13. PREGNANCY

RECOGNITION OF PREGNANCY

A crucial step in the reproductive process is the maternal recognition that the fertilized embryo is present in the uterus.

If fertilization has been successful, a signal has to be conveyed to the mother to prevent the destruction of the CL. By doing this, the system remains with high progesterone concentrations, which maintain the uterine environment suitable for implantation.

In ruminants, ewes and cows, protein of embryonic origin were identified as the signalling molecules. These proteins were called ovine and bovine trophoblastic protein 1 respectively (oTP-1 and bTP-1). Further characterization of these proteins revealed that both molecules belong to a family of proteins called interferons. Therefore, a new denomination of ovine and bovine interferon λ, oINF-λ and bINF-λ, respectively, has been implanted (Fig. 13-1). These molecules are secreted by trophoblastic cells during a very restricted window of time, approximately within days 13 to 25 following ovulation.

The mechanism of action of these molecules is through the inhibition of the synthesis of Ot receptors in the endometrium, thus preventing the production of PGF_{α}.

In swine, estrogen produced by the elongating filamentous blastocyst during day 11 and 12, exerts changes in the endometrium. The PGF_{α} produced is redirected, towards the lumen of the uterus where it is catabolized instead of allowing it to move towards the uterine vein and then, through the counter current mechanism reach the ovarian artery.

In the bitch, no signal of pregnancy is needed given that the life of the CL is prolonged even if the animal does not get pregnant.

ENDOCRINE PATTERNS

Regardless of the variations in length of gestation, sources, concentration and specific hormone profile observed among species; there are some general common endocrine patterns which characterize all pregnancies (Fig. 13-2).

Estrogens. Estrogens are present throughout pregnancy with a tendency to increase towards the end of gestation.

Progesterone. At the beginning, this hormone increases normally in concentration. The highest concentration observed during the luteal phase of the estrous cycle. It is maintained at high levels during most of the pregnancy and, with the exception of the mare, its levels are significantly decreased before the initiation of parturition.
SOURCE AND FUNCTION OF HORMONES DURING PREGNANCY

Throughout pregnancy, the source of a particular hormone may change. For example, the CL is essential to maintain pregnancy in the ewe, because of its progesterone secreting capabilities. During the last third of gestation, the P₄ required can be fully supplied by the placenta. The function of a particular hormone can be diverse and even opposite during different stages of gestation. Steroids play an important role during pregnancy and parturition.

**Estrogen**

The main role of estrogen in early gestation is to prime tissues for progesterone action. Part of this function is exerted in the uterus by enhancing epithelial cell multiplication and hypertrophy of uterine smooth muscle. It also facilitates deposition of glycogen in uterine muscle and increases vascularization of the uterus. Later in gestation, it increases uterine metabolism to cope with the increasing growth requirements of the fetus (Fig. 13-3).

The most potent estrogen is estradiol-17β, followed by estrone. They are initially produced in the ovary and then the yolk sac, as well as, the chorioallantois of the conceptus. Estrogens are antiluteolytic in the pig and luteotropic in the rabbit. By day 12, the pig embryo produces a large amount of estrogens which are believed to modify catabolism of PGF₂α in such a way that the CL does not regress; therefore, serving as the indicator of parturition to the sow. Neither the fetus nor the placenta have the full complement of enzymes to synthesize estrogens, therefore, some steps are carried out in each section of what is known as the feto-placental unit.

**Progesterone**

The initial role of progesterone is to stimulate development of endometrial glands and production of histotroph or uterine milk. This action is only successful if the uterus has previously been exposed to estrogens. Close to parturition it stimulates lobulo-alveolar growth in the mammary gland (Fig. 13-4).

Finally, it produces modifications in the behaviour of the pregnant animal, that are conducive to maintenance of pregnancy. The other essential action is to maintain uterine quiescence to permit attachment and maintenance of pregnancy. Progesterone is initially produced by the CL during the estrous cycle. If the CL is removed from the cow, sow, rabbit, goat, rat or mouse at any point during gestation, pregnancy would be terminated. These species are therefore called CL dependent (Fig. 13-5).
In late pregnancy, the mare, ewe, queen, and bitch are supplied with sufficient progesterone by the feto-placental unit to sustain pregnancy. Therefore, the CL is not essential throughout gestation, thus these are placental-dependent species (Fig. 13-6).

The mare uses yet another mechanism to supply enough progesterone to support early gestation by generating secondary CL to sustain pregnancy between the second and fifth month.

Secondary CL develops from follicles which grow after day 35 of pregnancy as a result of stimulation by PMSG (eCG) which is produced by endometrial cups. All CL become atretic in the mare by day 150. A negligible amount of progesterone is produced by the maternal and eventually fetal adrenal cortex (Fig. 13-7).

**Gonadotrophins**

**Follicle stimulating hormone**

FSH does not play any significant role during pregnancy in most species. The only exception is with the mare where FSH initially supports the development of the follicles which will become secondary CL. This occurs before the endometrial cups are capable of producing eCG (Fig. 13-8).

**Lutenizing hormone**

During early pregnancy, LH is required as a luteotropin to maintain the CL functioning properly. After the LH surge associated with ovulation, the secretion of this hormone by the gonadotropes in the pituitary is low but constant. Suppression of the tonic secretion of LH results in CL regression and the end of pregnancy. The luteotrophic effects of LH are supported by prolactin and later in pregnancy by choriomammotropins or placental lactogens (Fig. 13-8).
Relaxin

Very low levels can be detected in most animals during the first half of pregnancy. In the second half of pregnancy, a small elevation has been noted in the cow, sow, queen and rabbit doe. The source of relaxin during this period is not clearly known, but it is believed that the placenta produce it. Neither is clearly understood its physiological role during gestation. Close to parturition when the CL starts to regress, it releases a significant amount of relaxin. Relaxin acts on fibrocartilaginous ligaments of the pubic symphysis and other bones of the birth canal making them relax to allow dilation and expulsion of the fetus at parturition. The effects of this hormone are potentiated by previous exposure to estrogens (Fig. 13-9).

Oxytocin

Produced by the posterior pituitary, it has no identified role during gestation. Its role is to stimulate smooth muscle contractility. This function contributes to uterine motility, facilitating the transport of sperm to the fertilization site, and at parturition, speeding expulsion of the fetus. A luteolytic role, through stimulation of uterine PGF2α, has been determined in the cow, ewe and nanny. In the pregnant animal, this is blocked by the conceptus, by the mechanisms involving the reduction in oxytocin receptors in smooth muscle. Otherwise, every time a female is milked, it would compromise pregnancy (Fig. 13-10).

Steroids

Steroid production by the feto-placental unit is carried out by a complement of enzymes available either in the placenta or in the liver of the fetus, thus the name feto-placental unit.

The main characteristic of this functional association between the fetus and the temporary organ placenta, is based on the fact that all steroids follow a clearly defined sequence of synthesis. This starts with cholesterol, which has 27 carbons. These molecules are then converted to pregnanes with 21 carbons. The 21 carbon structures are further converted to 19 and 18 carbon respectively (Fig. 13-11).

The problem lies in the fact that the placenta lacks the enzyme to convert 21 carbon structures into 19 carbon structures. These enzymes are only present in the fetus in sufficient quantities. In turn, the fetus has a very low capacity to aromatize 19 carbon steroids into 18 carbon steroids. This step takes place in the placenta.
The cholesterol is usually of maternal origin and reaches the placental tissue through circulation where it is converted to pregnenolone. Pregnenolone can diffuse back to the maternal circulation or to the fetal circulation. In the maternal circulation, it can be further catabolized in the liver of the mother.

Alternatively, the pregnenolone in the placenta can be converted to progesterone, which in turn can diffuse to either the fetal or the maternal compartment. In the fetal compartment, usually progesterone is further used as substrate to synthesize glucocorticoids. The pregnenolone which diffuses into the fetal compartment can then be converted to pregnenolone sulphate or to dehydroepiandrosterone (DHEA), (a 19 carbon structure). The DHEA can then be returned to the placental tissue where it can be converted to an intermediary, androstenedione before it is aromatized to estrone (E₁), (an 18 carbon structure). The pregnenolone—sulphate in the fetus can in turn be converted to dehydroepiandrosterone sulphate (DHEAS), which is then returned to the placental tissue where the sulphate is removed and converted to DHEA or it can be directly aromatized to estriol (E₃). Alternatively, the fetal DHEAS can be further hydroxylated to 15, 16-di-OH-DHEAS before moving to the placenta where it can be converted to estetrol (E₄). Finally, the estrone in the placenta can be either converted to estradiol (E₂) in the placenta or returned to the fetal or maternal compartment where it can be also converted to E₂ (Fig. 13-12).

PLACENTA

The placenta is a transient organ which serves the purpose of lung, kidney, GI and heart to the fetus. As such, it permits the oxygenation of the blood, removes the waste products, and provides the nutrients and their movements (Fig. 13-13).

CLASSIFICATION OF PLACENTAS

The placenta can be classified according to the type of attachment of the chorionic villi to the uterus or based on the number of layers separating the maternal and the fetal circulation. The first classification is more adapted to describing the anatomical appearance of the placenta while the second classification better describes aspects of its functionality. More specifically the second classification addresses the issues of the barrier placed on the translocation of macromolecules between maternal and fetal circulation, in particular, immunoglobulins (Fig. 13-14, 13-15).
PARTURITION

The process of parturition can be divided into three distinct stages: labour, fetal and placental expulsion including uterine involution (Fig. 13-16).

Labour

The exact signal which starts the process of labour and subsequent expulsion of the fetus is not well identified. The general principle is that some type of stress triggers the initiation of the chain of events leading to parturition. There are proponents who believe that after reaching certain size, the placenta is unable to supply sufficient nutrients and oxygen to the fetus. Others suggest that physical pressure within the uterus may serve as a stressor. However, the variability in body size at birth, still maintaining the same gestation length suggests that there must be other contributing mechanism yet unknown.

What is clearly understood is that labour commences when the uterus starts contracting. Uterine motility is prevented during gestation by the "progesterone block" which promotes uterine quiescence. Uterine mobility is regained when progesterone levels decrease. There is increasing evidence, however, that the absolute concentration of progesterone is not as important as the ratio progesterone:estrogen (Figs. 13-17, 13-18).

Whatever signal or stress exists, it is detected by the hypothalamus of the fetus which activates the fetal pituitary-adrenal axis to elevate fetal cortisol. The cortisol then reaches
the placenta and, through several steps, is converted to estrogens. Thereafter, estrogens stimulate prostaglandin production by the endometrial tissue of the uterus. Prostaglandin \( F_{2 \alpha} \), reaches the ovary and exerts its luteolytic effect destroying the CL (Fig. 13-18).

In CL dependent species this mechanism is mainly responsible for lowering circulatory concentration of progesterone, thus modifying the progesterone:estrogen ratio and starting labour (Figs. 13-19, 13-20).

In those species in which progesterone is mainly produced by the placenta the elevation of cortisol triggers a shift in feto-placenta steroid synthesis from progesterone to estrogen. This biosynthetic shift results in an overall decrease in the progesterone:estrogen ratio which starts uterine motility (Figs. 13-21, 13-22).

**Fetal expulsion**

Once labour commences, the uterine contractions push the fetus towards the cervix. Physical stimulation of the cervix results in oxytocin production. Oxytocin reaches myoepithelial cells in the uterus which further strengthens the uterine contractions until the fetus is expelled through the birth canal, which has been enlarged by the relaxation of the pubic bones.

In litter bearing species, the passage of the fetus through the birth canal and the stimulation of the mammary gland by the suckling action further stimulate oxytocin production with resulting subsequent expulsion of other fetuses.

**Placental expulsion and uterine involution**

After the last fetus is expelled, the uterus continues contracting until all placentas are expelled from the uterine cavity. Simultaneously, the uterus shrinks to a smaller size at a very fast rate.
Figure 13-20. Endocrine changes leading to the initiation of parturition in CL dependent species

PLACENTA DEPENDENT
(THE FPU IS RESPONSIBLE FOR MOST PROGESTERONE PRODUCTION)

- Fetal P-A axis Cortisol
- Switch production of P₄ to Es by FP unit
- Destroy CL, Increase relaxin

Labour starts

Figure 13-21. Endocrine changes leading to the initiation of parturition in placental dependent species
Figure 13-22. Endocrine changes leading to the initiation of parturition in placental dependent species