Journal of Infertility and Reproductive Biology

Journal of Infertility and Reproductive Biology, 2014, Volume 2, Issue 2, Pages: 50-55

Physiological and molecular determinants of embryo-uterine interactions in ruminants

Tripathi SK[1], Farman M[2*], Nandi S[1], Gupta PSP[1], Girish Kumar V[2]

1. National Institute of Animal Nutrition and Physiology (NIANP), Bangalore, India
2. College of Veterinary Sciences, KVAFSU, Bangalore campus, Hebbal, Bangalore, India

Abstract

Maternal recognition of pregnancy (MRP) manifests the various ways in which the mother responds to the presence of a conceptus within her reproductive tract. Corpus luteum produces progesterone, the hormone of pregnancy, which is required to stimulate and maintain endometrial functions that are permissive to early embryonic development, implantation, placentation, and successful fetal and placental development. The interaction between a competent embryo and a receptive uterine environment is responsible for successful growth and development of the post-hatching blastocyst and pregnancy establishment. Certain hormones, enzymes, cytokines, interleukins and gene transcripts contribute and regulate the bidirectional channel of communication during the pregnancy period in ruminants. During Maternal recognition of pregnancy some genes like interferon-tau (IFNT), Ubiquitin Cross Reactive Protein (UCRP), Ghrelin, Aldoketoreductase-1B5 (AKR1B5), SERPINA14 are appear to have role in successful establishment of pregnancy and expression of the cascade of signaling molecules. These genes regulate the endometrial environment to establish pregnancy in farm animals. MRP in ruminants requires that the conceptus elongates from a spherical to a tubular and then filamentous form to produce IFNT which is the pregnancy recognition signal. Genetic factors of both embryo and mother's endometrium are also responsible for successful embryo development.

Keywords: Maternal recognition of pregnancy, Corpus luteum, Gene, Conceptus

1. Introduction

Giving birth to offspring simply defined as reproduction. Species survival largely depends on its ability to reproduce its own kind. Fertility is a vital function, complex process and polygenic in all living organisms. Sheep and goats are considered to be the most prolific of all domestic ruminants. Fertility is high in sheep (85%) and pigs (90%), moderate in beef cattle (45%), and low in dairy cattle (35% or less in high-producing cattle) and in humans (25%) (1). Pregnancy establishment in domestic ruminants (i.e., sheep, cattle, and goats) start at the conceptus stage and includes pregnancy recognition signaling, implantation, and placentation.

[*]**Corresponding Address:** Dr. Mohammad Farman, College of Veterinary Sciences, KVAFSU, Bangalore campus, Hebbal, Bangalore, India. Email: drfarmanmvsc@gmail.com

According to Guillomot et al (1988), the phases of implantation include shedding of the zona pellucid, pre-contact and blastocyst orientation, apposition, adhesion and endometrial invasion. Each of these phases occurs in domestic ruminants, but endometrial invasion is very limited. Unlike to rodent and human true endometrial invasion not occur in ruminants (2, 3). The present paper describes the events during early pregnancy in ruminants.

2. Events during early pregnancy

In the oviduct fertilization occur and the morula stage embryo enters the uterus on day 4$^{th.}$ By the day 7th blastocyst formed and on the day 9th it hatches from the zona pellucida. At the day 13th blastocyst develops from a spherical to a tubular and then elongates to a filamentous conceptus between days 14 and 19 (1).

The entry of blastocysts into the uterine cavity in cows, sheep and pig are on days 5, 5 and 2-3 respectively (3). The corresponding values for hatching are on days 9-10, 7-9 and 5-6 in cows, sheep and pig (3). Likewise, the implantations are on days 19-25, 15-20 and 14 in cows, sheep and pig (3) Elongation of the conceptus marks the beginning of implantation. According to Bazer *et.al* 2011 and 1991 (2,4) respectively elongation of the conceptus is initiated on days 11–12 in the pig, days 12– 13 in sheep, and days 13–14 in cattle and is concomitant with pregnancy recognition and implantation. Embryo uterus interaction is physiological process where the maternal system receives the signal for the presence of conceptus and prolongs the lifespan of corpus luteum (CL) of ovary (2,4). On the day 13^{th} to 18^{th} apposition and transient attachment will take place and on 19^{th} day there will be synthesis and secretion of prostaglandins and interferon tau by trophectoderm. Many molecules (hormones, cytokines, growth factors etc., Table 1) take part in the dialogue between the blastocyst and the maternal endometrium before the implantation was achieved (13-15)

Maternal recognition is the physiological process whereby the conceptus signals its presence to the maternal system and prolongs lifespan of the corpus luteum. In most of the mammals progesterone secreted by CL is required for successful establishment of pregnancy. There should be reciprocal interaction between conceptus and maternal endometrium for pregnancy maintenances. Hormones from the placenta act directly on the uterine endometrium to regulate cell differentiation and function. During pregnancy morphogenesis of endometrial gland increases the secretion of proteins that are transported to fetus by aerolae (specific area of placenta).

Table 1. Molecules involved in embryo-uterine interactions (Ref. 5-12).

Factors	Name
Hormones	Estrdiol (E2), Progesteron (P4), GnRH, FSH, LH, Progestamedins, Estramedins, Corticoids and Prostaglandins
Growth Factor	Epidermal growth factor
	Transforming growth factor-α and β (TGF-α and TGF-β)
	β- Cellulin (BTC)
	Epiregulin (Er)[ERbB-1,ErbB-2,ErbB-3,ErbB-4]
	Neuro-differentiating factor (NDFS)
	Heaprin binding EGF (HB-EGF)
	Vascular endothelial growth factor (VEGF)
Cytokines	Leukemia inhibitory factor (LIF),
	Colony stimulating factor-1 (CSF-1),
	Cyclooxygenase-2 (Cox-2),
	alpha (IFNA1-IFNA10, IFNA13, IFNA14, IFNA16, IFNA17 and IFNA21),
	interferon beta (IFNB), interferon delta (IFND), interferon epsilon (IFNE), interferon kappa (IFNK), interferon tau (IFNT) and interferon omega (IFNW1-IFNW3)
Modulators of cell adhesion	Mucin 1 (Muc1), Integrins, Basigin (Bsg)
Developmental factors	Homeobox (Hox) Genes, Wnt Genes

3. Maternal environment required for embryo implantation

When CL fully formed after ovulation it leads to implantation in mammals (15). In rodents, during the diestrous phase of the estrous cycle while in humans, during the luteal phase of the menstrual cycle implantation occurs (16). During implantation estrogen and progesterone are the principle hormones which are in fluctuating dynamics. During follicular phase (proestrous) estrogen level is very high, during estrous phase level of both hormones is low, while during the luteal phase both hormones are secreted from CL. The requirement of progesterone for implantation in all mammals is widely accepted, while the role of two estrogen surges at the proestrous and luteal phase prior to embryo implantation remains controversial (15-19). Earlier evidences show that there is requirement of solely progesterone for implantation (20) but according to other reports there are evidences of another hormone role during implantation. These finding were supported by inducing implantation in lactating rats by injection of small dose of estrogen (16). Estrogen requirement during implantation is species specific.

In species such as guinea pig, rhesus monkey, rabbit and golden hamster, progesterone alone is adequate for implantation (21-23). The maternal recognition of pregnancy starts on day 16-17, day 12-13, day 17, day 14-16 and day 12 of pregnancy in cows, sheep, goat, mare and sow respectively.

4. Establishment of Pregnancy

Progesterone is required for maintenance and stimulation of endometrial functions necessary for conceptus growth, implantation, and placentation (24, 25). Progesterone is also required for blastocyst survival and growth. Concentrations of progesterone in pregnancy affect embryonic survival during early pregnancy in cattle (26). Lower concentrations of progesterone in the early luteal phase had retarded conceptus which leads to less secretion of IFNT. Increasing concentrations of progesterone enhanced conceptus development and size, while animals with lower concentrations during the early luteal phase had retarded embryonic development (27). In endometrium positive and negative gene expression regulates by progesterone while progesterone along with IFNT stimulates number of gene in endometrium epithelium. In early luteal phase progesterone receptor (PR) is expressed in the endometrial epithelia and stroma, allowing direct regulation of a number of genes by progesterone via activation of the PR. Spencer *et al.* (1995) (28) reported that after 11 and 13 day of pregnancy expression of PR protein is not detectable in endometrial luminar epithelium (LE) and glandular epithelium (GE) in sheep, respectively. This loss of PR prior to implantation is common to sheep, cattle, mice and humans (25,29). Fibroblast growth factor-10 (FGF-10) hepatocyte growth factor (HGF) express by endometrial stromal cells while FGF2IIIb and c-met express by endometrial epithelium and trophectoderm respectively regulates epithelial function (30). These are ovine uterine milk protein (UTMP) and osteopontin (OPN). UTMP are serine protease inhibitors and act as marker for pregnancy in sheep. OPN is phophorylated glycoprotein (acidic) that is found in epithelia and in secretions of many tissues, including the uterus. OPN promotes cell adhesion, spreading and migration through binding with integrin heterodimers expressed by trophoectoderun and uterus. These bindings are responsible for changes in extra-embryonic morphology of conceptus and also for adhesion between LE and trophectoderm which is mandatory for implantation and pregnancy (25).

5. Genes involve in maternal recognition of pregnancy

Interferon *tau* (IFN- τ) is type 1 interferon family member, and is the pregnancy recognition signal in ruminants. It is secreted by the elongating conceptus (IFN-τ is produced exclusively by the ruminant placenta) which is going to acts on the endometrium (paracrine mode) resulting regress the development of the luteolytic mechanism required for rhythmic release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). This is necessary for continued production of progesterone by corpus luteum (31); along with this function it induces many gene expressions in a cell specific manner responsible for uterine receptivity and development of conceptus (31,32). IFN-τ is produced between days 10 and 25 of gestation with peak activity on days 14 to 16 by small and large ruminant placenta. In cattle, sheep and goat (ungulates ruminates) IFN- τ is essential for pregnancy maintenance from the period of blastocyst formation to attachment of elongated conceptus to uterine wall (33,34). LGALS15, CTSL, and CST3 genes expression induced by progesterone and further stimulated by IFNT. There is indirect effect of progesterone on the conceptus via the endometrium that leads to regulate blastocyst growth and conceptus elongation (35,36).

Few other genes like Ubiquitin cross reactive protein (UCRP), SERPINA 14 and Ghrelin play role in maternal recognition during pregnancy in case of animals. UCRP function in response to IFN- τ and responsible for proteosomal mediated degradation of cytosolic uterine protein involves in release of PGF2a. Under the influence of progesterone, uterine endometrium releases serine protease inhibitor (SERPINA 14) in bovine, ovine and in porcine (37-39). SERPINA 14 involve in providing direct nutrition to the conceptus, responsible for growth control, proteolytic activities inhibition and suppression of the maternal immune system for sustaining pregnancy. SERPINA 14 involves in inhibition of lymphocyte proliferation in ovine (40) during the estrous cycle (days 13-15) and in pregnancy (days 15-50). SERPINA 14 is recognized as an important factor (41) on 25^{th} days of pregnancy in goat. Ghrelin acts as a ligand for growth hormone secretagouge receptor (GSH). It also has a role in regulating the secretion of reproductive hormones and in embryo implantation; however its presence in goat and buffalo is not reported (34). The genes up and down regulated during embryo uterine interactions are given in Table 2.

Table 2. Genes up / down regulated during embryo uterine interactions (Ref. 42-44).

Genes up-regulated Gene name (symbol)	Genes down-regulated Gene name (symbol)
Calpain 6 (CAPN6)	alpha 1,4-galactosyltransferase (A4GLTA)
CD74 molecule, major histocompatibility complex, class II invariant chain (CD74)	actin, beta (ACTB)
CD81 molecules (CD81)	Annexin A2(ANX2)
CNDP dipeptidase 2 (CNDP2)	collagen, type I, alpha 1(COL1A1)
CyclinM3 (CNM3)	collagen, type I, alpha 2 (COL1A2)
Endothelial PAS domain protein 1(EPAS1)	collagen, type III, alpha 1 (COL3A1)
guanylate binding protein 4(GBP4)	collagen, type VI, alpha 1(COL6A1)
glutamic-pyruvate transaminase (GPT)	collagen, type VI, alpha 2 (COL6A2)
interferon, gama-inducible protein 16 (IFI16)	collagen, type VI, alpha 3 (COL6A3)
interferon, alpha-inducible protein 27 (IFI27)	estrogen receptor 1(ESR1)
interferon induced with helicase C domain 1(IFIH1)	growth arrest and DNA-damage-inducible, gamma (GADD45G)
interferon-induced protein with tetratricopeptide repeats 1(IFIT1)	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5)
interferon induced transmembrane protein 1 (9-27)(IFITM1)	Insulin-like growth factor 1 (IGF1)
interferon induced transmembrane protein 3 (1-8U) (IFITM3)	splicing factor, arginine/serine-rich 10 (transformer 2 homolog, Drosophila) (SFRS10)
Immunoglobulin heavy locus (IGH)	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1(SERPINH1)
Immunoglobulin lambda locus (IGL)	SMEK homolog 1, (SMEK1)
interferon regulatory factor 9 (IRF9)	SPARC related modular calcium binding 2 (SMOC2)
ISG15 ubiquitin-like modifier (ISG15)	SRY (sex determining region Y)-box 4(SOX4)
lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP)	signal transducer and activator of transcription 1, 91kDa (STAT1)
receptor (chemosensory) transporter protein 4 (RTP4)	Metallothionein 2A (MT2A)
spermidine/spermine N1-acetyltransferase 1(SAT1)	retinoic acid receptor responder (RARRES1)
scavenger receptor class A, member 5 (SCARA5)	Retinol binding protein 4 (RBP4)
serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary (SERPING1)	Thrombospondin1 (THBS1)
solute carrier family 16, member 11 (SLC16A11)	Thrombospondin2 (THBS2)
solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1 (SLC1A1)	TIMP metallopeptidase inhibitor 1 (TIMP1)
signal transducer and activator of transcription 1, 91kDa (STAT1)	Tublin,alpha 1b (TUBA1B)
Metallothionein 2A (MT2A)	Tublin beta (TUBB)
nuclear receptor subfamily 2, group F, member 2 (NR2F2)	Zyxin (ZYX)
nucleoporin 93kDa (NUP93)	
poly (ADP-ribose) polymerase family, member 12 (PARP12)	
poly (ADP-ribose) polymerase family, member 9 (PARP9)	
polyribonucleotide nucleotidyltransferase 1(PNPT1)	
proteasome (prosome, macropain) subunit, alpha type, 3 (P3)	
proteasome (prosome, macropain) inhibitor subunit 1 (PSMF1)	
Ran GTPase activating protein 1(RANGAP1)	
transporter 1, ATP-binding cassette, sub-family B (TAP1)	
Thioredoxin interacting protein (TXNIP)	
Ubiquitin-activating enzyme E1-like(UBE1L)	

6. Conclusions

Uterine microenvironment influenced directly or indirectly the survivability of successful development of an embryo. The survivability of embryo during early embryonic life is mostly dependent on the efficiency with which the maternal recognition of pregnancy is established. Early conceptus development, implantation and maintenance of pregnancy are critically dependent upon a precisely orchestrated interaction between the conceptus and the uterine environment. This maternal-conceptus interaction, if successful, prompts continued progestin support leading to a receptive uterine environment, which in turn, is essential for the conceptus' development. Though immense knowledge of the mechanisms and factors regulating conceptus implantation in mammals has been gathered but much remains to be discovered about the interactions responsible for blastocyst implantation. Exploring the factors that regulate uterine receptivity and implantation can be used to diagnose and identify the cause of recurrent pregnancy loss and improve pregnancy rates in domestic animals

References

1. Spencer TE. Early pregnancy: Concepts, challenges, and potential solutions. Anim Front. 2013; 3(4): 48-55.
2. Bazer FW, Spencer TE, Johnson GA, et al. Uterine receptivity to implantation of blastocysts in mammals. Front Biosci. 2011; 3:745-767.
3. Guillomot M, Reinaud P, Charpigny G, et al. Embryo-uterine interactions during early stages of pregnancy in domestic mammals. Reprod Nutr Dev. 1988 ; 28(6B): 1629-1648.
4. Bazer FW, Thatcher WW, Hansen PJ, et al. Physiological mechanisms of pregnancy recognition in ruminants. J Reprod Fertil Suppl. 1991; 43: 39-47.
5. Lee KY & DeMayo FJ. Focus on Implantation - Animal models of implantation. Reproduction. 2004; 128(6): 679-695.
6. Miller C, Sassoon DA. Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract. Dev.1998; 125(16): 3201-3211.
7. Wiley AA, Bartol FF, Barron DH. Histogenesis of the ovine uterus. J Anim Sci. 1987; 64(4): 1262-1269.
8. Reynolds LP, Kirsch JD, Kraft KC, et al. Time-course of the uterine response to estradiol-17beta in ovariectomized ewes: expression of angiogenic factors. Biol Reprod. 1998; 59(3): 613-620.
9. Albrecht ED, Aberdeen GW, Niklaus AL, et al. Acute temporal regulation of vascular endothelial growth/permeability factor expression and endothelial morphology in the baboon endometrium by ovarian steroids. J Clin Endocrinol Metab. 2003; 88(6): 2844-2852.
10. Robb L, Li R, Hartley L, et al. Infertility in female mice lacking the receptor for interleukin 11 is due to a defective uterine response to implantation. Nat Med. 1998; 4: 303-308.
11. Shinetugs B, Runesson E, Bonello NP, et al. Colony stimulating factor-1 concentrations in blood and follicular fluid during the human menstrual cycle and ovarian stimulation: possible role in the ovulatory process. Human Reprod. 1999; 14(5): 1302-1306.
12. Bazer FW. Pregnancy recognition signalling mechanisms in ruminants and pigs. *J Anim Sci Biotech.* 2013**;** 4(1)**:** 23.
13. Aitken RJ. Aspects of delayed implantation in the roe deer (*Capreolus capreolus*). J Reprod Fertil Suppl. 1981; 29: 83-95.
14. Valles CS. Embryo- Endometerial interaction. Special section From the Department of Obstetrics and Gynecology. 2005; Valencia University, Spain.
15. Finn CA, Martin L. The control of implantation. J Reprod Fertil. 1974; 39 (1): 195-206.
16. Zhang S, Lin H, Kong S, et al. Physiological and molecular determinants of embryo implantation. Mol Aspects Med. 2013; 34(5): 939-980.
17. Dey SK, Lim H, Das SK, et al. Molecular clues to implantation. Endocr Rev. 2004; 25(3): 341-373.
18. Tranguch S, Smith DF, Dey SK. Progesterone receptor requires a co-chaperone for signalling in uterine biology and implantation. Reprod Biomed Online. 2006; 13(5): 651-660.
19. Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. Nat Rev Genet. 2006; 7(3): 185-199.
20. Spencer TE & Bazer FW. Conceptus signals for establishment and maintenance of pregnancy. Reprod Biol Endocrin. 2004; 2: 49.
21. Heap RB, Deanesly R. The increase in plasma progesterone levels in the pregnant guinea-pig and its possible significance. J Reprod Fertil. 1967; 14(2): 339-341.
22. Harper MJ, Dowd D, Elliott AS. Implantation and embryonic development in the ovariectomized-adrenalectomized hamster. Biol Reprod. 1969; 1: 253-257.
23. Heap RB, Flint AP, Hartmann PE, et al. Oestrogen production in early pregnancy. J Endocrin Supp. 1981; 89: 77-94.

24. Spencer TE, Bazer FW. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. Fronts Biosci. 2002; 7: 879-898.
25. Spencer TE, Johnson GA, Burghardt RC, et al. Progesterone and placental hormone actions on the uterus: insights from domestic animals. Biol of Reprod. 2004; 71(1): 2-10.
26. Mann GE & Lamming GE. The influence of progesterone during early pregnancy in cattle. Reprod Domest Anim. 1999; 34(3-4): 269-274.
27. Nephew KP, McClure KE, Ott TL, et al. Relationship between variation in conceptus development and differences in estrous cycle duration in ewes. Biol Reprod. 1991; 44(3): 536-539.
28. Spencer TE, Bazer FW. Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol Reprod. 1995; 53(6): 1527-1543.
29. Carson DD, Bagchi I, Dey SK, et al. Embryo implantation. Dev Biol. 2000; 223(2): 217-237.
30. Chen C, Spencer TE, Bazer FW. Expression of hepatocyte growth factor and its receptor c-met in the ovine uterus. Biol Reprod. 2000; 62(6): 1844-1850.
31. Spencer TE, Johnson GA, Bazer FW, Burghardt RC. Fetal–maternal interactions during the establishment of pregnancy in ruminants. Soc Reprod Fertil Suppl. 2007; 64: 379-396.
32. Hansen TR, Austin KJ, Perry DJ, et al. Mechanism of action of interferon-*t* in the uterus during early pregnancy. J Reprod Fertil. Suppl. 1999; 54: 329-339.
33. Ealy AD, Larson SF, Liu L, et al. Polymorphic forms ofexpressed bovine interferon-tau genes: relative transcript abundance during early placental development, promoter sequences of genes and biological activity of protein products. Endocrin. 2001; 142(7): 2906-29015.
34. Kumar R, Ramteke PW, Nath A, et al. Role of candidate genes in regulation of embryonic survival and maternal recognition of pregnancy in farm animal. Vet World. 2013; 6(5): 280-284.
35. Bauersachs S, Wolf E. Transcriptome analyses of bovine, porcine and equine endometrium during the pre-implantation phase. Anim Reprod Sci. 2012; 134(1-2): 84-94.
36. Dorniak P, Bazer FW, Spencer TE. Physiology and Endocrinology Symposium: Biological role of interferon tau in endometrial function and conceptus elongation. J Anim Sci. 2013; 91(4): 1627-1638.
37. Ing NH, Francis H, McDonnell JJ, et al. Progesterone induction of the uterine milk proteins: major secretory proteins of sheep endometrium. Biol Reprod. 1989; 41(4): 643-654.
38. Mathialagan N, Hansen TR. Pepsin-inhibitory activity of the uterine serpins, Proc Natl Acad Sci. 1996; 93(24): 13653-13658.
39. Tekin S, Padua MB, Newton GR, et al. Identification and cloning of caprine uterine serpin. Mol Reprod Dev. 2005; 70(3): 262-270.
40. Susanne EU, Thomas F, Katy S, et al. Evidence for estrogen dependent uterine serpin (serpina14) expression during estrus in the bovine endometrial glandular epithelium and lumen. Biol Reprod. 2009; 8(4): 795-805.
41. Stewart MD, Johnson GA, Gray CA, et al. Prolactin receptor and uterine milk protein expression in the ovine endometrium during the estrous cycle and pregnancy. Biol Reprod. 2000; 62(6): 1779-1789.
42. Bauersachs S, Ulbrich SE, Gross K, et al. Embryo-induced transcriptome changes in bovine endometrium reveal species-specific and common molecular markers of uterine receptivity. Reprod. 2006; 132(2): 319-331.
43. Chen C, Spencer TE, Bazer FW. Fibroblast growth factor-10: a stromal mediator of epithelial function in the ovine uterus. Biol Reprod. 2000; 63(3): 959-966.
44. Spencer TE, Sandra O, Wolf E. Genes involved in conceptus–endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. Reprod. 2008; 135(2): 165-179.