

Hormonal Changes in the Uterus During Pregnancy- Lessons from the Ewe: A Review

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ABSTRACT

Pregnancy is the main event in the life of a female mammal to reproduce its progeny and maintain the integrity of the species. It is a very coordinated process involving reproductive organs and changes in the tissue concentration of various hormones, cytokines, enzymes and growth factors, of which hormones are most important. Various hormones are involved in the preparation of the uterus of ewe for conception of embryo, growth and development of embryo and foetus, maintenance of pregnancy and birth of a healthy lamb. However, pregnancy mainly involved two hormones, namely progesterone and oestrogens. Other hormones involved in this process are prostaglandin, cortisone, relaxin and oxytocin, which are mainly important for parturition. The endocrine changes in the ewe during the oestrous cycle, pregnancy and parturition dramatically affect the structure of the endometrium as well as the uterine immune system. During the 17-day oestrus cycle, progesterone dominates for about 13 days and oestrogens dominate for 3-4 days. In the pregnant ewe, oestrogens are necessary for the growth and development of the ovarian follicles. However, once the ewe become pregnant progesterone become the key hormone to maintain the pregnancy and, therefore, known as 'pregnancy hormone'. Progesterone dominates until the onset of parturition when endometrium switches from a progesterone-dominated state to an oestrogen-dominated state. Therefore, this paper reviews the hormonal profiles and endocrinological changes that occur during oestrous cycle and different stages of pregnancy in the ewe. The paper also illustrates the important immunological changes of the uterus that occur with the endocrinological changes.

Key words: Oestrous cycle, pregnancy, parturition, endometrium, immune cells, ewe.

INTRODUCTION

Pregnancy is the most important event in the life of any female organism to reproduce its progeny. It is a very coordinated process among the mammalian species involving reproductive organs and hormones. The endocrine changes, *i.e.* changes in the profile of oestrogens and progesterone in the ewe (female sheep) during the oestrous cycle, pregnancy and parturition dramatically affect the structure of the endometrium as well as the uterine immune system. Hunter (1980), Pineda (1989), Liggins and Thorburn (1994), and Lye (1996) have significant contribution in the sheep reproductive endocrinology. It is to be mentioned here that the hormonal profiles in the ewe varies those from the cow, the buffalo cow and the doe (female goat). Hormonal changes in the uterus might also regulate the immune cells and immunity of the uterus. Therefore, it is important to understand the

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hormonal interaction that happens in the uterus of the ewe during oestrus cycle, pregnancy and parturition. Although, the sheep in Bangladesh are non-descriptive type, however, they provide us meat and carpet wool. So, if we can improve their productivity it may contribute to our national livestock economy. Therefore, we need to be aware of the scientific information on the hormonal profiles and changes in the uterus of ewe during pregnancy.

ENDOCRINE PROFILES OF THE OESTROUS CYCLE

Like other mammals, the uterus of the ewe is subject to morphological and functional changes which span a 17-day oestrous cycle. The stages of the oestrous and reproductive cycles of the ewe are briefly presented in Fig. 1.

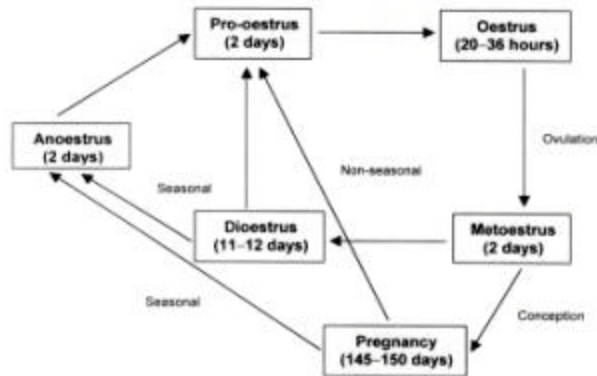


Fig. 1. The oestrous and reproductive cycles of the ewe. Adapted from Pineda (Pineda, 1989)

Cyclic changes in the circulating levels of the ovarian hormones, progesterone and oestrogen have direct effects on the growth and metabolism of cells in the reproductive tissues. The main events of the oestrous cycle are related to the periods of growth of the ovarian follicles and the corpus luteum. During the 17-day cycle, progesterone dominates for about 13 days and oestrogen dominates for 3–4 days. The endocrine patterns of lutenizing hormone (LH), oestrogen (oestradiol), progesterone and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during the oestrous cycle in the ewe are presented in Fig. 2.

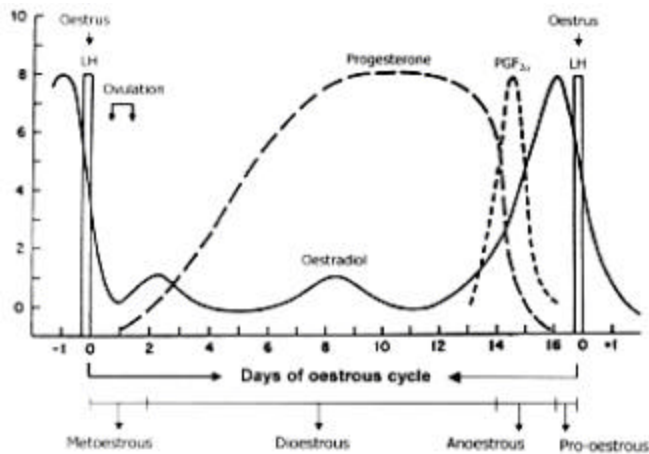


Fig. 2. Changes in the concentration of progesterone and oestradiol during the oestrous cycle in the ewe. Uterine $PGF_{2\alpha}$ pulses prior to oestrous cause the demise of the corpus luteum resulting in decreasing progesterone and elevated oestradiol levels and the occurrence of the oestrus. Adapted from Caldwell and colleagues (1972)

Day 0 of the cycle is generally designated as the first day of behavioural oestrus, which is the result of increasing oestrogen levels produced by the developing pre-ovulatory follicles. High oestrogen levels are believed to cause a surge of gonadotrophin releasing hormone (GnRH) and consequently an LH peak at oestrus resulting in spontaneous ovulation towards the end of oestrus. Following ovulation the ruptured follicle becomes a functional corpus luteum, which is the main source of progesterone in the cycling ewe. Blood levels of progesterone are low at oestrus (less than 1.0 ng/ml) through to day 3 of dioestrous, and then rapidly increase to maximal levels at day 8, and remain elevated until days 11–12 (Fig. 2). Regression of the corpus luteum (luteolysis), induced by PGF_{2α}, occurs if an embryo is not present in the uterus, resulting in a rapid drop in plasma progesterone. Ovarian oxytocin stimulates endometrial secretion and release of prostaglandins. The onset of the follicular phase of the next cycle is characterised by low progesterone levels and increasing GnRH and LH levels, while the follicle-stimulating hormone (FSH) levels present at the onset of this phase are progressively decreased. These events are controlled by oestrogen and inhibin, which are produced in increasing amounts by the developing follicles.

ENDOCRINE PROFILES DURING PREGNANCY

Once mating and fertilization are successfully completed the trophoblast must signal its presence to the maternal system to prevent luteolysis and maintain progesterone production, which is essential for the establishment of pregnancy (Geisert and Malayer, 2000). The blastocyst, before it attaches to the endometrium, secretes substances that directly or indirectly prolong the lifespan of the corpus luteum and prevent a return to ovarian cyclicity (Jainudeen and Hafez, 2000). The timing of this phenomenon is known as 'maternal recognition of pregnancy'. The substance/molecule that inhibits the synthesis and/or release of luteolytic PGF_{2α} from the endometrial cells and prevent corpus luteum regression (Bazer, 1989; Bazer *et al.*, 1989) is an embryonic protein first known as ovine trophoblast protein-1 (oTP-1) (Imakawa *et al.*, 1987). The oTP-1 was later classified as ovine interferon-tau (oIFN-τ) (Bazer *et al.*, 1997), when it was found to be a member of the interferon family. The oIFN-τ is a cytokine that acts locally on the uterine endometrium rather than systemically (Jainudeen and Hafez, 2000). The oIFN-τ has antiluteolytic, immunosuppressive, antiviral and possibly antiproliferative properties (Bazer, 1989; Bazer *et al.*, 1989). The anti-luteolytic effect of oTP-1 is dependant upon the presence of progesterone and endometrial progesterone receptors (Ott *et al.*, 1992). Granulocyte macrophage-colony stimulating factor (GM-CSF), which is produced in the maternal uterine endometrium, stimulates the production of oIFN-τ by the trophoblast (Imakawa *et al.*, 1993). Secretion of oIFN-τ from the conceptus trophoderm at 12–15 days post coitus (dpc) in the ewe (Bazer *et al.*, 1997) and 14–17 dpc in the cow and the doe (Bazer *et al.*, 1997; Gnatek *et al.*, 1989) is essential for maternal recognition of pregnancy.

Once the trophoblast established the 'maternal recognition of pregnancy' then it proceeds to the next step for implantation into the luminal epithelium of the endometrium of the ewe. The implantation event is accompanied by significant changes in the tissue concentration of various cytokines, adhesion molecules, hormones, enzymes and growth factors, all of which may be crucial in initiating the feto-maternal relationship (Rice and Chard, 1998; Saito, 2000). Like hormones, cytokines are very important for maintaining pregnancy. While IFN-τ is important for 'maternal recognition of pregnancy' other cytokines such as interleukin-1 beta (IL-1β), tumor growth factor beta (TGF-β), IL-6, leukaemia inhibition factor (LIF), IL-10 are important for embryo growth and development, and IL-6 and LIF are important for elongation of embryo and placentation (Rahman, 2002; Rahman *et al.*, 2004). The reproductive hormones believed to interact and regulate these cytokines in the uterus of the ewe (Rahman, 2002).

Progesterone is the key hormone of pregnancy and is thus often called the 'pregnancy hormone'. It acts to prevent the resumption of cyclicity, prepares the uterus for implantation and maintains myometrial quiescence (Lye, 1996). Actually, myometrial quiescence during pregnancy is achieved by the combined action of progesterone, relaxin, prostacyclin and nitric oxide (Lye, 1996). In the cow, progesterone is found to stimulate the production of a variety of endometrial secretions, which are required for the successful development of embryos (Geisert *et al.*, 1992). Low concentrations of progesterone in the ewe can lead to poor embryo development (Nephew *et al.*, 1991) and

progesterone supplementation enhances embryo growth in both the bovine (Garrett *et al.*, 1988) and the ovine (Kleemann *et al.*, 1994) species. Together with oestrogen, progesterone acts to transform the endometrium into a secretory tissue capable of supporting both the pre- and post-implantation conceptus. The plasma concentration of progesterone during pregnancy in the ewe is depicted in Fig. 3. The progesterone concentration in the peripheral plasma gradually rises from the luteal phase level during the first half of pregnancy, markedly increases at about 90 dpc, peaks at about 125 dpc (Bassett *et al.*, 1969; Liggins and Thorburn, 1994; Thorburn *et al.*, 1977) and falls in the last few days before parturition (Bassett *et al.*, 1969; Challis and Lye, 1994; Liggins and Thorburn, 1994; Stabenfeldt *et al.*, 1972). In the ewe, during the first third of pregnancy, progesterone is produced by the corpus luteum, and as with humans (Lye, 1996), production is taken over by the placenta at about 50 dpc and subsequent removal of the ovaries does not compromise development of the foetus (Casida and Warwick, 1945; Denamur and Martinet, 1955).

Progesterone also believed to aid in the maintenance of uterine immune system during pregnancy in the ewe (Rahman, 2002). It has been reported that a systemic hormonal signal of maternal or foetal origin activates and increase the number of a specific immune cells in the uterine endometrium of ewe (Majewski *et al.*, 2001) which protect the foetus from maternal immune rejection. These cells are known as intraepithelial large granulated lymphocytes (LGL) or gamma-delta TcR positive large granulated lymphocytes ($\gamma\delta$ TcR⁺ LGLs). Progesterone could be a prime candidate for this systemic hormonal signal (Hansen, 1998). These $\gamma\delta$ TcR⁺ LGLs remain highest level in the uterus of the ewe throughout the final stage of pregnancy and even just before the onset of parturition (Rahman *et al.*, 2002).

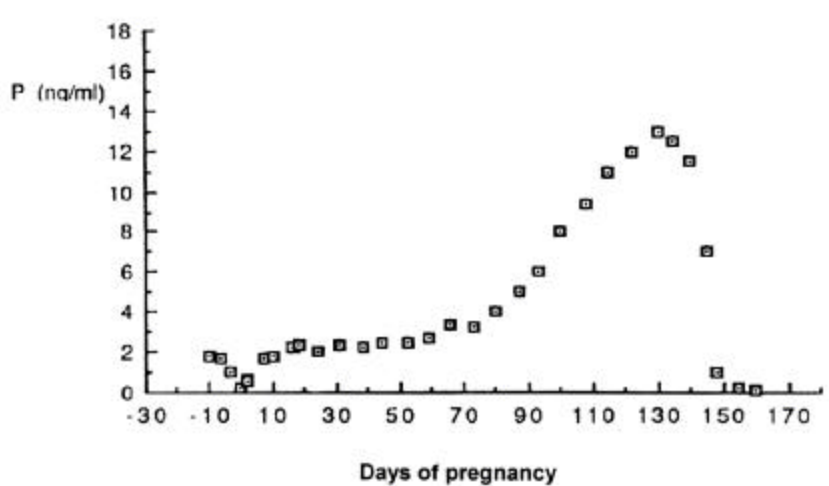


Fig. 3. Progesterone levels in the peripheral plasma of the ewe throughout the pregnancy period. Adapted from Bassett and colleagues (1969)

The major oestrogens present in the maternal plasma of the ewe are the sulpho-conjugates of oestrone and oestradiol-17 α , the concentration of which increases progressively from 70 dpc to parturition (Carnegie and Robertson, 1978; Currie *et al.*, 1973). Until 120 dpc, the concentration of free oestrone and oestradiol-17 β in the maternal plasma remains at a low level, but then increases gradually, before undergoing a sudden and rapid rise in the last days of pregnancy (reviewed in Liggins and Thorburn, 1994). This dramatic increase in free oestrogens coincides with a major increase in the oestrogen sulpho-conjugates in the foetal and maternal plasma (Liggins and Thorburn, 1994).

Prostaglandins, particularly PGF, are very important in the parturition of the ewe and the doe, with increasing levels in the venous plasma in the last days before parturition (Liggins *et al.*, 1972). The concentration of progesterone, oestradiol-17 α and PGF in the venous plasma of a doe during the last week of pregnancy, parturition and post-partum is depicted in Fig. 4.

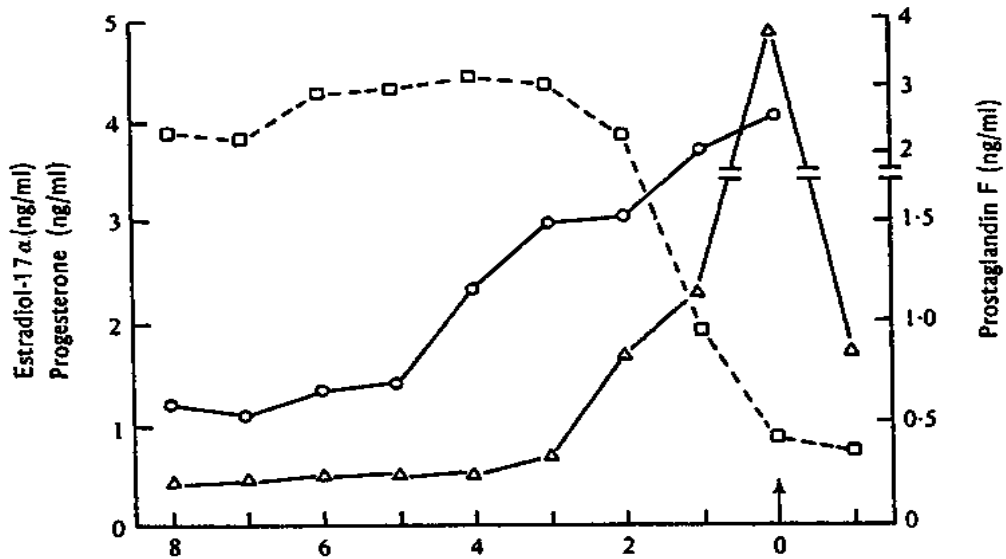


Fig. 4. Changes in the concentration of oestradiol-17 α ($\frac{3}{4}$ O $\frac{3}{4}$), progesterone (---□---) and PGF ($\frac{3}{4}$ D $\frac{3}{4}$) in the uterine venous plasma of a doe before and after parturition. Adapted from Umo and colleagues (1976)

ENDOCRINE PROFILES AT PARTURITION

Parturition is the physiological process by which the pregnant uterus delivers the foetus/foetuses and placenta from the mother. The onset of parturition is associated with a switch from a progesterone-dominated state to an oestrogen-dominated state (Liggins and Thorburn, 1994). The foetus of an ewe attains a weight of about 4 kilograms close to parturition. Parturition is triggered by the foetus and is completed by a complex interaction of endocrine, neural, and mechanical factors (Jainudeen and Hafez, 2000) in which both the foetal and maternal mechanisms play essential roles. In the ewe, the foetal endocrine system plays a major role. During the final stage of gestation, the production and secretion of cortisol by the foetal adrenal gland (Bazer and First, 1983; Thorburn, 1991) induces a fall in the maternal progesterone concentration, which initiates parturition. This cortisol induces the placental 17 α -hydroxylase to catalyse the conversion of progesterone to oestrogen (Liggins and Thorburn, 1994) resulting in increased oestrogen:progesterone ratios, which play an important role in the increased synthesis and release of prostaglandins, activation of the myometrium and ripening of the cervix (Challis and Lye, 1994). Prostaglandins, particularly PGF, play a central role in myometrial contraction, which begins 6–18 hours before delivery (Lye, 1996). Along with relaxin, oestrogen causes a relaxation of the birth canal, especially the cervix and the vagina (McDonald, 1989), and helps to facilitate the birth of the foetus. Oxytocin is not a pre-requisite for the parturition in the ewe (Liggins and Thorburn, 1994) but it facilitates the delivery of the foetus and placenta by inducing forceful uterine contractions (Glatz *et al.*, 1981).

It has been reported that at the onset of parturition – when progesterone concentration decline in the blood plasma – a dramatic decline in the proportion of $\gamma\delta$ TcR $^+$ LGLs occur (Fox *et al.*, 1998, Rahman *et al.*, 2002). This is mainly due to the apoptosis of LGL in the luminal epithelium and migration of these cells to the uterine lumen (Rahman *et al.*, 2002). Withdrawal of progesterone during the onset of parturition (Basset *et al.*, 1969; Wooding and Flint, 1994), which could in turn cause the onset of apoptosis, would lead to the disappearance of the LGLs (Rahman *et al.*, 2002). It has also been reported that the LGLs seem to play a major role through de-granulation to facilitate detachment of fetal membranes and to protect the uterus from microbial infection at a time when the uterine cervix is open to the environment (Rahman, 2002; Rahman *et al.*, 2002).

Therefore, the interaction of hormones with immune cells have very important roles in the healthy birth of a lamb as well as for the protection of infection and maintenance of uterine integrity in the ewe.

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