., Lessey B.A., Yoshinaga K. (2000). Embryo implantation. *Developmental Biology* 223, 217-237. [This paper describes basic mechanism for implantation in mammals.]

Carson D.D., Lagow E., Thathiah A., Al-Shami R., Farach-Carson M.C., Vernon M., Yuan L., Fritz M.A., Lessey B. (2002). Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. *Molecular Human Reproduction* 8, 871-879. [This paper describes changes in gene expression associated with opening of the window of implantation in women.]

Cencič A., Guillomot M., Koren S., LaBonnariére C. (2003). Trophoblastic interferons: Do they modulate uterine cellular markers at the time of conceptus attachment in the pig? *Placenta* 24, 862-869. [This paper describes effect of interferons from pig conceptuses on uterine epithelia during the peri-implantation period.]

Cochet M., Vaiman D., Lefèvre F. (2009). Novel interferon delta genes in mammals: cloning of one gene from the sheep, two genes expressed by the horse conceptus and discovery of related sequences in several taxa by genomic database screening. *Gene* 433, 88-99. [This paper describes discovery of expression of interferon delta by equine conceptuses.]

Cunha G.R., Cooke P.S., Kurita T. (2004). Role of stromal-epithelial interactions in hormonal responses. *Archives of Histolology and Cytology* 67, 417-434. [This classical paper details evidence that uterine stromal cells regulate function of epithelial cells with respect to proliferation and differentiation.]

Dey S.K., Lim H., Das S.K., Reese J., Paria B.C., Daikoku T., Wang H. (2004). Molecular cues to implantation. *Endocrine Reviews* 25, 341-373. [This paper reviews key events in implantation in mammals.]

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.]

Erikson D., Burghardt R.C., Bayless K.J., Johnson G.A. (2009). Secreted phosphoprotein 1 (SPP1, osteopontin) binds to integrin alphaVbeta6 on porcine trophectoderm cells and integrin alphaVbeta3 on uterine luminal epithelial cells, and promotes trophectoderm cell adhesion and migration. *Biology of Reproduction* 81, 814-825. [This paper describes roles for secreted phosphoprotein 1 on adhesion and migration of pig trophectoderm cells.]

Evertts, A.G., Zee B.M.,Garcia B.A. (2010). Modern approaches for investigating epigenetic signaling pathways. *Journal of Applied Physiology* 109, 927-933. [This paper describes methods for investigating pathways by which epigenetic events may be induced by environmental factors.]

Farmer J.L., Burghardt R.C., Jousan F.D., Hansen P.J., Bazer F.W., Spencer T.E. (2008). Galectin 15 (LGALS15) functions in trophectoderm migration and attachment. *Federation of the American Society for Experimental Biology* 22, 548-560. [This paper reports on the discovery of function for galectin 15 in the peri-implantation period of pregnancy in sheep.]

Fazleabas A.T. (2007). Physiology and pathology of implantation in the human and nonhuman primate. *Seminars in Reproductive Medicine* 25, 405-409. [This paper details key events in the implantation process for humans and nonhuman primates.]

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.]

Franco H.L., Jeong J.W., Tsai S.Y., Lydon J.P., DeMayo F.J. (2008). In vivo analysis of progesterone receptor action in the uterus during embryo implantation. *Seminars in Cell and Developmental Biology* 19, 178-186. [This paper provides unique insights into the role of progesterone in implantation in a rodent model.]

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.]

Golding, M.C., Williamson G.L., Stroud T.K., Westhusin M.E., Long C.R. (2011). Examination of DNA methyltransferase expression in cloned embryo reveals an essential role for Dnmt1 in bovine development. *Molecular Reproduction and Development* 78, 306-317. [This paper provides an example of how cloning leads to inappropriate methylation of DNA leading to epigenetic outcomes in development of the conceptus.]

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.]

Gray C.A., Abbey C.A., Beremand P.D., Choi Y., Farmer J.L., Adelson D.L., Thomas T.L., Bazer F.W., Spencer T.E. (2006). Identification of endometrial genes regulated by early pregnancy, progesterone, and interferon tau in the ovine uterus. *Biology of Reproduction* 74, 383-394. [This paper identifies genes expressed during early pregnancy and in response to interfereon tau that affect establishment of pregnancy and conceptus development.]

Greenwood P.L., Hunt A.S., Hermanson J.W., Bell A.W. (2000). Effects of birth weight and postnatal nutrition on neonatal sheep: II. Skeletal muscle growth and development. *Journal of Animal Science* 78, 50-61. [This paper describes differential growth and development of the neonate as affected by its growth and development *in utero*.]

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.]

Grunder C., Hrabe A., Kirchner C. (1994). Chorionic gonadotropin-like proteins in the placental giant cells of the rabbit. *Cell Tissue Research* 278, 573-578. [This paper describes the discovery of chorionic gonadotrophin in cells of the rabbit placenta that might be involved in pregnancy recognition signaling.]

Guillomot M. (1995). Cellular interactions during implantation in domestic ruminants. *Journal of Reproduction and Fertility* Suppl 49, 39-51. [This is a classic paper detailing implantation in domestic ruminants.]

Harrison M., Langley-Evans S.C. (2009). Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. *British Journal of Nutrition* 101, 1020-1030. [This paper describes adverse effects of dietary protein restriction during pregnancy on kidney functions and hypertension in the rat model.]

Hess A.P., Hamilton A.E., Talbi S., Dosiou C., Nyegaard M., Nayak N., Genbecev-Krtolica O., Mavrogianis P., Ferrer K., Kruessel J., Fazleabas A.T., Fisher S.J., Giudice L.C. (2007). Decidual stromal cell response to paracrine signals from the trophoblast: amplification of immune and angiogenic modulators. *Biology of Reproduction* 76, 102-117. [This paper first describes that trophectoderm of women are producing a factor or factors that induce expression of interferon stimulated genes in uterine decidual tissue suggesting that the trophectoderm is secreting an unidentified interferon.]

Horcajadas J.A., Pellicer A., Simón C. (2007). Wide genomic analysis of human endometrial receptivity: new times, new opportunities. *Human Reproduction Update* 13, 77-86.

Johnson G.A. (2008). Uterine Stromal Cell Differentiation in Non-Decidualizing Species, in *The Endometrium: Molecular, Cellular and Clinical Perspectives*. (eds. J.D. Aplin, A.T. Fazleabas, S.R. Glasser, and L.C. Giudice), United States of America: Informa Healthcare Limited; pp 409-421. [This paper details key events in the implantation process for domestic animals that do not have an invasive form of implantation.]

Johnson G.A., Bazer F.W., Burghardt R.C., Spencer T.E., Wu G., Bayless K.J. (2009) Conceptus-uterus interactions in pigs: Endometrial gene expression in response to estrogens and interferons from conceptuses. *Society for Reproduction and Fertility* Supplement 66:321-332. [This paper provides state of knowledge on effects of interferons produced by pig conceptuses on gene expression either independently or in concert with estrogens that are also produced by pig conceptus trophectoderm.]

Johnson G.A., Burghardt R.C., Bazer F.W., Spencer T.E. (2003). Minireview: Osteopontin: roles in implantation and placentation. *Biology of Reproduction* 69, 1458-1471. [This paper reviews the roles of secreted phosphoprotein 1 (osteopontin) in implantation in mammals.]

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 trophectodermal 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 trophectoderm to affect proliferation and differentiation of those cells.]

Kim J., Erikson D.W., Burghardt R.C., Spencer T.E., Wu G., Bayless K.J., Johnson G.A., Bazer F.W. (2010). Secreted phosphoprotein 1 binds integrins to initiate multiple cell signaling pathways, including FRAP1/mTOR, to support attachment and force-generated migration of trophectoderm cells. *Matrix Biology* 29, 369-382. [This paper provides insight into effects of secreted phosphoprotein 1 on activation of the mechanistic target of rapamycin cell signaling pathway that affects proliferation, migration, mRNA translation and focal adhesion assembly in sheep conceptus trophectoderm.]

Kirn-Safran C.B., D'Souza S.S., Carson D.D. (2008). Heparan sulfate proteoglycans and their binding proteins in embryo implantation and placentation. *Seminars in Cell and Developmental Biology* 19,187-193. [This paper provides state of knowledge on effects of proteoglycans in implantation and placentation in mammals.]

Kliman H.J. (2000). Uteroplacental blood flow. The story of decidualization, menstruation, and trophoblast invasion. *American Journal of Pathology* 157, 1759-1768. [This paper provides knowledge of changes in uteroplacental blood flows during pregnancy and during the menstrual cycle.]

Lash F.W., Otun H.A., Innes B.A., Kirkley M., De Oliveira L., Searle R.F., Robson S.C., Bulmer J.N. (2006). Interferon-γ inhibits extravillous trophoblast cell invasion by a mechanism that involves both changes in apoptosis and protease levels. *Federation of the American Society for Experimental Biology Journal* 20, 2512-2518. [This paper describes a role for interferon gamma in implantation of the blastocyst.]

Lefèvre F., Guillomot 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 andhorse conceptuses.]

Lefèvre F., Martinat-Botté F., Locatelli A., De Niu P., Terqui M., LaBonnardière C. (1998). Intrauterine infusion of high doses of pig trophoblast interferons has no antiluteolytic effect in cyclic gilts. *Biology of Reproduction* 58, 1026-1031. [This report indicated that pig trophoblast interferons are not part of pregnancy recognition signaling.]

Lessey B., Ilesanmi A.O., Lessey B.A., Riben A., Harris J.W., Chwalisz K. (1996). Luminal and glandular endometrial epithelium express integrins differentially throughout the menstrual cycle: implications for implantation, contraception, and infertility. *American Journal of Reproductive Immunology* 35,195-204. [This paper describes the discovery that integrins are expressed during the window of uterine receptivity to implantation of the blastocyst.]

Lessey B.A. (2002). Adhesion molecules and implantation. *Journal of Reproductive Immunology* 55,101-112. [This paper describes the discovery of cell adhesion molecules associated with implantation in women.]

Li Q., Zhang M., Kumar S., Zhu L.J., Chen D., Bagchi M.K., Bagchi I.C. (2001). Identification and implantation stage specific expression of an interferon-alpha-regulated gene in human and rat endometrium. *Endocrinology* 142, 2390-2400. [This paper describes temporal and cell-specific expression of interferon alpha regulated genes in human and rat endometrium.]

Marcinkiewicz J.J., Bahr J.M. (1993). Identification and preliminary characterization of luteotropic activity in the rabbit placenta. *Biology of Reproduction* 48, 403-408. [This paper provides insight into mechanisms for pregnancy recognition in rabbits.]

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 trophectoderm of mouse blastocysts.]

Monk J.M., Leonard S., McBey B.A., Croy B.A. (2005). Induction of murine spiral artery modification by recombinant human interferon-gamma. *Placenta* 26, 835-838. [This paper describes effects of interferon gamma on development of uterine arteries in pregnant mice.]

Newton G.R., Ott T.L., Woldesenbet S., Shelton A.M., Bazer F.W. (1996). Biochemical and immunological properties of related small ruminant trophoblast interferons. *Theriogenology* 46, 703-716. [This paper describes the discovery of interferon tau as the pregnancy recognition signal in goats.]

Peterson C.L., Laniel M.A. (2004). Histones and histone modifications. *Current Biology* 14, R546-R551. [This paper describes how histones modify gene expression associated with epigenetic events.]

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.]

Reynolds F.L.P., Borowicz P.P., Vonnahme K.A., Johnson M.L., Grazul-Bilska A.T., Wallace J.M., Caton J.S., Redmer D.A. (2005). Animal models of placental angiogenesis. *Placenta* 26,689-708. [This paper reviews the process of angiogenesis that is critical to uterine and placental blood flows affecting delivery of nutrients and gases to the fetus, as well removal of metabolic products such as carbon dioxide and urea.]

Reynolds L.P., Borowicz P.P, Caton J.S., Vonnahme K.A., Luther J.S., Hammer C.J., Maddock K.R., Carlin, A. T., Grazul-Bilska A.T., Redmer D.A. (2010). Developmental programming: The concept, large animal models, and the key role of uteroplacental vascular development. *Journal of Animal Science* 88, E61-72. [This paper describes developmental programming and epigenetic events associate with large offspring syndrome that is often an outcome of techniques such as long-term culture of embryos and cloning.]

Robertson H.A., King G.J. (1974). Plasma concentrations of progesterone, oestrone, oestradiol-17-beta and of oestrone sulfate in the pig at implantation, during pregnancy and at parturition. *Journal of Reproduction and Fertility* 40, 133-141. [This paper describes the endocrinology of pregnancy in pigs.]

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.]

Salamonsen L. (1999). Role of proteases in implantation. *Reviews in Reproduction* 4, 11-22. [This paper describes the important roles of proteases and protease inhibitors in tissue remodeling during implantation.]

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.]

Satterfield M.C., Hayashi K., Song G., Black S.G., Bazer F.W., Spencer T.E. (2008). Progesterone regulates FGF10, MET, IGFBP1, and IGFBP3 in the endometrium of the ovine uterus. *Biology of Reproduction* 79, 1226-1236. [This paper describes regulation of expression of growth factors from uterine stromal cells and epithelia that affect conceptus development.]

Satterfield M.C., McKnight J.R., Li X.L., Wu G. (2011). Nutrition, epigenetics, and vascular function, in *Nutrition, Epigenetic Mechanisms, and Human Disease*. (eds. N. Maulik and G. Maulik). New York: CRC Press. pp. 125-139. [This chapter is a valuable reference to understanding the link between over- and under-nutrition, epigenetic events leading to reprogramming of the genome and adult onset of disease.]

Sharp D.C., McDowell K.J., Weithenauer J., Thatcher W.W. (1989). The continuum of events leading to maternal recognition of pregnancy in mares. *Journal of Reproduction and Fertility* (Suppl) 37, 101-107. [This paper describes what is know about pregnancy recognition signaling in the mare and the fact that the mechanism remains to be elucidated.]

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.]

Simmons R.M., Satterfield M.C., Welsh T.H., Bazer F.W., Spencer T.E. (2010). HSD11B1, HSD11B2, PTGS2 and NR3C1expression in the peri-implantation ovine uterus: effects of pregnancy, progesterone and interferon tau. *Biology of Reproduction* 82, 35-43. [This paper indicates that interfereon tau induces expression of genes that encode for enzymes that allow for local intra-uterine production of cortisol, an anti-inflammatory steroid, by uterine epithelia and conversion of cortisol to inactive cortisone by the conceptus.]

Slayden O.D., Keator C.S. (2007). Role of progesterone in nonhuman primate implantation. *Seminars in Reproductive Medicine* 25, 418-430. [This paper describes progesterone induction of progestamedins by stromal cells of the primate uterus that then regulate function of conceptus trophectoderm and uterine epithelia.]

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 are 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., Bazer F.W. (2002). Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Frontiers in Bioscience* 7, 1879-1898. [This review is a valuable reference to understanding pregnancy recognition signaling in primate and subprimate species.]

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. *Biology of 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 and endogenous retrovirus in uterine epithelia and conceptus trophectoerm that is important in successful establishment and maintenance of pregnancy in ewes.]

Spencer T.E., Sandra O., Wolf E. (2008). Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. *Reproduction* 135, 165-179. [This paper provides insight into gene expression by the conceptus and uterus during the peri-implantation period of pregnancy in domestic animals.]

Symonds M.E., Sebert S.P., Hyatt M.A., Budge H. (2009). Nutritional programming of the metabolic syndrome. *Nature Reviews Endocrinology* 5, 604-610. [This paper provides insight into nutritional programming leading to adult onset of metabolic syndrome.]

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.]

Thatcher W.W. Guzeloglu A., Mattos R., Binelli M., Hansen T.R., Pru J.K. (2001). Uterine-conceptus interactions and reproductive failure in cattle. *Theriogenology* 56,1435-1450. [This paper provides insight into pregnancy recognition signaling and conceptus development in cattle.]

Vallet, J.L., K.A. Leymaster, Christenson R.K. (2002). The influence of uterine function on embryonic and fetal survival. *Journal of Animal Science* 80 (E. Suppl. 2), E115-E125. [This paper reviews evidence that uterine capacity if a limiting factor affected growth, development and survival of pig conceptuses.]

Verstegen-Onclin K., Verstegen J. (2008). Endocrinology of pregnancy in the dog: A review. *Theriogenology* 70, 291-299. [This paper reviews the endocrinology of pregnancy in dogs.]

Vonnahme K.A., Hess B.W., Hansen T.R., McCormick R.J., Rule D.C., Moss G.E., Murdoch W.J., Nijland M.J., Skinner D.C., Nathanielsz P.W., Ford S.P. (2003). Maternal undernutrition from early- to mid-gestation leads to growth retardation, cardiac ventricular hypertrophy, and increased liver weight in the fetal sheep. *Biology of Reproduction* 69, 133-140. [This paper provides insight into links between under-nutrition, epigenetic events leading to reprogramming of the genome and adult onset of disease.]

Wang, J.J., Chen L.X., Li D.F., Yin Y.L., Wang X.Q., Li P., Dangott L.J., Hu W.X., Wu G. (2008). Intrauterine growth restriction affects the proteomes of the small intestine, liver and skeletal muscle in 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.]

Warner M.J., Ozanne S.E. (2010). Mechanisms involved in the developmental programming of adulthood disease. *Biochem J* 427: 333-347. [An excellent review of mechanisms for developmental programming of the fetal genome and adult onset of disease.

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.]

Wu, G., Bazer F.W., Burghardt R.C., Johnson G.A., Kim S.W., Li X.L., Satterfield M.C., Spencer T.E. (2010). Impacts of amino acid nutrition on pregnancy outcome in pigs: mechanisms and implications for swine production. *Journal of Animal Science* 88, E195-E204. [This paper provides insight into the critical roles of amino acids in the developing conceptus and successful outcomes of pregnancy.]

Wu, G., Bazer F.W., Cudd T.A., Meininger C.J., Spencer T.E. (2004). Maternal nutrition and fetal development. *Journal of Nutrition* 134, 2169-2172. [This paper provides valuable insights into the critical role of nutrition in determining growth and development of the fetus and successful outcomes of pregnancy.]

Wu, G., Bazer F.W., Johnson G.A., Knabe D.A., Burghardt R.C., Spencer T.E., Li X.L, Wang J.J. (2011). Important roles for L-glutamine in swine nutrition and production. *Journal of Animal Science* 89, 2017-2030. [This paper provides insight into the critical roles of glutamine in the developing conceptus for successful outcomes of pregnancy.]

Ye X., Hama K., Contos J.J., Anliker B., Inoue A., Skinner M.K., Suzuki H., Amano T., Kennedy G., Arai H., Aoki J. (2005). LPA3-meditated lysophosphatidic acid signaling in embryo implantation and spacing. *Nature* 435, 34-35. [The use of gene knockout to silence expression of lysophophatidic acid demonstrated that intra-uterine migration and spacing of embryos in mice is essential for high rates of embryonic survival.]

Youngson N.A., Whitelaw E. (2008). Transgenerational epigenetic effects. *Annual Review of Genomics and Human Genetics* 9, 233–57. [This paper reviews transgenerational effects resulting from epigenetic events.]

Ziecik A.J., Waclawik A., Bogacki M. (2008). Conceptus signals for establishment and maintenance of pregnancy in pigs–lipid signaling system. *Experimental and Clinical Endocrinology and Diabetes* 116, 443-449. [This paper provides new insights into mechanisms for pregnancy recognition signaling in pigs.]

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.

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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.