—Mini Review—

Mechanisms for Establishment of Pregnancy in Mammalian Species

*Koji Kimura[1]**

[1]Reproductive Physiology Laboratory, Department of Animal Breeding and Reproduction, National Institute of Livestock and Grassland Science, 768 Senbonmatsu, Nasushiobara, Tochigi 329-2793, Japan

Abstract: *Cross-talk between the embryo and mother is necessary for the establishment of successful pregnancy. In the mouse, cervical stimuli during mating induce prolactin secretion from the pituitary and formation of the corpus luteum (CL) of pregnancy. Placental lactogen supports the maintenance of pregnancy after implantation. In the human, the embryo secretes chorionic gonadotropin (hCG), which has lenbinizing hormone (LH) activity and maintains pregnancy before the luteal-placental shift occurs. In cattle and sheep, the mechanism underlying the establishment of pregnancy is unique. Their embryos secrete interferon (IFN)-τ, which is related to type I IFNs. IFN-τ prevents the secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterine endometrium by suppressing the expression of estrogen and oxytocin receptor. In the pig, estrogen secreted from the conceptus is a factor for maternal recognition of pregnancy. Estrogen leads to a shift in the direction of $PGF_{2\alpha}$ secretion from endocrine to exocrine and inhibits the regression of CL. Thus, each species has specific mechanisms for the establishment of pregnancy.*

Key words: *Mechanism, Pregnancy, Implantation*

Introduction

In mammals, the fertilization and development of a fetus to term occur in the female reproductive tract. The events of the early stage of embryo development are similar in many species. For instance, oocytes are fertilized in the oviduct and then zygotes cleave and develop into blastocysts. However, after the embryo hatches from the zona pellucida, the process of embryonic development differs from species to species. In some species, such as the mouse and human, embryos start to attach to the uterine wall immediately after hatching and provoke stromal decidualization. On the other hand, in cattle, sheep, and porcine, embryos do not implant immediately after hatching, they float in the uterine lumen and develop without any attachment to the uterus. Moreover, the placentas have different morphologies depending on the species. In cattle, sheep, porcine, and equine, an epitheliochorial placenta is formed. In the human, mouse, and rat, the placenta is a hemochorial type. These differences imply a large number of diversities exist among these species in the mechanisms underlying the establishment of pregnancy.

In this article, the mechanisms for the establishment of early stage of pregnancy in mammalian species that have been intensively researched, mouse, human, cattle, sheep, and pig, are discussed.

Received: August 26, 2005
Accepted: September 2, 2005
*To whom correspondence should be addressed.
e-mail: kimurak@affrc.go.jp

Mouse

The mouse is widely used for research into embryonic development and implantation. One reason for this is the similarities in the mechanisms of pregnancy between the mouse and human. In both species, embryo implantation leads to stromal decidualization. Moreover, both have a hemochorial type of placentation. In this section, the literature on the factors involved in the establishment of pregnancy in the mouse are reviewed.

Prolactin and related hormones

The estrous cycle in the mouse lasts four days (proestrus, estrus, metoestrus, and diestrus). The embryo migrates from the oviduct to the uterus on day 3 of pregnancy (day 1 = occurrence of vaginal plug) and attaches to the surface of the endometrium on day 4.5. Therefore, until the establishment of direct cell-to-cell communication between the fetus and the mother

(implantation), some mechanisms that prevent the beginning of the next estrus cycle are necessary for maintaining pregnancy.

Prolactin (PRL) has luteotrophic activity in rodents [1]. During mating, cervical stimuli induce a semicircadian surge of PRL secretion from the pituitary. These PRL surges in turn stimulate the production of progesterone from the corpus luteum (CL), thus transforming the CL of the cycle into the CL of pregnancy, which secretes enough progesterone to establish early pregnancy [2].

Moreover, numerous PRL-related hormones secreted from the placenta have been identified. Two of these hormones were initially found in research for placental proteins that bind to the PRL receptor [3]. These two hormones are placental lactogen I (PL-I) and II (PL-II), both of which have lactogenic activity [3]. One of the most important targets of these hormones is the ovary. As described previously, the hypothalamus-pituitary PRL is involved in the establishment of early pregnancy. However, PL-I replaces PRL secreted from the pituitary after implantation [4]. At mid-pregnancy, the secretion of PL-II begins and increases as the PL-I secretion declines [4]. Therefore, PRL and PRL-related hormones contribute to the establishment of pregnancy in the mouse by maintaining CL. These PRL-related hormones share the same receptor, PRL receptor (PRLR) [5, 6]. The PRLR-gene-null mouse has been generated by a gene targeting technique and experiments with this mouse have revealed that PRLR is multifunctional [7]. Homozygous PRLR-gene-null ($PRLR^{-/-}$) female mice show multiple reproductive abnormalities. They mate irregularly, every 3 to 4 days, and never become pregnant (they are sterile) [8]. Moreover, the survival rate of embryos and the fertilization rates of oocytes from $PRLR^{-/-}$ female mice are lower than in the wild types [9].

Implantation window and uterine receptivity

The endometrium of the uterus acquires the ability to support blastocyst growth, attachment, and subsequent events of implantation. This state of the uterus, termed 'uterine receptivity', is limited to a specific period of pregnancy, called the 'implantation window' [10, 11]. The implantation window is influenced by numerous factors.

Ovarian steroid hormones, progesterone (P_4) and estradiol-17β (E_2), regulate the proliferation and differentiation of uterine endometrial cells in the mouse [12, 13]. E_2 induces the proliferation of uterine epithelial cells, while both E_2 and P_4 are required for the proliferation of stroma cells [14]. The coordinated actions of these hormones are involved in the process of establishing an implantation window. On the day when the vaginal plug is detected (first day of pregnancy: day 1), the proliferation of uterine epithelial cells is induced by preovulatory ovarian E_2 secretion. On day 3, P_4 secreted from the newly formed CL induces the proliferation of P_4-primed stroma cells. On day 4, the proliferation of stroma cells is further stimulated by a small amount of E_2 secreted from the ovary, while epithelial cells stop proliferating and become differentiated. Therefore, the uterus reaches the receptive state on day 4 of pregnancy and proceeds to the non-receptive (refractory) state on day 5 [10, 15]. The uterus in the refractory state fails to respond to the presence of blastocysts. A similar event occurs in the mouse during lactation. While the mouse is lactating, implantation is delayed after postpartum fertilization. However, the implantation process resumes after lactation ends [16]. Moreover, ovariectomy before the E_2 surge on day 4 of pregnancy results in the failure of implantation and induces dormancy of blastocysts [17]. This state persists for many days as long as P_4 treatment continues. When E_2 is given to mice in these conditions, the uteri become receptive to blastocyst implantation. Recent research suggests that the concentration of E_2 within a very narrow range determines the duration of the window of uterine receptivity. The window of uterine receptivity remains open for an extended period at lower E_2 levels. At higher levels, however, the window closes very rapidly, accompanied by aberrant uterine expression of implantation-related genes [18]. Thus it is suggested that the synergistic function of E_2 and P_4 is essential for uterine receptivity for implantation of mouse embryo.

Role of LIF in implantation

Recently, besides ovarian steroid hormones, many factors in implantation and uterine receptivity have been discovered via technologies such as gene manipulation (gene knockout mice) and RNA differential display. One such factor is leukemia inhibitory factor (LIF), a member of interleukin-6 (IL-6) family of cytokines.

LIF is a cytokine and has pleiotrophic effects in various physiological systems, including cell survival, proliferation, and differentiation [19, 20]. LIF is widely used for regulating the undifferentiated proliferation of mouse embryonic stem cells [21], and transcripts of LIF are present in inner cell mass cells of mouse blastocysts [22]. Moreover, high levels of LIF expression are detected in the uterine endometrial glands of the mouse throughout the reproductive cycle

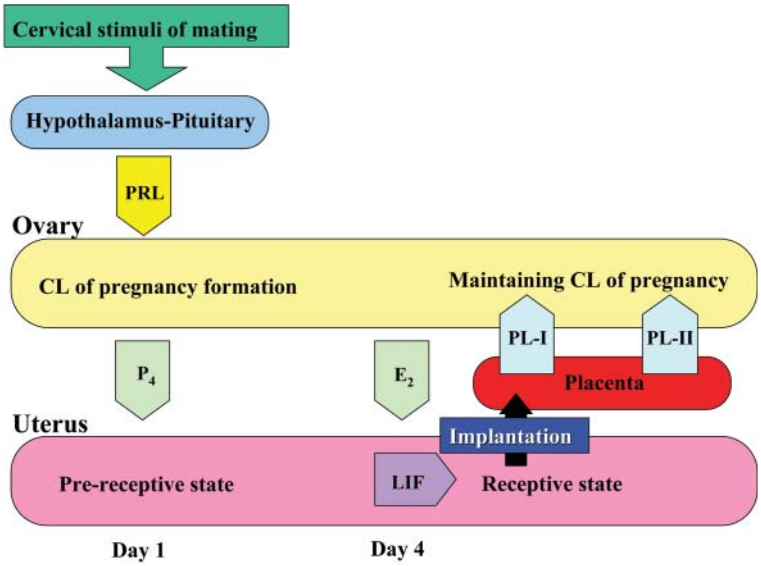

Fig. 1. Diagram of establishment of pregnancy in the mouse. Prolactin (PRL) secretion from the pituitary is induced by cervical stimuli during mating and forms the CL of pregnancy. On day 4 of pregnancy, estradiol-17β (E_2) leads to the expression of leukemia inhibitory factor (LIF) in the uterus. LIF turns the uterus to a receptive state. After implantation, placental lactogen I (PL-I) and II (PL-II) contribute to the maintenance of pregnancy.

[23]. A significant level of LIF is expressed in the endometrial glands on day 1 (when the vaginal plug occurs) of pregnancy [24]. Another transient high level of LIF expression is observed on day 4 of pregnancy, the day of implantation. Afterwards, the expression declines and is present at a low level throughout the remainder of pregnancy. This expression pattern of LIF coincides with that of E_2, and thus E_2 regulates the expression of LIF in the uterus [23–25].

LIF's involvement in the implantation of the mouse embryo has been revealed by studies using LIF gene knockout mice [26]. Homozygous LIF-gene-null ($LIF^{-/-}$) female mice are sterile. However, when the blastocysts from $LIF^{-/-}$ female mice mated with $LIF^{-/-}$ males are transferred to wild-type recipient female mice, they implant normally and develop successfully to term [26]. When the blastocysts from wild-type female mice are transferred to $LIF^{-/-}$ female recipient mice, they cannot implant. These results suggest that the failure of implantation in $LIF^{-/-}$ female mice is not due to some specific defects in their embryos but rather to the unreceptive state of the maternal uterine environment [26]. The location of $LIF^{-/-}$ embryos in $LIF^{-/-}$ female uteri on days 5 to 7 is identical to that in a normal pregnancy. However, the initiation of implantation does not take place in $LIF^{-/-}$ female uteri. The embryos in $LIF^{-/-}$ female uteri do not establish strong contact with uteri, and $LIF^{-/-}$ uteri are unresponsive to decidualizing stimuli [25]. When LIF is injected intraperitoneally to $LIF^{-/-}$ female mice on days 4 or 5 of pregnancy, embryo implantation is successfully induced [25]. Moreover, when LIF is injected into ovariectomized mice on day 3 of pregnancy and the mice are maintained for 3 to 4 days on P_4 (no nidatory E_2), LIF efficiently induces implantation [25]. These findings suggest that the transient expression of LIF on day 4 of pregnancy is the primary requirement for the initiation of embryo implantation, and that LIF can substitute for the nidatory E_2 surge at implantation.

Moreover, LIF affects embryonic development after implantation. The LIF receptor gene and gp130 gene are expressed at the blastocyst stage [27, 28]. Either the LIF receptor or the gp130 gene knockout mouse embryo can develop to the blastocyst stage and implant normally but die during the perinatal period [29, 30].

Summary

The schematic diagram of possible molecular cascades of events occurring in the early pregnancy of the mouse is depicted in Fig. 1. Recently, numerous genes that seem to be involved in the mechanisms

underlying the establishment of pregnancy have been discovered comprehensively using microarray techniques and mouse genome databases. However, their mechanisms have not been fully elucidated and further progress in this area is expected.

Human

Recently, assisted reproductive technologies (ART) have been developed and used for clinical objectives. For successful pregnancy, it is important to understand and elucidate the mechanisms underlying the establishment of pregnancy in humans.

The human ovarian cycle is uterine-independent and lasts approximately 28 days (the menstrual cycle). The cycle is composed of three phases: menstrual, proliferative, and secretory. After menstruation, the level of estradiol increases and multiple endometrial cell types undergo proliferation (the proliferative phase). Ovulation occurs approximately in the middle of the cycle, after which the CL is formed and starts to secrete progesterone (the secretory phase). Regardless of the presence of a living embryo, the level of progesterone, which is secreted from the newly formed CL, remains high for several days after ovulation, followed by regression of CL and menstruation. For a successful pregnancy, the embryo has to establish cross-talk with its mother and inhibit both the regression of CL and menstruation.

In this section, studies on the mechanisms underlying the establishment of pregnancy in the human are reviewed.

Implantation window and uterine receptivity

For human embryo transfer following ART, it is important to clarify the timing of the implantation window and the factors involved in uterine receptivity. The maternal endometrium does not accept the embryo at all times in the cycle. As with the mouse uterus, described above, the human uterus can accept an embryo only during a limited period of the cycle (the implantation window). An early report suggested that the human implantation window occurs in the middle of the secretory phase [31]. Recently it was suggested that the window exists around day 6 post-ovulation and lasts for 5 days [32–34]. Moreover, a high pregnancy success rate (84%) is observed when the embryo is implanted 8 to 10 days post-ovulation, compared with 18% when implantation occurs 11 days or more post-ovulation [35]; it is also reported that the window must last at least 3.5 days [36]. However, Formigli *et al.* suggests that the period may be as long as 7 days [37]. Taking these findings together, it is hypothesized that the implantation window is 7–11 days post-ovulation.

In the case of the mouse, the implantation window or uterine receptivity is influenced by steroid hormones secreted from the ovary [17, 18]. Progesterone is required for implantation in all species, but the need for estradiol in all species is controversial. In rhesus monkeys, implantation occurs when exogenous progesterone administration alone is performed following ovariectomy 2-6 days post-ovulation [38], a finding confirmed by other groups [39]. However, in studies using anti-estrogen antibody or anti-estrogen chemicals to inhibit endogenous estrogen action, the pregnancy rate was compromised [40–42]. These results strongly suggest that estrogen is necessary for the establishment of uterine receptivity in primates.

In humans, the level of hormones during the menstrual cycle has been investigated [43]. It was clearly shown that an estrogen peak exists when implantation would occur, simultaneous with peak progesterone and 17α-hydroxy-proge sterone levels. Moreover, the levels of progesterone and estrogen in women within the implantation window during conception are not different from levels during non-conception cycles [44]. When gonadotropin administration or steroid hormone replacement was performed, the implantation window shifted [45–48]. These results suggest that the implantation widow is flexible.

A diagnosis of uterine receptivity or an open implantation window is important for successful pregnancy following ART. The molecular events involved in the implantation of the human embryo remain obscure. Recently microarray [49–52], differential display PCR [53], and subtractive cDNA hybridization [54] profiling have revealed candidate genes for uterine receptivity. Those studies identified many genes that are up- or down-regulated during the implantation window. The involvement of these genes in the mechanisms of the implantation window will be investigated.

Human chorionic gonadotropin (hCG): its structure and functions

In the non-conception cycle, the level of progesterone declines gradually in the late secretory phase. However, in the conception cycle, no decrement of progesterone is found. This implies that the embryo inhibits luteolysis directly or indirectly. The human embryo secretes a peptide hormone, human chorionic

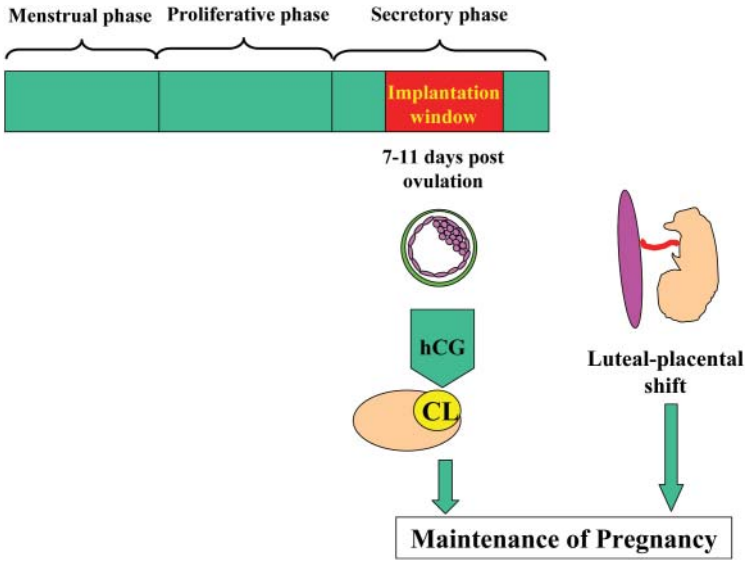

Fig. 2. Diagram of establishment of pregnancy in the human. The implantation window in the human occurs in the secretory phase of the menstrual cycle (7–11 days post-ovulation). When the human embryo develops to the blastocyst stage, it produces human chorionic gonadotropin (hCG). hCG has a luteotrophic effect and maintains the CL up to the luteal-placental shift.

gonadotropin (hCG), which appears to inhibit luteolysis and maintain the functional CL for the establishment of pregnancy [55–57]. The human embryo develops to blastocyst stage on day 5 and hatches from the zona pellucida on day 6 post-fertilization. Then trophectoderm differentiates into two types of cells, the cytotrophoblast and the syncytiotrophoblast. The latter, a multinucleated non-dividing cell that develops from cytotrophoblast, secretes hCG [58]. Measurable hCG in embryo culture medium is first detectable at approximately day 7 [59–62]. However, its transcript is found as early as the 8-cell stage embryo [63]. This hormone is also detected in the blood of women as early as 8–12 days post-ovulation [64, 65], and then it doubles at intervals of 1.2–2.0 days over the next 8–9 days [65, 66].

hCG is a member of the glycoprotein family, which includes FSH, LH, and TSH [67]. It is composed of two subunits, α and β, that are non-covalently linked [67]. All glycoprotein hormones in this family have the same α subunit, which is a peptide of 92 amino acids that has two sites for N-linked glycosylation [67]. The β subunit is specific to each hormone. It is a peptide of 145 amino acids in length that has two N-linked glycosylation and four O-linked glycosylation sites [67]. The hCG β subunit is encoded by a cluster of six transcriptionally active genes evolved from the LH β subunit gene [68–71]. DNA sequence studies have demonstrated approximately 96% identity between the hCG β and LH β genes. The remarkable differences between these two molecules are the number of glycosylation sites and patterns [67]. The hCG β subunit oligosaccharides terminate with sialic acid, while the LH β subunit contains sulfate. The sialic acid residues in O-linked glycosylation of the hCG β subunit are involved in the longer half-life of hCG *in vivo* compared with LH [67].

hCG acts through the same receptor (LH/hCG receptor) as LH, and its gene has been identified [72]. The LH/hCG receptor is a member of the subfamily of glycoprotein hormone receptors belonging to the superfamily of G protein-coupled receptors [73, 74]. The pattern of the LH/hCG receptor concentration in the CL is similar to that of progesterone production during the menstrual cycle (a decline in the late stage of the menstrual cycle) [75]. However, during early pregnancy, the down-regulation of the receptor expression is inhibited and both the LH/hCG receptor gene expression and binding activity are maintained [76].

Via binding to the LH/hCG receptor in the CL, hCG maintains acute regulatory protein (StAR), cytochrome P450 cholesterol side-chain cleavage (P450scc), and

3β-hydroxysteroid dehydrogenase (3β-HSD), which are all involved in steroidogenesis [77]. Moreover, hCG increases the expression of the tissue inhibitors of metalloproteinases (TIMPs), which are some of the major products of the CL and regulate the tissue remodeling and activity of metalloproteinases (MMPs) [78].

In addition to the role of rescuing CL from regression, hCG directly or indirectly affects the receptive uterine endometrium. The LH/hCG receptor is also found in human endometrium [79] and myometrium [80]. Development of the endometrial stroma is delayed during the cycle of conception, unlike the case during the non-conception cycle [81–83]. A study on the effect of hCG infusion into the human uterus revealed that IGF binding protein-1 (IGFBP-1) and macrophage colony-stimulation factor (M-CSF) are down-regulated, while LIF, vascular endothelial growth factor (VEGF), and MMP-9 are up-regulated [84]. This suggests that hCG also influences the production of proteins in the uterus that are involved in the regulation of the uterine environment, such as endometrial differentiation, vascularization, and tissue invasion.

Summary

hCG, which is secreted from the embryo, is a signal for embryo-maternal cross-talk in humans. It acts not only on the ovary but also on the uterus to establish successful pregnancy. The mechanisms underlying the establishment of human pregnancy are illustrated in Fig. 2. hCG is involved in the maintenance of CL in early pregnancy. Anti-hCG antiserum given in early pregnancy decreases progesterone levels in blood and induces abortion. However, it has no effect on later stages of pregnancy [85]. These results suggest that the pregnancy-maintenance mechanisms shift from the ovary to the fetus/placenta (the luteal-placental shift) as pregnancy progresses [86].

Cattle and Sheep

As discussed above, in mice and humans the embryo can implant to the uterine wall immediately after it moves into the uterine cavity then hatches from the zona pellucida. This implies that direct cross-talk between the embryo (fetus) and its mother is established several days after fertilization. However, in cattle and sheep, both of which are ungulate ruminants, the embryo floats in the uterine cavity after it moves into the uterus and hatches. During this period, the embryo can grow to more than several centimeters in length (elongation). Implantation occurs approximately 30 days after fertilization in cattle, 20 days in sheep. The estrous cycles are 21 days in cattle and 16 days in sheep. The level of progesterone that regulates the uterine environment to support embryo development declines at estrus. Therefore, the embryo must avoid first estrus post-fertilization by establishing communication with the mother and by maintaining the CL.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a luteolytic factor secreted from the uterine endometrium. The endometrium of cyclic ewes releases $PGF_{2\alpha}$ in a pulsatile manner between days 15 and 17 of the cycle, and five episodes of $PGF_{2\alpha}$ in 24 h are required for luteolysis [87, 88]. In cattle, $PGF_{2\alpha}$ in uterine venous blood starts to increase on day 14 post-estrus and peaks on days 15–17 [89]. However, in pregnant ewes and cattle, the manner in which PGF is secreted from the uterus is modified [90, 91]. These results suggest that the embryo can regulate $PGF_{2\alpha}$ secretion from the uterus.

In fact, the embryo can produce signal molecules to notify its presence to the mother (maternal recognition of pregnancy) and thus maintain pregnancy. In this section, studies on the maternal recognition of pregnancy in sheep and cattle are reviewed.

Progesterone: its importance for survival of the embryo

Progesterone is a key factor for regulating the uterine environment and establishing uterine receptivity for embryo implantation in many species. In cattle and sheep, progesterone induces the production of uterine secretion, affects the length of the estrous cycle, and alters the uterine environment to support embryo development [92–95]. In cattle, administration of progesterone in the first 4 days of the estrous cycle advances the uterine environment and accelerates the growth of the embryo [92]. In sheep, progesterone treatment in the first several days of the cycle also increases embryo growth [93, 96].

In embryo transfer (ET) in cattle, a technique used by the livestock industry, the progesterone level is positively correlated with the pregnancy rate after ET [97]. The administration of hCG, GnRH analog (Buserelin), or LH, all of which are luteotrophic hormones, induces accessory CL formation and increases the plasma progesterone concentration [97]. These results demonstrate that progesterone is important for the uterine environment supporting embryo development and pregnancy in cattle and sheep.

The period of maternal recognition of pregnancy

The period in which the uterus can accept embryonic signals to inhibit CL regression is limited in cattle and sheep. Moor and Rowson demonstrated that transferring the sheep embryo to a non-pregnant uterus before days 11–12 of the cycle can prolong luteal function. However, removal of the embryo from the uterus of the pregnant ewe before this time does not extend CL lifespan [98, 99].

In cattle, experiments in embryo removal by uterine flushing and intrauterine infusion on days 16–18 of conceptus homogenates or secretions to cyclic heifers demonstrated the critical role of conceptuses on days 16–18 in extending the CL lifespan in cattle [100–102]. These studies suggest that the periods of maternal recognition of pregnancy in sheep and cattle are post-estrus days 11–12 and 16–18, respectively.

Interferon-tau (IFN-τ): an embryonic signal for maternal recognition of pregnancy in cattle and sheep

1) Discovery of IFN-τ

To survive, sheep and cattle embryos have to make their presence known to their mothers and rescue CL during the period of maternal recognition of pregnancy. It was previously believed that the embryo produced a signal to the mother for the establishment of pregnancy. The homogenates of sheep conceptus rescue CL after intrauterine infusion [98, 99]. Intrauterine infusion of conceptus homogenates or secretions to cyclic heifers on days 16–18 post estrus also prolongs the CL lifespan [100–102]. Martal *et al*. suggested that the embryo secretes an antiluteolytic component that, when infused into the uterus, prevents CL regression. Moreover, they showed that this component, called trophoblastin, is thermolabile and inactivated by pronase [103]. Godkin *et al*. purified a protein secreted on days 13–21 by sheep concepti and named it ovine trophoblast protein-1 (oTP-1). oTP-1 is the same component as trophoblastin, described above [104]. In cattle, the same substance was identified using an immunological cross-reaction with antiserum against oTP-1 [105].

The cDNAs of oTP-1 have been cloned and their sequences determined. The sequences are very similar to those of interferon, and so the protein is called interferon-τ (IFN-τ) [106, 107]. The IFN-τ genes of ruminants share approximately 70% homology with IFN-ω. In the amino acid sequences, the homology with IFN-ω is approximately 80% [106, 107]. The IFN-τ genes have a 595-bp open reading frame (ORF) that encodes a 195-bp amino acid protein containing a 23-bp amino acid signal sequence [106, 107].

2) Properties of IFN-τ

The molecular weight of ovine IFN-τ (oIFN-τ) is approximately 18 kDa, and its isoelectric point ranges from 4.7–5.4 [104]. This protein is not glycosylated [108]. Bovine IFN-τ (bIFN-τ) has multiple isoforms and an isoelectric point range of 5.6–6.8. Its molecular weight is either 22 or 24 kDa, depending on its form; this variation is due to differential glycosylation. The 22-kDa form has a single high-mannose oligosaccharide chain, and the 24-kDa form has a single complex-type oligosaccharide chain [109]. These oligosaccharide chains are N-linked and do not have sialic acid ends [109].

Since IFN-τ is structurally similar to type I IFN, this molecule has the biological properties of type I IFN. IFN-τ can slow the proliferation of bovine kidney epithelial cells (antiproliferative activity) [110]. Moreover, IFN-τ protects several cell types that possess the type I receptor from lysis by viruses (antiviral activity) [111, 112]. These effects of IFN-τ are achieved with less cytotoxicity than IFN-α, β, or γ [113].

3) Antiluteolytic mechanisms of IFN-τ

In sheep and cattle, the CL regression is mediated by $PGF_{2\alpha}$ secreted from the uterine endometrium. The release of $PGF_{2\alpha}$ is influenced by steroid hormones such as estrogen and progesterone via binding to their respective endometrial receptors. During the early and mid-luteal phases, estrogen receptor expression is suppressed in the endometrium by the increase of progesterone [114, 115]. Continuous exposure of the uterus to progesterone inhibits the expression of the progesterone receptor and prevents its negative effect on the expression of estrogen receptor [116–118]. Then, the increase of ovarian estrogen secretion facilitates the increase in oxytocin receptor expression [119–121]. Finally, the oxytocin secreted from CL and the posterior pituitary induces the release of $PGF_{2\alpha}$ from the uterine endometrium [122, 123]. In pregnant ewes, the pulsatile release of $PGF_{2\alpha}$ is prevented by the conceptus. The expression levels of estrogen and oxytocin receptor are low or absent in the endometrium [114, 124–126]. These results demonstrate that IFN-τ appears to interrupt the cascade of CL regression. Intrauterine infusion of oIFN-τ in cyclic ewes during the period of maternal recognition of pregnancy decreases endometrial estrogen receptor mRNA and protein expression [127]. IFN-τ can also suppress estrogen receptor mRNA and protein expression in the luminal epithelium of the endometrium in response to estradiol administration [128]. Moreover, the promoter region of

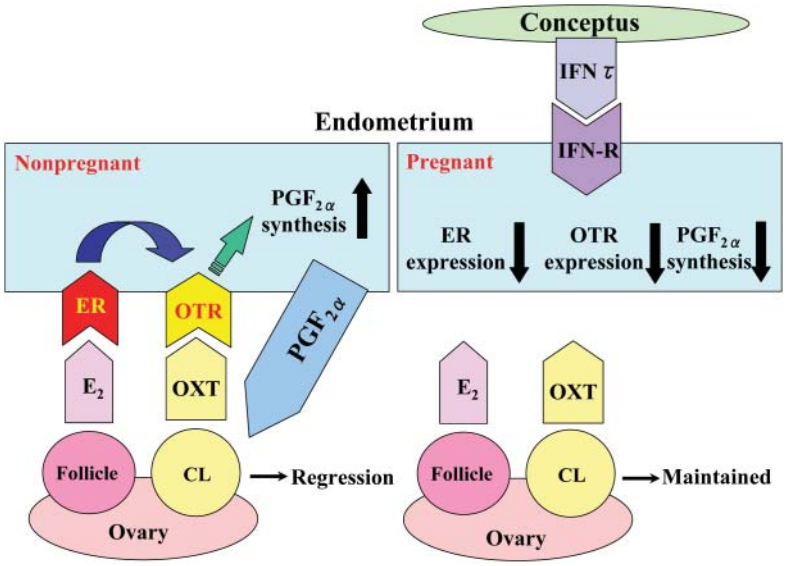

Fig. 3. Diagram of luteolysis and establishment of pregnancy in sheep and cattle. Without pregnancy, estradiol-17β (E_2) from follicles binds to its receptor (ER) on the endometrium and induces the expression of oxytocin receptor (OTR). Oxytocin (OXT) secreted from CL binds to its receptor and in turn the synthesis of $PGF_{2\alpha}$ is stimulated. $PGF_{2\alpha}$ leads to CL regression. In pregnancy, IFN-τ secreted from the conceptus down-regulates the expression of ER and OTR by binding to the type I IFN receptor (IFN-R). Consequently, the synthesis of $PGF_{2\alpha}$ is prevented. The expression of IFN-τ disappears when the conceptus starts to attach to the endometrium.

the estrogen receptor gene has IFN-stimulated response elements (ISREs) and IFN regulatory factor elements (IRFEs) [129]. These observations suggest that IFN-τ inhibits oxytocin receptor up-regulation by preventing a rise in estrogen receptor expression.

In unilaterally pregnant ewes on day 16, the expression of estrogen receptor increases in pregnant horns but not in non-pregnant horns [130]. This result suggests that IFN-τ action is local (not in an endocrine manner). IFN-τ binds to the type I IFN receptor [111, 112, 131, 132]. The genes for the type I IFN receptor have been identified from the cDNA of ovine and bovine endometria [133, 134]. Moreover, the existence of type I IFN receptor protein in the ovine uterus has been demonstrated [135]. These findings suggest that IFN-τ inhibits $PGF_{2\alpha}$ secretion from the uterus via binding type I IFN receptor to the endometrium.

4) Regulation of IFN-τ gene expression

The expression of the IFN-τ gene is very unique. It occurs during a limited period of embryo development before implantation. Furthermore, unlike other members of the type I IFN family, the expression of this gene is not induced by viruses [136]. The expression is regulated by factors secreted from the uterus primed by progesterone. Although IFN-τ expression is induced outside an *in vivo* environment, *in vivo* factors are required for its sustained expression [137]. The addition of granulocyte-macrophage colony-stimulation factor (GM-CSF), which is expressed in the uterus, to culture medium enhances the expression of IFN-τ in the ovine embryo [138, 139]. Colony-stimulating factor 1 also enhances IFN-τ gene expression via Ras-responsive enhancer [140]. These observations suggest that IFN-τ expression is influenced by the uterine environment.

Analysis of the promoter region of the IFN-τ gene has revealed the transcription factors involved in the regulation of IFN-τ gene expression. Ets-2, a transcription factor widely expressed in embryo tissue, strongly enhances the expression of the IFN-τ gene [141]. This effect of Ets-2 is repressed by Oct-4, a POU-domain transcription factor [142]. Since the expression of Oct-4 protein is limited to trophoblast cells

of bovine blastocysts [143], Oct-4 is involved in the unique expression pattern of IFN-τ limited to trophoblastic lineage cells in the embryo. Moreover, the production of IFN-τ is sexually dimorphic (the female blastocyst produces more IFN-τ than that of the male) [144, 145], and it is suggested that the X-chromosomal factor is involved in the occurrence of the sexual dimorphism of IFN-τ production [146].

5) Summary

In ruminants, the secretion of $PGF_{2\alpha}$ from the uterus regulates CL regression. Maternal recognition of pregnancy in these species requires an embryonic antiluteolytic molecule, IFN-τ. Figure 3 illustrates the mechanisms underlying IFN-τ's inhibition of CL regression.

Pig

As with the ruminants described above, pig embryos do not attach to the uterine wall just after hatching. During days 9–14 of pregnancy, the morphology of the pig embryo changes dramatically: from a spherical to an ovoid shape, then to a tubular and finally a thin filamentous form [147, 148]. Implantation is completed on day 20 of pregnancy [149]. During the pregnancy period, the level of progesterone is maintained and the regression of CL is inhibited. In this section, studies of maternal recognition and establishment of pregnancy in the pig are reviewed.

Maternal recognition of pregnancy

The estrus interval of the pig is 21 days, and the cycle is regulated by the presence of CL. The destruction of the uterine endometrial epithelium or hysterectomy before day 14 of the estrous cycle extends the CL lifespan beyond 30 days [150]. It has been suggested that the source of luteolysin is the uterine endometrium. In ruminants, the endometrium is a source of $PGF_{2\alpha}$, a luteolysin [87, 88]. When $PGF_{2\alpha}$ was administered to cyclic, hysterectomized, or pregnant gilts, CL regression was induced [151–153]. The concentration of $PGF_{2\alpha}$ in utero-ovarian vein plasma is elevated on days 12–13 of the estrous cycle, and it is related to both CL regression and the decline in progesterone level [154]. These observations demonstrate that $PGF_{2\alpha}$ secreted from the uterine endometrium is also a luteolysin in the pig.

Since the implantation, which accomplishes direct communication between embryo and uterus, is not completed during the early stage of pregnancy [149], the pig embryo has to produce substances that inhibit CL regression and start cross-talk with its mother (maternal recognition of pregnancy). The period of maternal recognition of pregnancy in the pig is between days 11 and 18 of pregnancy, according to embryo removal experiments [155–157].

Estrogen is a signal for maternal recognition of pregnancy in the pig

In ruminants, the signal molecule for maternal recognition of pregnancy is IFN-τ, and this protein is secreted from trophoblast cells in the embryo [106, 107]. However, when total secretory protein from the pig conceptus was infused into the uterine lumen of cyclic sows during the period of maternal recognition of pregnancy, the estrous cycle was not prolonged and CL regression was not inhibited [158]. The pig embryo does not secrete an anti-luteolytic protein such as IFN-τ, which in maternal recognition of pregnancy in ruminants.

On the other hand, it is known that the pig embryo on days 11–18 of pregnancy produces and releases estrogen [159]. Pig embryos can convert progesterone to estradiol-17β when they develop to the spherical stage [160], and its production increases as the embryos develop into the tubular and filamentous forms [161]. Estrogens are localized in the trophectoderm and endoderm of the conceptus on day 12 of pregnancy [161]. When estrogen is administered into the blood stream or uterus, the lifespan of CL is prolonged and CL regression is inhibited [162–167]. These observations support the hypothesis that estrogen is an embryonic signal for maternal recognition of pregnancy in pig.

The production of estrogen in the pig embryo is biphasic. When the uterine content of estrogen is measured, it increases rapidly on days 11–12 of pregnancy, declines on days 13–14, and then increases again [147, 168, 169]. The plasma estrone sulphate concentration of pregnant gilts also demonstrates a biphasic pattern [170, 171]. A single administration of exogenous estrogen between days 9.5 and 15.5, or on days 11 and 14 of the estrus cycle, failed to extend the inter-estrus interval beyond 30 days. However, estrogen treatment on days 11 and 14–16 can prolong the extension of CL function beyond 60 days [166]. These results indicate that the complete establishment of pregnancy in the pig requires both phases of estrogen stimulation.

Effect of estrogen on endometrium in maternal recognition of pregnancy

In non-pregnant gilts, $PGF_{2\alpha}$ is secreted from the

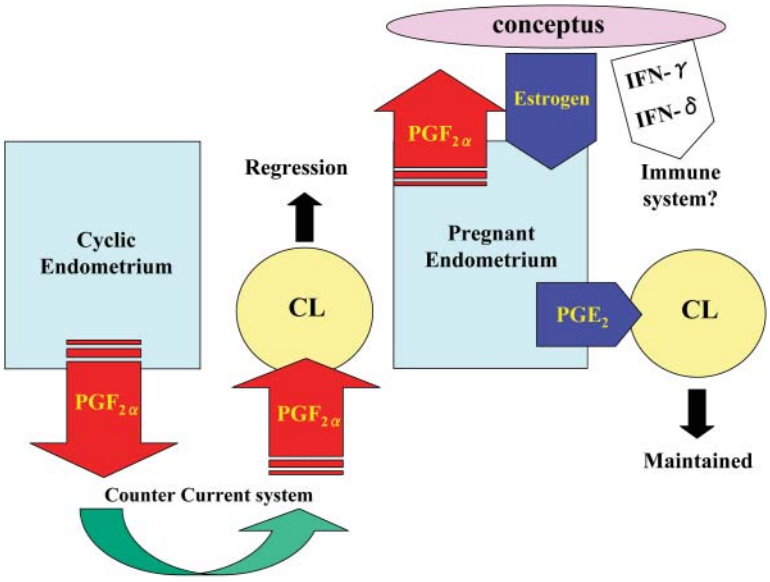

Fig. 4. Diagram of luteolysis and establishment of pregnancy in the pig. As in the other species, $PGF_{2\alpha}$ is a luteolytic signal that induces the regression of CL. In pregnancy, the conceptus produces estrogen as a factor for maternal recognition of pregnancy. Estrogen alters the direction of $PGF_{2\alpha}$ secretion from towards the uterine venous system (endocrine) to towards the uterine lumen (exocrine). Additionally, the conceptus produces PGE_2, which has luteotrophic activity, and IFN-γ and δ, which may be involved in immunoregulation of the uterus.

uterine endometrium toward the endometrial stroma (in the endocrine direction) during the period of CL regression. $PGF_{2\alpha}$ is secreted into the uterine venous system and transported into the ovarian artery by a counter-current exchange effect. Finally, $PGF_{2\alpha}$ reaches the CL and induces its regression [172, 173]. The concentration of utero-ovarian vein $PGF_{2\alpha}$ is significantly elevated between days 13 and 17 of the estrous cycle, when plasma progesterone concentrations decline [154]. However, in pregnant gilts, there is no change in the concentration of $PGF_{2\alpha}$ in the utero-ovarian vein between days 12 and 25 of pregnancy [154]. In contrast, uterine flushings of pseudopregnant and pregnant gilts have significantly higher amounts of $PGF_{2\alpha}$ than do those from cyclic gilts [168]. Based on these observations, Bazer and Thatcher hypothesized that estrogen secreted from the embryo is a key factor for the maternal recognition of pregnancy in pig, and that the secretion of $PGF_{2\alpha}$ towards the uterine lumen (exocrine) rather than towards the uterine veins (endocrine) is involved in the establishment of pregnancy in the pig [174].

When estrogen was administered to gilts on day 14–20 of the estrous cycle, the luminal content of $PGF_{2\alpha}$ was greater in estrogen-treated gilts than in control gilts [163]. Moreover, an *in vitro* experiment showed that the secretion of $PGF_{2\alpha}$ and PGE_2 is greater from the myometrial side (endocrine) for day 10 in pregnant and day 14 in cyclic gilts, whereas the secretion shifts toward the luminal side (exocrine) for day 12 and day 14 in pregnant gilts and for day 14 in estrogen-treated gilts [175]. These observations strongly suggest that estrogen from the pig embryo induces the transition from endocrine to exocrine secretion of $PGF_{2\alpha}$.

The mechanism by which estrogen shifts the uterine secretion of $PGF_{2\alpha}$ toward the uterine lumen during pregnancy has not been elucidated completely. Estrogens, whether secreted from the embryo or injected, induce a transient release of calcium into the uterine lumen. When the endometrium from day 14 cyclic gilts is treated with calcium ionophore A23187, estradiol and prolactin change the secretion of $PGF_{2\alpha}$ from an endocrine to an exocrine direction. This observation suggests that the reorientation of $PGF_{2\alpha}$ secretion of endometrium in pigs during the establishment of pregnancy involves interactive effects of estrogens and prolactin through increased calcium cycling across the uterine epithelium [176].

Estrogen has various effects on uterine endometrium. Estrogen also increases the PGE:PGF ratio [177–179]. PGE_2 has a luteotrophic effect and is involved in the maintenance of progesterone secretion from the CL [178]. Furthermore, estrogen maintains high LH receptor levels in both the uterus and CL [180, 181].

Interferons secreted from the pig embryo

The infusion of pig conceptus secretory proteins into the uterus extends the CL lifespan [158]. This suggests that the pig embryo does not produce a protein, such as IFN-τ in ruminants, that inhibits CL regression. However, a significant antiviral activity is found in uterine flushings and in embryo-conditioned culture medium on days 11–17 of pregnancy in pig [182]. IFN secretion by the pig embryo begins on days 12–13 of pregnancy and reaches a maximum on days 15–16 [183]. Two types of IFNs have been identified. One is type II IFN, IFN-γ, and the other is a novel type I IFN, IFN-δ, previously known as sp-1 IFN [184–186]. IFN-γ is secreted by the pig embryo in substantial amounts. The roles of these IFNs secreted from the pig embryo in pregnancy have not been revealed. They may be involved in the immunoregulation of the uterus during implantation.

Summary

In the pig, estrogen secreted from the embryo plays important roles in maternal recognition of pregnancy. The concept of the mechanism is illustrated in Fig. 4. However, the complete mechanisms underlying estrogen's induction of the shift in $PGF_{2\alpha}$ secretion from endocrine to exocrine, or the mechanisms by which the maintenance of CL is supported, are not fully understood.

Conclusions

This article has review the mechanisms by which pregnancy is established in mouse, human, sheep, cattle, and pig, and shows that these mechanisms vary widely among these species. Recently, the development of assisted reproductive technologies has contributed a great deal to medical science and livestock industries. Successful pregnancy using these techniques requires the control of the maternal environment and embryo development. However, since the mechanisms of pregnancy have not been fully elucidated, further studies are needed.

References

1) Armstrong, D.T., Knudsen, K.A. and Miller, L.S. (1970): Effects of prolactin upon cholesterol metabolism and progesterone biosynthesis in corpora lutea of rats hypophysectomized during pseudopregnancy. Endocrinology, 86, 634–641.
2) Gunnet, J.W. and Freeman, M.E. (1984): Hypothalamic regulation of mating-induced prolactin release. Effect of electrical stimulation of the medial preoptic area in conscious female rats. Neuroendocrinology, 38, 12–16.
3) Kelly, P.A., Tsushima, T., Shiu, R.P.C. and Friesen, H.G. (1976): Lactogenic and growth hormone-like activities in pregnancy determined by radioreceptor assays. Endocrinology, 99, 765–764.
4) Linzer, D.I.H. (1998): Placental lactogen. In: Encyclopedia of Reproduction (Knobil, E. and Neil, J.B., eds.), vol. 3, pp. 874–888, Academic Press, London.
5) Harigaya, T., Smith, W.C. and Talamantes, F. (1988): Hepatic placental lactogen receptors during pregnancy in the mouse. Endocrinology, 122, 1366–1372.
6) Macleod, K.R., Smith, W.C., Ogren, L. and Talamantes, F. (1989): Recombinant mouse placental lactogen-I binds to lactogen receptors in mouse liver and ovary: partial characterization of the ovarian receptor. Endocrinology, 125, 2258–2266.
7) Ormandy, C.J., Camus, A., Barra, J., Damotte, D., Lucas, B.K., Buteau, H., Edery, M., Brousse, N., Bavinet, C., Binart, N. and Kelly, P.A. (1997): Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. Genes Dev., 11, 167–178.
8) Gunnet, J.W. and Freeman, M.E. (1983): The mating-induced release of prolactin: a unique neuroendocrine response. Endocr. Rev., 4, 44–61.
9) Bole-Feysot, C., Goffin, V., Edery, M., Binart, N. and Kelly, P.A. (1998): Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr. Rev., 19, 225–268.
10) Psychoyos, A. (1973): Hormonal control of ovoimplantation. Vitam. Horm., 31, 201–256.
11) Paria, B.C., Huet-Hudson, Y.M. and Dey, S.K. (1993): Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. Proc. Natl. Acad. Sci. USA., 90, 10159–10162.
12) Huet-Hudson, Y.M., Andrews, G.K. and Dey, S.K. (1989): Cell type-specific localization of c-myc protein in the mouse uterus: modulation by steroid hormones and analysis of the periimplantation period. Endocrinology, 125, 1683–1690.
13) Huet-Hudson, Y.M. and Dey, S.K. (1990): Requirement for progesterone priming and its long-term effects on implantation in the mouse. Proc. Soc. Exp. Biol. Med., 193, 259–263.
14) Pollard, J.W. (1990): Regulation of polypeptide growth factor synthesis and growth factor-related gene expression in the rat and mouse uterus before and after implantation. J.

Reprod. Fertil., 88, 721–731.
15) Paria, B.C., Reese, J., Das, S.K. and Dey, S.K. (2002): Deciphering the cross-talk of implantation: advances and challenges. Science, 296, 2185–2188.
16) McLaren, A. (1968): A study of blastocysts during delay and subsequent implantation in lactating mice. J. Endocrinol., 42, 453–463.
17) Yoshinaga, K. and Adams, C.E. (1966): Delayed implantation in the spayed, progesterone treated adult mouse. J. Reprod. Fertil., 12, 593–595.
18) Ma, W.G., Song, H., Das, S.K., Paria, B.C. and Dey, S.K. (2003): Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. Proc. Natl. Acad. Sci. USA., 100, 2963–2968.
19) Hilton, D.J. (1992): LIF: lots of interesting functions. Trends Biochem. Sci., 17, 72–76.
20) Metcalf, D. (1992): Leukemia inhibitory factor — a puzzling polyfunctional regulator. Growth Factors, 7, 169–173.
21) Williams, R.L., Hilton, D.J., Pease, S., Willson, T.A., Stewart, C.L., Gearing, D.P., Wagner, E.F., Metcalf, D., Nicola, N.A. and Gough, N.M. (1988): Myeloid leukemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature, 336, 684–687.
22) Nichols, J., Chambers, I., Taga, T. and Smith, A. (2001): Physiological rationale for responsiveness of mouse embryonic stem cells to gp130 cytokines. Development, 128, 2333–2339.
23) Bhatt, H., Brunet, L.J. and Stewart, C.L. (1991): Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. Proc. Natl. Acad. Sci. USA., 88, 11408–11412.
24) Shen, M.M. and Leder, P. (1992): Leukemia inhibitory factor is expressed by the preimplantation uterus and selectively blocks primitive ectoderm formation *in vitro*. Proc. Natl. Acad. Sci. USA., 89, 8240–8244.
25) Chen, J.R., Cheng, J.G., Shatzer, T., Sewell, L., Hernandez, L. and Stewart, C.L. (2000): Leukemia inhibitory factor can substitute for nidatory estrogen and is essential to inducing a receptive uterus for implantation but is not essential for subsequent embryogenesis. Endocrinology, 141, 4365–4372.
26) Stewart, C.L., Kaspar, O., Brunet, L.J., Bhatt, H., Gadi, I., Kontgen, F. and Abbondanzo, S.J. (1992): Blastocst implantation depends on maternal expression of leukemia inhibitory factor. Nature, 359, 76–69.
27) Fry, R.C. (1992): The effect of leukemia inhibitory factor (LIF) on embryogenesis. Reprod. Fertil. Dev., 4, 449–458.
28) Nichos, J., Davidson, D., Taga, T., Yoshida, K., Chambers, I. and Smith, A. (1996): Complementary tissue-specific expression of LIF and LIF-receptor mRNAs in early mouse embryogenesis. Mech. Dev., 57, 123–131.
29) Ware, C.B., Horowitz, M.C., Tenshaw, B.R., Hunt, J.S., Liggitt, D., Koblar, S.A., Gliniak, B.C., McKenna, H.J., Papayannopoulou, T. and Thoma, B. (1995): Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. Development, 121, 1283–1299.
30) Yoshida, K., Taga, T., Saito, M., Suematsu, S., Kumanogoh, A., Tanaka, T., Fujiwara, H., Hirata, M., Yamagami, T., Nakahata, T., Hirabayashi, T., Yoneda, Y., Tanaka, K., Wang, W.Z., Mori, C., Shiota, K., Yoshinaga, N. and Kishimoto, T. (1996): Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. Proc. Natl. Acad. Sci. USA., 93, 407–411.
31) Hertig, A. and Rock, J.A. (1956): A description of 34 human ova within the first 17 days of development. Am. J. Anat., 98, 435–494.
32) Martel, D., Monier, M.N., Roiche, D. and Psychoyos, A. (1991): Hormonal dependence of pinopode formation at the uterine luminal surface. Human Reprod., 6, 597–603.
33) Nikas, G. (1999): Cell surface morphological events relevant to human implantation. Human Reprod., 14 (Suppl. 2), 37–44.
34) Nikas, G., Makrigiannakis, A., Hovatta, O. and Jones, H.W.Jr. (2000): Surface morphology of the human endometrium: basic and clinical aspects. Ann. N. Y. Acad. Sci., 900, 316–324.
35) Wilcox, A.J., Baird, D.D. and Weinberg, C.R. (1999): Time of implantation of the conceptus and loss of pregnancy. New England J. Med., 340, 1796–1799.
36) Psychoyos, A. (1993): The implantation window: basic and clinical aspects. Ares Serono Symposia, 4, 57–62.
37) Formigli, L., Formigli, G. and Roccio, C. (1987): Donation of fertilized uterine ova to infertile women. Fertil. Steril., 47, 162–165.
38) Meyer, R.K., Wolf, R.C. and Arslan, M. (1978): Implantation and maintenance of pregnancy in progesterone-treated ovariectomized monkeys (*Macaca mulatta*). In: Recent Advances in Primatology (Chivers, D.J. and Lane-Petter, W., eds.), vol. 2, pp. 30–35, Academic Press, London.
39) Bosu, W.T.K. and Johansson, E.D.B. (1975): Implantation and maintenance of pregnancy in mated rhesus monkeys following bilateral oophorectomy or luteectomy with and without progesterone replacement. Acta Endorinologica, 79, 598–609.
40) Sankaran, M.S., Prahalada, S. and Hendrickx, A. (1984): Effects of zuclomihene citrate on the nonfertile and fertile menstrual cycles in the rhesus monkey (*Macaca mulatta*). An experimental approach to the understanding of the role of periovulatory estrogens in implantation in primates. Am. J. Primatology, 7, 309.
41) Ravindranath, N. and Moudgal, N.R. (1987): Use of tamoxifen, an antiestrogen, in establishing a need for oestrogen in early pregnancy in the bonnet monkey (*Macaca radiata*). J. Reprod. Fert., 81, 327–336.
42) Ravindranath, N. and Moudgal, N.R. (1990): Effect of a specific estrogen antibody on pregnancy establishment in the bonnet monkey (*Macaca radiata*). Fertil. Steril., 54, 1162–1167.
43) Yen, S.S.C. (1986): The human menstrual cycle. In: Reproductive Endocrinology. Physiology, Pathophysiology

and Clinical Management, 2nd ed., pp. 200–236, WB Saunders, Philadelphia.
44) Lenton, E.A., Sulaiman, R., Sobowale, O. and Cooke, I.D. (1982): The human menstrual cycle: plasma concentrations of prolactin, LH, FSH, oestradiol and progesterone in conceiving and non-conceiving women. J. Reprod. Fert., 65, 131–139.
45) Martel, D., Frydman, R., Glissant, M., Maggioni, C., Roche, D. and Psychoyos, A. (1987): Scanning electron microscopy of postovulatory human endometrium in spontaneous cycles and cycles stimulated by hormone treatment. J. Endocrinol., 114, 319–324.
46) Nikas, G., Develioglu, O.H., Toner, J.P. and Jones, H.W. Jr. (1999): Endometrial pinopodes indicate a shift in the window of receptivity in IVF cycles. Human Reprod., 14, 787–792.
47) Develioglu, O.H., Hsiu, J.G., Nikas, G., Toner, J.P., Oehninger, S. and Jones H.W. Jr. (1999): Endometrial estrogen and progesterone receptor and pinopode expression in stimulated cycles in oocyte donation. Fertil. Steril., 71, 1040–1047.
48) Navot, D., Scott, R.T., Droesch, K., Veeck, L.L., Liu, H.C. and Rosenwaks, Z. (1991): The window of embryo transfer and the efficiency of human conception *in vitro*. Fertil. Steril., 55, 114–118.
49) Kao, L.C., Tulac, S., Lobo, S., Imani, B., Yang, J.P., Germeyer, A., Osteen, K., Taylor, R.N., Lessey, B.A. and Giudice, L.C. (2002): Global gene profiling in human endometrium during the window of implantation. Endocrinology, 143, 2119–2138.
50) Borthwick, J.M., Charnock-Jones, D.S., Tom, B.D., Hull, M.L., Teirney, R., Phillips, S.C. and Smith, S.K. (2003): Determination of the transcript profile of human endometrium. Mol. Hum. Reprod., 9, 19–33.
51) Carson, D.D., Lagow, E., Thathiah, A., Al-Shami, R., Farach-Carson, M.C., Vernon, M., Yuan, L., Fritz, M.A. and 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. Mol. Hum. Reprod., 8, 871–879.
52) Riesewijk, A., Martin, J., van Os, R., Horcajadas, J.A., Polman, J., Pellicer, A., Mosselman, S. and Simon, C. (2003): Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. Mol. Hum. Reprod., 9, 253–264.
53) Vaisse, C., Atger, M., Potier, B. and Milgrom, E. (1990): Human placental protein 14 gene: sequence and characterization of a short duplication. DNA Cell Biol., 9, 401–413.
54) Higuchi, T., Kanzaki, H., Nakayama, H., Fujimoto, M., Hatayama, H., Kojima, K., Iwai, M., Mori, T. and Fujita, J. (1995): Induction of tissue inhibitor of metalloproteinase 3 gene expression during *in vitro* decidualization of human endometrial stromal cells. Endocrinology, 136, 4973–4981
55) Bradbury, J.T., Brown, W.E. and Gray, L.A. (1950): Maintenance of the corpus luteum and physiological actions of progesterone. Recent Prog. Horm. Res., 5, 151–194.
56) Segaloff, A., Sternberg, W.H. and Gaskill, C.J. (1951): Effects of luteotropic doses of chorionic gonadotropin in women. J. Clin. Endocrinol. Metab., 11, 936–944.
57) Stevens, V.C. (1975): Fertility control through active immunization using placenta proteins. Acta Endocrinol. (Copenh.), 194 (Suppl.), 357–375.
58) Bentin-Ley, U., Horn, T., Sjogren, A., Sorensen, S., Falck Larsen, J. and Hamberger, L. (2000): Ultrastructure of human blastocyst-endometrial interactions *in vitro*. J. Reprod. Fertil., 120, 337–350.
59) Lopata, A. and Hay, D.L. (1989): The surplus human embryo: its potential for growth, blastulation, hatching, and human chorionic gonadotropin production in culture. Fertil. Steril., 51, 984–991.
60) Dorkas, A., Sargent, I.L., Gardner, R.L. and Barlow, D.H. (1991): Human trophectoderm biopsy and secretion of chorionic gonadotropin. Hum. Reprod., 6, 1453–1459.
61) Dimitriadou, F., Phocas, I., Mantzavinos, T., Sarandakou, A., Rizos, D. and Zourlas, P.A. (1992): Discordant secretion of pregnancy specific beta-1-glycoprotein and human chorionic gonadotropin by human pre-embryo cultured *in vitro*. Fertil. Steril., 57, 631–636.
62) Lopata, A. and Oliva, K. (1993): Chorionic gonadotrophin secretion by human blastocysts. Hum. Reprod., 8, 932–938.
63) Bonduelle, M.L., Dodd, R., Liebaers, I., Van Steirteghem, A., Williamson, R. and Akhurst, R. (1988): Chorionic gonadotrophin-beta mRNA, a trophoblast marker, is expressed in human 8-cell embryos derived from tripronucleate zygotes. Hum. Reprod., 3, 909–914.
64) Vaitukaitis, J.L., Braunstein, G.D. and Ross, G.T. (1972): A radioimmunoassay which specially measures human chorionic gonadotropin in the presence of human luteinizing hormone. Am. J. Obstet. Gynecol., 113, 751–758.
65) Braunstein, G.D., Grodin, J.M., Vaitukaitis, J.L. and Ross, G.T. (1973): Secretory rates of human chorionic gonadotrophin by normal trophoblast. Am. J. Ostet. Gynecol., 115, 447–450.
66) Marshall, J.R., Hammond, C.B., Ross, G.T., Jacobson, A., Rayford, P. and Odell, W.D. (1968): Plasma and urinary chorionic gonadotropin in human gestation. Am. J. Obstet. Gynecol., 37, 760–764.
67) Pierce, J. and Parsons, T.F. (1981): Glycoprotein hormones: structure and function. Annual Rev. Biochem., 50, 465–469.
68) Boorstein, W., Vanvakopoulos, N. and Fiddes, J.C. (1982): Human chorionic gonadotropin β subunit is encoded by at least eight genes arranged in tandem and inverted pairs. Nature, 300, 419–422.
69) Policastro, P., Ovitt, C., Hoshina, M., Fukuoka, H., Boothby, M. and Boime, I. (1983): The β subunit of human chorionic gonadotropin is encoded by multiple genes. J. Biol. Chem., 258, 11492–11499.
70) Fiddes, J.C. and Talmadge, K. (1984): Structure, expression, and evolution of the genes for the human glycoprotein hormones. Recent Prog. Hormone Res., 40, 43–78.
71) Bo, M. and Moime, I. (1992): Identification of the transcriptionally active genes of the human chorionic

gonadotropin β gene cluster *in vivo*. J. Biol. Chem., 267, 3179–3184.
72) Minegishi, T., Nakamura, K., Takakura, Y., Miyamoto, K., Hasegawa, Y., Ibuki, Y., Igarashi, M. and Minegishi, T. (1990): Cloning and sequencing of human LH/hCG receptor cDNA. Biochem. Biophys. Res. Commun., 172, 1049–1054.
73) McFarand, K.C., Sprengel, R., Phillips, H.S., Kohler, M., Rosemblit, N., Nikolics, K., Segaloff, D.L. and Seeburg, P.H. (1989): Lutoropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. Science, 245, 494–499.
74) Ascoli, M., Fanelli, F. and Segaloff D.L. (2002): The lutoropin-choriogonadotropin receptor: a 2002 perspective. Endocr. Rev., 23, 141–174.
75) Yeko, T.R., Khan-Dawood, F.S. and Dawood, M.Y. (1989): Human corpus luteum: human chorionic gonadotropin receptors during the menstrual cycle. J. Clini. Endocrinol. Metab., 68, 529–534.
76) Duncan, W.C., McNeilly, A.S., Fraser, H.M. and Illingworth, P.J. (1996): Luteinizing hormone receptor in the human corpus luteum: lack of down regulation during maternal recognition of pregnancy. Hum. Reprod., 11, 2291–2297.
77) Duncan, W.C., Cowen, G.M. and Illingworth, P.J. (1999): Steroidogenic enzyme expression in human corpola lutea in the presence and absence of exogenous human chorionic gonadotropin (HCG). Mol. Hum. Reprod., 5, 291–298.
78) Duncan, W.C., McNeilly, A.S. and Illingworth, P.J. (1996): Expression of tissue inhibitor of metalloproteinases 1 in the human corpus luteum after luteal rescue. J. Endocr., 148, 59–67.
79) Reshef, E., Lei, Z.M., Rao, C.V., Pridham, D.D., Chegini, N. and Luborsky, J.L. (1990): The presence of gonadotropin receptors in non-pregnant human uterus, human placenta, fetal membrane, and deciduas. J. Clin. Endocrinol. Metab., 70, 421–429.
80) Környei, J., Lei, Z.M. and Rao, C.V. (1993): Human myometrial smooth muscle cells are novel targets of direct regulation by human chorionic gonadotropin. Biol. Reprod., 49, 1149–1157.
81) Hertig, A.T. (1964): Gestational hyperplasia of endometrium, a morphologic correlation of ova, endometrium, and corpora lutea during early pregnancy. Lab. Invest., 13, 1153–1191.
82) Karow, W.G., Gentry, W.C., Skeels, R.F. and Payne, S.A. (1971): Endometrial biopsy in the luteal phase of the cycle of conception. Fertil. Steril., 22, 482–495.
83) Wentz, A.C., Herbert, C.M. 3rd., Maxson, W.S., Hill, G.A. and Pittaway, D.E. (1986): Cycle of conception endometrial biopsy. Fertil. Steril., 46, 196–199.
84) Licht, P., Russu, V. and Mildt, L. (2001): On the role of human chorionic gonadotropin (hCG) in the embryo-endometrial microenvironment: implications for differentiation and implantation. Semn. Reprod. Med., 19, 37–47.
85) Hearn, J.P. (1976): Immunization against pregnancy. Proc. R. Soc. London B. Biol. Sci., 195, 149–160.
86) Souffer, R.L. (1998): Corpus luteum of pregnancy. In: Encyclopedia of Reproduction (Knobil, E. and Neil, J.B., eds.), vol. 1, pp. 709–717, Academic Press, London.
87) Zardo, L., Stabenfeld, G.H., Quirke, J.F., Kindahl, H. and Bradford, G.E. (1988): Release of prostaglandin F-2α and the timing of events associated with luteolysis in ewes with oestrous cycles of different lengths. J. Reprod. Fertil., 83, 517–526.
88) Zarco, L., Stabenfeld, G.H., Basu, S., Bradford, G.E. and Kindahl, H. (1988): Modification of prostaglandin F-2α synthesis and release in the ewe during the initial establishment of pregnancy. J. Reprod. Fertil., 83, 527–536.
89) Shemesh, M. and Hansel, W. (1975): Levels of prostaglandin F (PGF) in the bovine endometrium, uterine venous, ovarian artery and jugular plasma during the oestrous cycle. Proc. Soc. Exp. Biol. Med., 148, 123–126.
90) Hooper, S.B., Watkins, W.B. and Thorburn, G.D. (1986): Oxytocin, oxytocin-associated neurophysin, and prostaglandin $PGF_{2\alpha}$ concentrations in the utero-ovarian vein of pregnant and nonpregnant sheep. Endocrinol., 119, 2590–2597.
91) Wolfenson, D., Thatcher, W.W., Drost, M., Caton, D., Foster, D.B. and Leblanc, M.M. (1985): Characteristics of prostaglandin F measurements in the ovarian circulation during the oestrous cycle and early pregnancy in the cow. J. Reprod. Fertil., 75, 491–499.
92) Garrett, J.E., Geisert, R.D., Zavy, M.T. and Morgan, G.L. (1988): Evidence for maternal regulation of early conceptus growth and development in beef cattle. J. Reprod. Fertil., 84, 437–446.
93) Geisert, R.D., Lee, C.Y., Simmen, F.A., Zavy, M.T., Fliss, A.E., Bazer, F.W. and Simmen, C.M. (1991): Expression of messenger RNAs encoding insulin-like growth factor-I, II, and insulin-like growth factor binding protein-2 in bovine endometrium during the estrous cycle and early pregnancy. Biol. Reprod., 45, 975–983.
94) Lawson, R.A.S. and Cahill, L.P. (1983): Modification of the embryo-maternal relationship in ewes by progesterone treatment early in the oestrus cycle. J. Reprod. Fertil., 67, 473–475.
95) Moore, F.W. (1975): Progesterone requirements for the maintenance of pregnancy in the ovariectomized ewe. J.Reprod. Fertil., 43, 386–387.
96) Kleemann, D.O., Walker, S.K. and Seamark, R.F. (1994): Enhanced fetal growth in sheep administered progesterone during the first three day of pregnancy. J. Reprod. Fertil., 102, 411–417.
97) Binelli, M., Thatcher, W.W., Mattos, R. and Baruselli, P.S. (2001): Antiluteolytic strategies to improve fertility in cattle. Theriogenology, 56, 1451–1463.
98) Moor, R.M. and Rowson, L.E.A. (1966): The corpus luteum of the sheep: effect of the removal of embryos on luteal function. J. Endocrinol., 34, 497–502.
99) Moor, R.M. and Rowson, L.E.A. (1966): The corpus luteum of the sheep: functional relationship between the embryo and the corpus luteum. J. Endocrinol., 34, 233–239.

100) Northey, D.L. and French, L.R. (1980): Effect of embryo removal and intrauterine infusion of embryonic homogenates on the lifespan of the bovine corpus luteum. J. Anim. Sci., 50, 298–302.
101) Humblot, P. and Dalla Porta, M.A. (1984): Effect of conceptus removal and intrauterine administration of conceptus tissue on luteal function in the cow. Reprod. Nutr. Dev., 24, 529–541.
102) Knickerbocker, J.J., Thatcher, W.W., Bazer, F.W., Drost, M., Barron, D.H., Fincher, K.B. and Roberts, R.M. (1986): Proteins secreted by Day-16 to -18 bovine conceptuses extend corpus luteum function in cows. J. Reprod. Fertil., 77, 381–391.
103) Martal, J., Lacroix, M. C., Saunier, M. and Wintenberger-Torrès, S. (1979): Trophobalstin, an antiluteolytic protein present in early pregnancy in sheep. J. Reprod. Fertil., 56, 63–73.
104) Godkin, J.D., Bazer, F.W., Moffat, R.J., Sessions, F. and Roberts, R.M. (1982): Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts at Day 13–21. J. Reprod. Fertil., 65, 141–150.
105) Helmer, S.D., Hansen, P.J., Anthony, R.V., Thatcher, W.W., Bazer, F.W. and Roberts, R.M. (1987): Identification of bovine trophoblast protein-1, a secretory protein immunologically related to ovine trophoblast protein-1. J. Reprod. Fertil., 79, 83–91.
106) Imakawa, K., Anthony, R.V., Kazemi, M., Marotti, K.R., Polites, H.G. and Roberts, R.M. (1987): Interferon-like sequence of bovine trophoblast protein secreted by embryonic trophectoderm. Nature, 330, 337–379.
107) Imakawa, K., Hansen, T.R., Malathy, P.V., Anthony, R.V., Polites, H.G., Marotti, K.R. and Roberts, R.M. (1989): Molecular cloning and characterization of complementary deoxyribonucleic acids corresponding to boine trophoblast protein-1: a comparison with ovine trophoblast protein-1 and bovine interferon αII. Mol. Endocrinol., 3, 127–139.
108) Anthony, R.V., Helmer, S.D., Sharif, S.F., Roberts, R.M., Hansen, P.J., Thatcher, W.W. and Bazer, F.W. (1988): Synthesis and processing of ovine trophoblast protein-1 and bovine trophoblast protein-1, conceptus secretory proteins involved in the maternal recognition of pregnancy. Endocrinol., 58, 1274–1280.
109) Helmer, S.D., Hansen, P.J. and Thatcher, W.W. (1988): Differential glycosylation of the components of the bovine trophoblast protein-1 complex. Mol. Cell. Endocrinol., 58, 103–107.
110) Roberts, R.M., Imakawa, K., Niwano, Y., Kazemi, M, Malathy, P.V., Hnasen, T.R., Glass, A.A. and Kronenbery, L.H. (1989): Interferon production by preimplantation sheep embryo. J. Interferon Res., 9, 175–187.
111) Klemann, S.W., Li, J., Imakawa, K., Cross, J.C., Francis, H. and Roberts, R.M. (1990): The production, purification and bioactivity of bovine trophoblast protein-1 (bovine trophoblast interferon). Mol. Endocrinol., 4, 1506–1514.
112) Pontzer, C.H., Torres, B.A., Vallet, J.L., Bazer, F.W. and Johnson, H.M. (1988): Antiviral activity of the pregnancy recognition hormone ovine trophoblast protein-1. Biochem. Biopys. Res. Commun., 152, 801–807.
113) Soos, J.M. and Johnson, H.M. (1999): Interferon-tau-prospects for clinical use in autoimmune. Biodrugs, 11, 125–135.
114) Wathes, D.C. and Hamon, M. (1993): Localisation of oestradiol, progesterone and oxytocin receptors in the uterus during the oestrous cycle and early pregnancy of the ewe. J. Endocrinol., 138, 479–491.
115) Spencer, T.E. and Bazer, F.W. (1995): Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy. Biol. Reprod., 53, 1527–1543.
116) McCraken, J.A., Schramm, W. and Okulicz, W.C. (1984): Hormone receptor control of pulsatile secretion of PGF-2 alpha from the ovine uterus during luteolysis and its abrogation in early pregnancy. Animal Reprod. Sci., 7, 31–55.
117) Vallet, J.L., Lamming, G.E. and Batten, M. (1990): Control of the endometrial oxytocin receptors and uterine response to oxytocin by progesterone and oestradiol in the ewe. J. Reprod. Fertil., 90, 625–634.
118) Wathes, D.C., Mann, G.E., Payne, J.H., Riley, P.R., Stevenson, K.R. and Lamming, G.E. (1996): The regulation of oxytocin, oestradiol and progesterone receptor in different uterine regions by oestradiol, progesterone and oxytocin in ovariectomized ewe. J. Endocrinol., 115, 375–393.
119) Soloff, M.S. (1975): Uterine receptor for oxytocin: effects of estrogen. Biochem. Biophys. Res. Commun., 65, 205–212.
120) Hixon, J.E. and Flint, A.P.F. (1987): Effects of luteolytic dose of oestradiol benzoate on uterine oxytocin receptor concentrations, phosphoinositide turnover and prostaglandin F-2α secretion in sheep. J. Reprod. Fertil., 78, 457–467.
121) Beard, A.P. and Lamming, G.E. (1994): Oestradiol concentration and the development of the uterine oxytocin receptor and oxytocin-induced $PGF_{2\alpha}$ release in ewes. J. Reprod. Fertil., 100, 469–475.
122) Wathes, D.C. and Lamming, G.E. (1995): The oxytocin receptor, luteolysis and the maintenance of pregnancy. J. Reprod. Fertil. Suppl., 49, 53–67.
123) Wathes, D.C. and Denning-Kendall, P.A. (1992): Control of synthesis and secretion of ovarian oxytocin in ruminants. J. Reprod. Fertil. Suppl., 45, 39–52.
124) Cherny, R.A., Salamonsen, L.A. and Findlay, J.K. (1991): Immunocytochemical localization of oestrogen receptors in the endometrium of the ewe. Reprod. Fertil. Dev., 3, 321–331.
125) Stevenson, K.R., Riley, P.R., Stewart, H.J., Flint, A.P.F. and Wathes, D.C. (1994): Localization of oxytocin receptor mRNA in the ovine uterus during the oestrus cycle and early pregnancy. M. Molec. Endocrinol., 12, 93–105.

126) Robinson, R.S., Mann, G.E., Lamming, G.E. and Wathes, D.C. (1999): The effect of pregnancy on the expression of uterine oxytocin, oestrogen and progesterone receptors during early pregnancy in the cow. J. Endocrinol., 160, 21–33.
127) Spencer, T.E., Ing, N.H., Ott, T.L., Mayes, J.S., Becker, W.C., Watson, G.H., Mirando, M.A. and Bazer, F.W. (1995): Intrauterine injections of ovine interferon-τ alters oestrogen receptor and oxytocin receptor expression in the endometrium of cyclic ewe. J. Molec. Endocrinol., 15, 203–320.
128) Spencer, T.E., Becker, W.C., George, P., Mirando, M.A., Ogle, T.F. and Bazer, F.W. (1995): Ovine interferon-tau (IFN-τ) inhibits estrogen receptor up-regulation and estrogen-induced luteolysis in cyclic ewes. Endocrinol., 136, 4932–4944.
129) Lamming, G.E., Wathes, D.C., Flint, A.P.F., Payne, J.H., Stevenson, K.R. and Vallet, J.L. (1995): Local actions of trophoblast interferons in suppression of the development of oxytocin and oestradiol receptors in ovine endometrium. J. Reprod. Fertil., 105, 165–175.
130) Fleming, F.G.W., Choi, Y.S., Johnson, G.A., Spencer, T.E. and Bazer, F.W. (2001): Cloning of the ovine estrogen receptor-α promoter and functional regulation by ovine interferon-τ. Endocrinol., 142, 2879–2887.
131) Stewart, H.J., McCann, S.H., Barker, P.J., Lee, K.E., Lamming, G.E. and Flint, A.P. (1987): Interferon sequence homology and receptor binding activity of ovine trophoblast antiluteolytic protein. J. Endocrinol., 115, R13–R15.
132) Li, J. and Roberts, R.M. (1994): Structure-function relationships in the interferon-tau (IFN-tau). Changes in receptor binding and in antiviral and antiproliferative activities resulting from site-directed mutagenesis performed near the carboxyl terminus. J. Biol. Chem., 269, 13544–13550.
133) Kaluz, S., Fisher, P.A., Kaluzova, M., Sheldrick, E.L. and Flint, A.P.F. (1996): Structure of an ovine interferon receptor and its expression in endometrium. J. Molec. Endocrinol., 17, 207–215.
134) Han, C.S., Mathialagan, N., Klemann, S.W. and Roberts, R.M. (1997): Molecular cloning of ovine and bovine type I interferon receptor subunits from uteri, and endometrial expression of messenger ribonucleic acid for ovine receptors during the estrous cycle and pregnancy. Endocrinol., 138, 4757–4767.
135) Rosenfeld, C.S., Han, C.S., Alexenko, A.P., Spencer, T.E. and Roberts, R.M. (2002): Expression of interferon receptor subunits, IFNAR1 and IFNAR2, in the ovine uterus. Biol. Reprod., 67, 847–853.
136) Guesdon, F., Stewart, H.J. and Flint, A.P.F. (1996): Negative regulatory domains in a trophoblast interferon promoter. J. Molec. Endocrinol., 16, 99–106.
137) Hernandez-Ledezma, J.J., Sikes, J.D., Murphy, C.N., Watson, A.J., Schultz, G.A. and Roberts, R.M. (1992): Expression of bovine trophoblast interferon in conceptuses derived by *in vitro* techniques. Biol. Reprod., 47, 374–380.
138) Imakawa, K., Helmer, S.D., Nephew, K.P., Meka, C.S.R. and Christenson, R.K. (1993): A novel role for GM-CSF: enhancement of pregnancy specific interferon production, ovine trophoblast protein-1. Endocrinol., 132, 1869–1871.
139) McGuire, W.J., Imakawa, K., Tamura, K., Meka, C.S.R. and Christenson, R.K. (2002): Regulation of endometrial granulocyte macrophage-colony stimulating factor (GM-CSF) in the ewe. Domestic Anim. Endocrinol., 23, 383–396.
140) Ezashi, T. and Roberts, R.M. (2000): Colony stimulating factor-1 enhances bovine interferon-tau gene transcription via a Ras-responsive enhancer. Biol. Reprod., 62, 289.
141) Ezashi, T., Ealy, A.D., Ostrowski, M.C. and Roberts, R.M. (1998): Control of interferon-tau gene expression by Ets-2. Proc. Natl. Acad. Sci. USA., 95, 7882–7887.
142) Ezashi, T., Ghosh, D. and Roberts, R.M. (2001): Repression of Ets-2 induced transactivation of the interferon-tau promoter by Oct-4. Molec. Cell. Biol., 21, 7883–7891.
143) Kurosaka, S., Eckardt, S. and McLaughlin, K.J. (2004): Pluripotent lineage definition in bovine embryos by Oct4 transcript localization. Biol. Reprod., 71, 1578–1582.
144) Larson, M.A., Kimura, K., Kubisch, H.M. and Roberts, R.M. (2001): Sexual dimorphism among bovine embryos in their ability to make the transition to expanded blastocysts and in the expression of the signaling molecule IFN-τ. Proc. Natl. Acad. Sci. USA., 98, 9677–9682.
145) Kimura, K., Spate, L.D., Green, M.P., Murphy, C.N., Seidel Jr., G.E. and Roberts, R.M. (2004): Sexual dimorphism in interferon-τ production by *in vivo*-derived bovine embryos. Molec. Reprod. Dev., 67, 193–199.
146) Kimura, K., Spate, L.D., Green, M.P. and Roberts, R.M. (2004): Effects of oxidative stress and inhibitors of the pentose phosphate pathway on sexually dimorphic production of IFN-τ by bovine blastocysts. Molec. Reprod. Dev., 68, 88–95.
147) Geisert, R.D., Renegar, R.H., Thatcher, W.W., Roberts, R.M. and Bazer, F.W. (1982): Establishment of pregnancy in the pig. I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol. Reprod., 27, 925–939.
148) Geisert, R.D., Zavy, M.T., Moffatt, R.J., Blair, R.M. and Yellin, T. (1990): Embryonic steroids and the establishment of pregnancy. J. Reprod. Fertil. Suppl., 40, 293–305.
149) Stroband, H.W.J. and Van der Lende, T. (1990): Embryonic and uterine development during early pregnancy in pigs. J. Reprod. Fertil. Suppl., 40, 261–267.
150) Melampy, R.M. and Anderson, L.L. (1968): Role of the uterus in corpus luteum fuction. J. Anim. Sci., 27, 77–96.
151) Hallford, D.M., Wettemann, R.P., Turman, E.J. and Omtvedt, I.T. (1974): Luteal function in gilts after prostaglandin $F_{2\alpha}$. J. Anim. Sci., 38, 213.
152) Moeljono, M.P.E., Bazer, F.W. and Thatcher, W.W. (1976): A study of prostaglandin $F_{2\alpha}$ as the luteolysin in

swine. I. Effect of prostaglandin $F_{2\alpha}$ in hysteretomized gilts. Prostaglandins, 11, 737–743.
153) Diehl, J.R. and Day, B.N. (1974): Effect of prostaglandin $F_{2\alpha}$ on luteal function in swine. J. Anim. Sci., 66, 404.
154) Moeljono, M.P.E., Thatcher, W.W., Bazer, F.W., Frank, M., Owens, L.J. and Wilcox, C.J. (1977): A study of prostaglandin $F_{2\alpha}$ as the luteolysin in swine: II. Characterization and comparison of prostaglandin F, estrogen and progestin concentrations in utero-ovarian vein plasma of nonpregnant and pregnant gilts. Prostaglandins, 14, 543–555.
155) Dhinsda, D.S. and Dziuk, P.J. (1968): Effect on pregnancy in the pig after killing embryos or fetuses in one uterine horn in early gestation. J. Anim. Sci., 27, 122–126.
156) Ford, S.P., Christenson, R.K. and Ford, J.J. (1982): Uterine blood flow and uterine arterial, venous and luminal concentrations of oestrogens on days 11, 13 and 15 after oestrus in pregnant and nonpregnant sows. J. Reprod. Fertil., 64, 185–190.
157) Van der Meulen, J., Kaufman, J.M. and Oudenaarden, C.P.J. (1988): Effect of flushing of blastocysts on day 10–13 on the life-span of the corpora lutea of the pig. J. Reprod. Fertil., 84, 157–162.
158) Harney, J.P. and Bazer, F.W. (1989): Effect of porcine conceptus secretory proteins on interestrous interval and uterine secretion of prostaglandins. Biol. Reprod., 41, 277–284.
159) Perry, J.S., Heap, R.B. and Amoroso, E.C. (1973): Steroid hormone production by pig blastocysts. Nature, 245, 45–47.
160) Fischer, H.E., Bazer, F.W. and Fields, M.J. (1985): Steroid metabolism by endometrial and conceptus tissues during early pregnancy and pseudopregnancy in gilts. J. Reprod. Fertil., 75, 69–78.
161) King, G.J. and Ackerley, C.A. (1985): Demonstration of oestrogens in developing pig trophectoderm and yolk sac endoderm between Days 10 and 16. J. Reprod. Fertil., 73, 361–367.
162) Kidder, H.E., Casida, L.E. and Grummer, R.H. (1955): Some effects of estrogen injections on estrual cycle of gilts. J. Anim. Sci., 14, 470–474.
163) Frank, M., Bazer, F.W., Thatcher, W.W. and Wilcox, C.J. (1977): A study of prostaglandin $F_{2\alpha}$ as the luteolysin in swine. III. Effects of estradiol valerate on prostaglandin F, progestins, estrone, and estradiol concentrations in the utero-ovarian vein of nonpregnant gilts. Prostaglandins, 14, 1183–1196.
164) Ford, S.P., Magness, R.R., Farley, D.B. and Van Orden, D.E. (1982): Local and systemic, effects of intrauterine estradiol-17β on luteal function of nonpregnant sows. J. Anim. Sci., 55, 657–664.
165) Saunders, M.J., Edgerton, L.A., Kagan, J.M., Stahly, T.S. and Cromwell, G.L. (1983): Comparison of intrauterine and subcutaneous sites of estrogen injection for luteal maintenance in swine. J. Anim. Sci., 57, 146–149.
166) Geisert, R.D., Zavy, M.T., Wettemann, R.P. and Biggers, B.G. (1987): Length of pseudopregnancy and pattern of uterine protein released as influenced by time and duration of oestrogen administration in the pig. J. Reprod. Fertil., 79, 163–172.
167) King, G.J. and Rajamahendran, R. (1988): Comparison of plasma progesterone profiles in cyclic, pregnant, pseudopregnant and hysterectomized pigs between 8 and 27 days after oestrus. J. Endocrinol., 119, 111–116.
168) Zavy, M.T., Bazer, F.W., Thatcher, W.W. and Wilcox, C.J. (1980): A study of prostaglandin $F_{2\alpha}$ as the luteolysin in swine. V. Comparison of prostaglandin $F_{2\alpha}$, progestins, estrone and estradiol in uterine flushings from pregnant and nonpregnant gilts. Prostaglandins, 20, 837–851.
169) Stone, B.A. and Seamark, R.F. (1985): steroid hormones in uterine washings and in plasma of gilts between Days 9 and 15 after oestrus and between Days 9 and 15 after coitus. J. Reprod. Fertil., 75, 209–221.
170) Robertson, H.A. and King, G.J. (1974): Plasma concentrations of progesterone, oestrone, oestradiol-17β and oestrone sulphate in the pig at implantation, during pregnancy and at parturition. J. Reprod. Fertil., 40, 133–141.
171) Stoner, C.S., Geisert, R.D., Bazer, F.W. and Thatcher, W.W. (1981): Characterization of estrogen patterns in early pregnant and estrous gilts. J. Anim. Sci., 53 (Suppl. 1), 370.
172) Krzymowski, T., Kotwica, J. and Stefanczyk-Krzymowska, S. (1990): Uterine and ovarian countercurrent pathways in the control of ovarian function in the pig. J. Reprod. Fertil. Suppl., 40, 179–191.
173) Stefańczk-Krzymowska, S., Krzymowski, T., Einer-Jensen, N., Kaminski, T. and Kotwica, J. (1990): Local transfer of prostaglandin $F_{2\alpha}$ from the uterine lumen into the venous and arterial blood and into the uterine, mesometrial and ovarian tissue on Day 18 of pregnancy in the pig. Anim. Reprod. Sci., 23, 223–235.
174) Bazer, F.W. and Thatcher, W.W. (1977): Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F2 by uterine endometrium. Prostaglandins, 14, 397–401.
175) Gross, T.S., Lacroix, M.C., Bazer, F.W., Thatcher, W.W. and Harney, J.P. (1988): Prostaglandin secretion by perifused porcine endometrium: Further evidence for an endocrine versus exocrine secretion of prostaglandin. Prostaglandins, 35, 327–341.
176) Gross, T.S., Mirando, M.A., Young, K.H., Beers, S., Bazer, F.W. and Thatcher, W.W. (1990): Reorientation of prostaglandin F secretion by calcium ionophore, estradiol, and prolactin in perifused porcine endometrium. Endocrinol., 127, 637–642.
177) Akinlosotu, B.A., Diehl, J.R. and Gimenez, T. (1988): Progstaglandin E_2 counteracts the effect of $PGF_{2\alpha}$ in indomethacin treated gilts. Prostaglandins, 35, 81–93.
178) Christenson, L.K., Farley, D.B., Anderson, L.H. and Ford, S.P. (1994): Luteal maintenance during early pregnancy in the pig: role for prostaglandin E_2. Prostaglandins, 47,

61–75.
179) Davis, D.L. and Blair, R.M. (1993): Studies of uterine secretions and products of primary cultures of endometrial cell in pigs. J. Reprod. Feril. Suppl., 8, 143–155.
180) Garverick, H.A., Polge, C. and Flint, A.P.F. (1982): Oestradiol administration raises luteal LH receptor levels in intact and hysterectomized pigs. J. Reprod. Fertil., 66, 371–377.
181) Ziecik, A.J., Jedlinska, M. and Rzucidlo, J.S. (1992): Effect of estradiol and progesterone on myometrial LH/hCG receptors in pigs. Acta Endocrinol., 127, 185–188.
182) Cross, J.C. and Roberts, R.M. (1989): Porcine conceptuses secrete an interferon during the preattachment period of early pregnancy. Biol. Reprod., 40, 1109–1118.
183) Mirando, M.A., Harney, J.P., Beers, S., Pontzer, C.H., Torres, B.A., Johnson, H.M. and Bazer, F.W. (1990): Onset of secretion of proteins with antiviral activity by pig conceptuses. J. Reprod. Fertil., 88, 197–203.
184) Lefevre, F., Martinat-Botte, F., Guillomot, M., Zouari, K., Charley, B., La Bonnardiere, C. (1990): Interferon-gamma gene and pretein are spontaneously expressed by the porcine trophectoderm early in gestation. Eur. J. Immunol., 20, 2485–2490.
185) Lefevre, F. and Boulay, V. (1993): A novel and atypical type one interferon gene expressed by trophoblast during early pregnancy. J. Biol. Chem., 268, 19760–19768.
186) Lefevre, F., Guillomot, M., D'Andrea, S., Battegay, S. and La Bonnardiere, C. (1998): Interferon-delta: the first member of a novel type I interferon family. Biochimie., 80, 779–788.