***Chapter 1*:**

GENERAL INTRODUCTION

Tremendous progress has been made in unravelling the complex endocrine relationships that control ovarian function in farm animals. This has provided the possibility of manipulating oestrus and ovulation in female animals with an associated opportunity to increase the efficiency of production. Oestrous synchronisation (implying artificial induction and synchronisation of oestrus and ovulation in a group of females) makes it possible to control the time of oestrus and ovulation and increase the proportion of females inseminated during a desired breeding period. Insemination can be carried out at a prearranged or fixed time(s) if the method is sufficiently reliable or performed according to detected oestrus with reduced labour required for routine daily observations. Therefore, oestrous synchronisation facilitates the use of artificial insemination (AI) and multiple ovulation and embryo transfer (MOET) programs, thereby allowing greater exploitation of genetically superior breeding animals, introduction of new bulls, blood lines or breeds and cross breeding, resulting in faster improvement in traits of economic importance. In addition, oestrus synchronisation may enhance production efficiency because initial conception could be induced at an earlier age and the calving to conception interval, hence the calving interval and possibly breeding and calving season, could be reduced.

Improvements have been made in enhancing the efficacy of oestrus synchronisation. However, the methods presently in use are rarely cost-effective and are associated with uncertainty as to level of success. Any method of oestrus synchronisation must be economical and feasible to manage. An ideal method of oestrus synchronisation should result in high rates of response to treatments initiated at any stage, tightly synchronised oestrus and ovulation, normal fertility at the regulated ovulation, and normal return to oestrus and fertility at repeated services. Such a method should be able to minimise the effect of factors that may influence the response.

The present thesis examines the efficacy of different regimes of oestrous synchronisation in which recent techniques to manipulate oestrus and ovulation in post-partum beef suckled cows were used. Factors influencing success of the synchronisation treatments are of concern. A selective review on the physiology of important reproductive processes related to the oestrous cycle and the post-partum period as well as follicular growth and development leading to ovulation in female cattle is presented to provide insights into the physiological regulation of oestrus and ovulation as the basis for artificial manipulation of these two events. Methods for inducing and synchronising oestrus and ovulation are also summarised to review the treatment protocols, advantages and disadvantages as well as the main factors influencing success of each method. Finally, three experiments which examine factors affecting reproductive responses to oestrous synchronisation on two comercial beef properties in New England, Australia are reported.

Chapter 2:

REPRODUCTIVE PHYSIOLOGY OF FEMALE CATTLE

(LITERATURE REVIEW)

2.1 INTRODUCTION

Artificial manipulation of the reproductive cycle of female cattle involves the use of exogenous hormone treatments to regulate oestrus and ovulation either in individual animals or in groups. Major advances in the understanding of reproduction in farm animals have brought about the possibility to artificially induce and synchronise the two important reproductive events. The essential basis for such artificial control of reproduction in cattle should include the physiological mechanisms governing the oestrous cycle and the post-partum period as well as the underlying dynamics of follicular growth and development leading to oestrus and subsequent ovulation. This chapter reviews the literature on the important aspects of the reproductive physiology and provides the physiological background for artificial control of oestrus and ovulation in general and for the following experiments in particular.

2.2 THE BOVINE OESTROUS CYCLE AND OVULATION

Following puberty, oestrus occurs many times a year at fairly regular intervals and is accompanied by spontaneous ovulation in female cattle. The interval from the beginning of one oestrus to the next is called the oestrous cycle. The average cycle length is 21 days with considerable variation within and between animals (Peters *et al.*, 1987a). Throughout the cycle, the reproductive tract undergoes changes as a result

of the action of the pituitary and ovarian hormones. In addition to initiating the period of behavioural receptivity to the male and subsequent ovulation, these hormones also regulate the tubular genitalia for its role in receiving sperm, producing eggs and supporting possible conception, attachment and nourishment of the embryo and the foetus (Salisbury *et al.*, 1978).

2.2.1 CHANGES DURING THE OESTROUS CYCLE

Many authors (Cupps *et al.*, 1969, McDonald, 1969, Salisbery *et al.*, 1978, Frandson, 1986, Peters *et al.*, 1987a, Arthur *et al.*, 1989) divide the oestrous cycle into several phases, namely *proestrus, oestrus, metoestrus* and *dioestrus* (Fig. 2.1). The morphological, secretory and behavioural characteristics of these phases can be briefly summarised as follows:

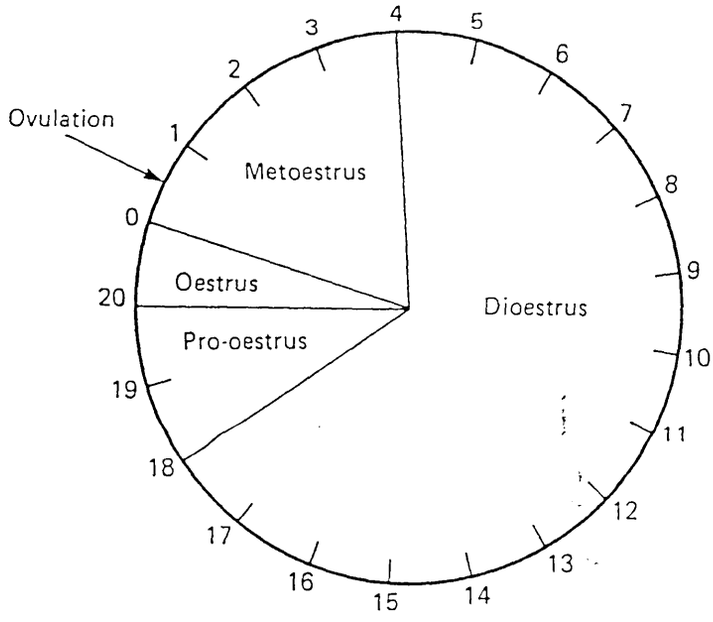

Figure 2.1: *The four phases of the bovine oestrous cycle* (from Peters *et al.*, 1987a)

2.2.1.1 Proestrus

This is the period that immediately precedes oestrus. During this phase, from the wave of large antral follicles,one follicle, which is destined to ovulate, begins to increase in size, primarily by increasing the volume of follicular fluid. This occurs as the *corpus luteum* (CL) regresses; the vaginal wall thickens; the uterine vascularity and cell growth of the tubular genitalia are increased; and genital glands increase secretary activity. All of these changes are in preparation for the forth coming oestrus, fertilisation and possible pregnancy .

2.2.1.2 Oestrus

Oestrus is the short period of sexual receptivity (heat) in the female during which the vulva is swollen, the mucous membrane of the cervix swells considerably, and clear, viscous and cohesive mucus often hangs in strings from the vulva. The female on heat shows restlessness, craves for companionship and stands to be mounted.

Oestrus occupies a short part of the cycle, which typically lasts for between 6 and 30 hours with an average of 17 hours. Shortly after this phase (10-12 hours) ovulation occurs. Immediately before ovulation, the follicle becomes larger and turgid, with the ovum inside undergoing maturation changes. The follicle then ruptures and the ovum is expelled from the follicle into the upper part of the oviduct.

2.2.1.3 Metoestrus

The phase that follows oestrus is known as metoestrus and is characterised by a sudden cessation of behavioural oestrus. The female is no longer receptive to the male and the uterine, cervical and vaginal glands reduce secretion.

After ovulation, the lining of the ruptured follicle begins to grow inward; the cells lining the cavity left by the rupturing of the follicle increase in size, multiply and become full of fat droplets. The reorganised structure is a new endocrine gland called the *corpus luteum*, or "*yellow body*".

2.2.1.4 Dioestrus

Dioestrus is the phase of quiescence between oestrous cycles dominated by the presence of the *corpus luteum*. If pregnancy does not occur, the *corpus luteum* reaches maturity about 8 days after ovulation and functions for a further 8-9 days before it finally regresses at the commencement of proestrus, thus initiating a new oestrous cycle.

Dioestrus may be replaced by gestation and post-partum anoestrus. If the ovum is fertilised after ovulation, secretions from a functional *corpus luteum* are necessary for the implantation and subsequent nourishment of the developing embryo(s) and the formation of the placenta; the endometrial lining of the uterus thickens, and the uterine gland and muscles increasingly develop throughout gestation, while the *corpus luteum* remains intact for the majority of the period. After calving the cow undergoes a post-partum anoestrous period before cycling again.

The following sections will elaborate on the mechanisms underlying the changes during the bovine oestrous cycle described above.

2.2.2 HORMONAL CONTROL OF THE OESTROUS CYCLE

In cattle, the oestrous cycle is controlled by complex feedback mechanisms involving steroids and proteins from the ovaries, gonadotrophins from the pituitary gland and hormones from the hypothalamus (Fig. 2.2.). This control system ensures that in more than 96% of females only one follicle ovulates per oestrous cycle (Webb *et al.*, 1992). Changes in blood plasma concentrations of hormones reflect their patterns of secretion and these are shown schematically in relation to the stage of the cycle in Figure 2.3.

From the viewpoint of hormonal interactions each oestrous cycle is composed of two phases: (1) the *luteal* (or *progesteronal*) *phase* that includes metoestrus and dioestrus and (2) the *follicular* (or *oestrogenic*) *phase* that includes proestrus and oestrus. However, there is some hormonal overlap between phases.

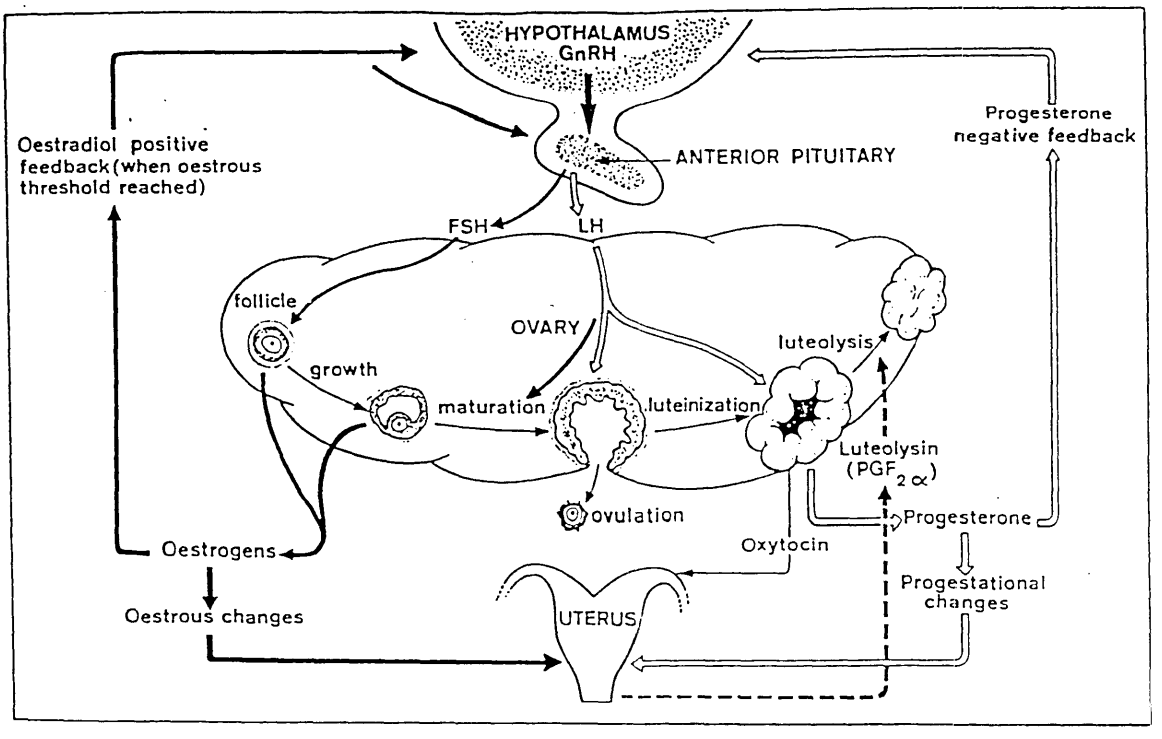

Figure 2.2: *Interrelationship between the hypothalamus, pituitary and ovary during the oestrous cycle and ovulation in the cow* (from Peters *et al.*, 1987a)

2.2.2.1 Luteal Phase

This phase is characterised by the presence of the *corpus luteum*. After ovulation the granulosa cells of the ruptured follicle proliferate and undergo structural and biochemical changes that result in the formation of the *corpus luteum* which secretes large amounts of progesterone (Niswensder *et al.*, 1988). Progesterone levels in plasma are closely correlated with the growth, maintenance, and regression of the *corpus luteum* (Hansel *et al.*, 1983, Jainudeen *et al.*, 1993). Plasma progesterone concentrations begin to rise from about day 4 of the cycle (oestrus is day 0), reaching a peak around day 8 and remaining high until day 17, then sharply decreasing back to basal levels in the following follicular phase (Peters *et al.*, 1987a).

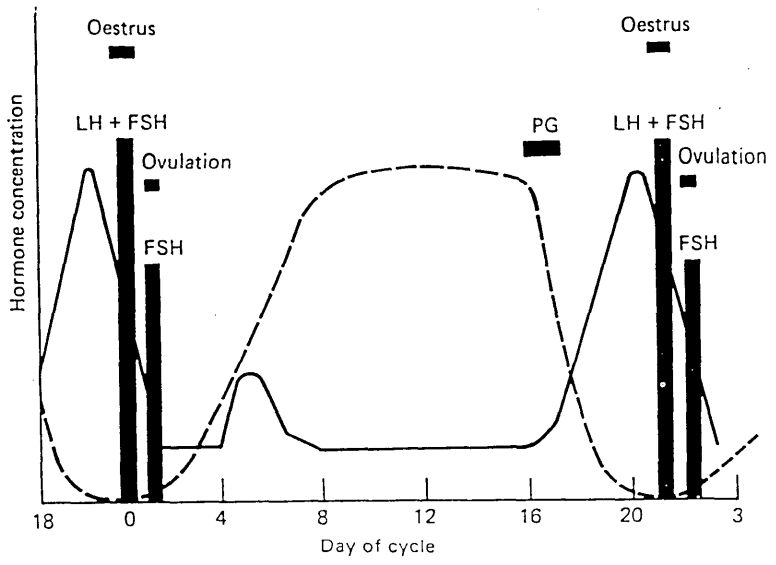

Figure 2.3: *Changes in plasma levels of hormones during the bovine oestrous cycle*: _____ oestradiol; --------- progesterone (from Peters *et al.*, 1987a)

Progesterone together with other ovarian hormones plays an important role in the control of the pituitary secretion of gonadotrophins so that during this phase FSH (*follicle-stimulating hormone*) and LH (*luteinizing hormone*) are only secreted at tonic (basal) levels (Fink, 1988). On the other hand, the pituitary gonadotrophins are importantly involved in controlling the secretion of ovarian hormones.

LH is considered the primary hormone responsible for regulating the synthesis and secretion of progesterone of the *corpus luteum*, while there is controversy regarding the role of pituitary prolactin in the regulation of luteal function. LH stimulates progesterone production by activating adenylate cyclase on luteal cells to produce cAMP which activates protein kinase. Increased protein kinase activity can influence the luteal cell via several different mechanisms involved in steroidogenesis (Hansel *et al.*, 1983, Niswender *et al.*, 1988). However, luteal secretion of progesterone seems to be also controlled by other mechanisms not involving LH, which have as yet to be clearly elucidated.

Under the influence of basal levels of FSH and LH, the latter being secreted in a pulsatile manner, the granulosa cells of the ovarian follicles secrete oestrogens, mainly oestradiol-17β, and inhibin (Fink, 1988). After fixation on the cell membrane receptors, FSH and LH also stimulate adenylcyclase activity, causing an increase of

intracellular cAMP. In the next step, cAMP activates protein kinase which enhances activity of specific enzymes (Gore-Langton *et al.*, 1988). With activated enzymes the granulosa cells of all growing follicles produce inhibin, a glycoprotein hormone with a long half-life (de Jong, 1987, Findlay *et al.*, 1990). In addition, the granulosa cells of the large antral (dominant) follicles secrete oestradiol under FSH influence. However, the production of oestradiol is dependent on the supply of androgenic precursors produced by the theca. The theca interna cells are stimulated by LH to produce androgens, which in turn traverse the follicular basement menbrane to be utilised for oestrogen synthesis in an FSH-stimulated aromatisation reaction within the granulosa cells (Gore-Langton *et al.*, 1988).

The basal concentration of FSH in peripheral plasma is determined by stimulatory effects of gonadotrophin releasing hormone (GnRH) from the hypothalamus and inhibitory effects of secretions from the ovaries, such as steroids (oestrogens and progesterone). GnRH stimulates gonadotrophin release, increases pituitary responsiveness, and stimulates gonadotrophin synthesis and the recruitment of pituitary cells into the gonadotrope pool (Fink, 1988). The levels of pituitary gonadotrophin in turn influence the secretion of GnRH in a "short loop" feedback mechanism (Hafez, 1993b).

During the luteal phase, both oestradiol and progesterone control the secretion of FSH and LH through a "long loop" of negative feedback by suppressing its release from dispersed pituitary cells via inhibition of GnRH secretion (Hansel *et al.*, 1983, Fink, 1988, Findlay *et al.*, 1991). In addition, inhibin, with its long half-life, can contribute to the overall level of negative feedback on FSH secretion so that the concentration of FSH is kept at a level below that required to activate or recruit small antral follicles, but above that necessary to maintain the development of the dominant follicle (Taya *et al.*, 1991). Injection of anti-inhibin serum markedly increases plasma concentrations of FSH (but not LH) and therefore stimulates the rapid growth of a large number of follicles (Kaneko *et al.*, 1993).

The secretion of oestradiol from dominant follicles seems to determine the fluctuation in the patterns of FSH which plays a crucial role in determining follicular recruitment.

Ovarian follicles then go through selection, dominance and atresia phases (see details in section 2.3.2) coincident with transient increases and decreases in FSH (Sunderland *et al.*, 1994). During the luteal phase, FSH rhythms continue underlying follicular waves. From these waves one follicle will normally progress through the final preovulatory maturation during the follicular phase after the concentration of progesterone falls due to the regression of *corpus luteum* (luteolysis) toward the end of the luteal phase (Jochle *et al.*, 1980).

Luteolysis, which occurs between day 17 and 18 of the normal cycle is caused by prostaglandin F2α (PGF2α) secreted episodically from the endometrium after a certain length of exposure to oestradiol, progesterone and oxytocin which is produced in the *corpus luteum* itself (Hansel *et al.*, 1983, Peters *et al.*, 1987a, Flint *et al.*, 1992). Lamming *et al.* (1995) demonstrated that progesterone and oestradiol control the development and responsiveness of endometrial receptors of oxytocin, which stimulates the release of PGF2α. The episodic nature of PGF2α, which is important for luteolysis, is thought to result from a positive feedback loop, in which oxytocin secreted by the *corpus luteum* acts on the uterus to stimulate PGF2α secretion, which in turn acts on the *corpus luteum* to release more oxytocin (Flint *et al.*, 1992).

Niswensder *et al.* (1988) proposed a number of different mechanisms to explain the luteolytic effects of PGF2α: (a) a rapid and dramatic decrease in luteal blood flow, (b) a reduction in the number of LH receptors, (c) an uncoupling of the LH receptors from adenylate cyclase, and (d) a cytotoxic effect. Although there is good evidence for each of these actions, they are not conclusive yet and further studies are necessary to clarify our understanding of these mechanisms. The process of luteolysis eliminates the barrier for the resumption of the subsequent follicular phase (Jochle *et al.*, 1980). However, the *corpus luteum* of pregnancy is resistant to the luteolytic effect of PGF2α until the end of gestation (Hafez, 1993d).

2.2.3.2 Follicular Phase

The follicular phase, the period from *corpus luteum* regression to the following ovulation, is short, being about 4 to 5 days in cows (Hafez, 1993d). During proestrus, gonadotrophins induce final follicular maturation, resulting in increased secretion of oestradiol. As the granulosa cells of the large antral follicles have already acquired maximal aromatase activity (induced by FSH), the increased secretion of LH (in the absence of progesterone) stimulates the progressive increase in secretion of oestradiol by the increased supply of androgen precursors from the theca cells. The rapid rise in oestrogen level is an essential factor for the initiation of behavioural oestrus (Fig. 2.4.) and the changes in the tubular genitalia through stimulating the nervous system and increased vascularity and cell growth (Jainudeen *et al.*, 1993). Allrich (1994) indicates that the effects of oestradiol appear to be "all or none", ie. once a threshold of oestradiol is achieved, oestrus is induced, and additional amounts of oestradiol above threshold do not further enhance the oestrous response (duration and intensity of oestrus).

Since the amount of oestradiol production is now determined not by FSH, which is still inhibited by inhibin, but by LH, which has a high concentration after the regression of the *corpus luteum*, the follicles can break out of the negative feedback loop involving FSH and ovarian steroids to secrete enough oestrogen to induce a massive preovulatory gonadotrophin surge by a positive feedback effect (Hansel *et al.*, 1983, Hafez, 1993b, Jainudeen *et al.*, 1993). The increased oestradiol secretion acts at two levels: the pituitary and the hypothalamus (Fig. 2.5.). Oestradiol increases the sensitivity of pituitary gonadotrophin-producing cells to the hypothalamic gonadotrophin-releasing hormone, GnRH. In addition, it causes the hypothalamus itself to discharge GnRH, which stimulates gonadotrophin secretion of the pituitary gland, through positive feedback on the central system. The termination of the gonadotrophin surge later on is due mainly to the fall in portal plasma GnRH concentrations and, to a lesser extent, to the decline in pituitary responsiveness to GnRH, which is not due simply to exhaustion of pituitary LH stores (Fink, 1988).

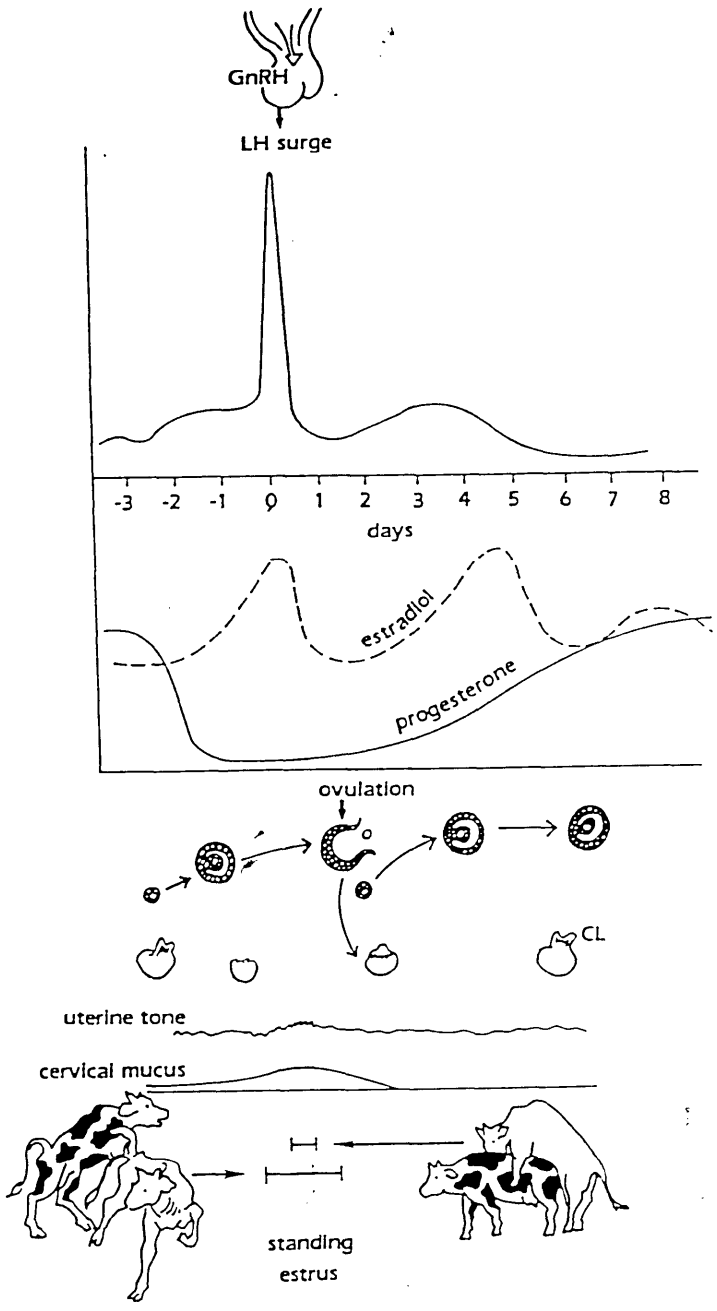

Figure 2.4: *The endocrine, physiological and behavioural changes associated with "standing oestrus" in the cow:* During proestrus, the preovulatory follicle secretes increasing amounts of oestradiol; at this time, the cow may ride other cows and begin to secrete cervical mucus. At the onset of oestrus, peak levels of oestradiol trigger a surge of LH that causes ovulation to occur about 10-12 h after the end of oestrus; uterine tone is maximum, and cervical mucus is copious and watery (from Jainudeen *et al.*, 1993)

The LH and FSH surges, lasting for 6 to 12 hours, induce the final stages of oocyte maturation and are responsible for ovulation (Hafez, 1993b). It is assumed that FSH is needed for the rapid increase in follicular development during this phase and LH is necessary for ovulation (Fink, 1988). On the other hand, the preovulatory LH surge induces the first steps of luteinization of the theca interna and particularly granulosa cells before ovulation (Jochle *et al.*, 1980, Niswender *et al.*, 1988).

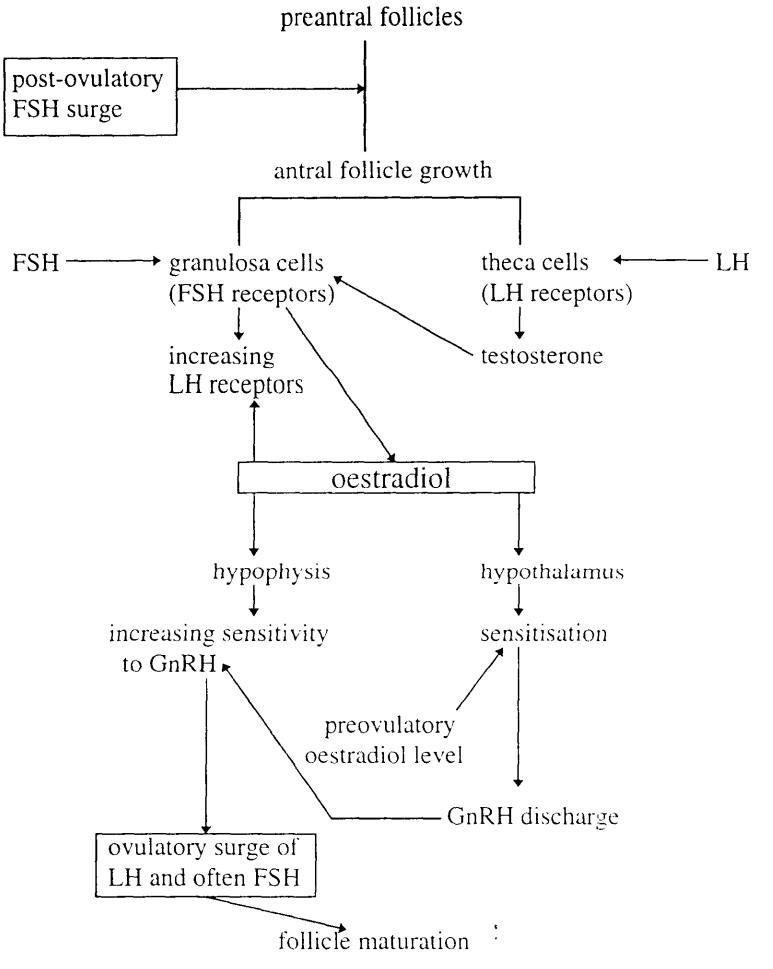

Figure 2.5: *Neuro-endocrine interactions in relation to preovulatory gonadotrophin surge* (from Hafez *et al.*, 1980)

Shortly after starting, the LH surge suppresses the secretion of oestradiol markedly by desensitisation of the theca cells and thus the behavioural manifestations of oestrus abate (Hafez, 1993b). Due possibly to the lack of negative feedback of oestradiol at the level of the anterior pituitary a small second peak of FSH occurs. This FSH rise may trigger antrum formation in the follicle population, which is necessary for the emergence of a new wave of folliclular development after ovulation (Hafez *et al.*, 1980 , Hansel *et al.*, 1983, Peters *et al.*, 1987a, Turzillo *et al.*, 1990).

2.2.3 CONTROL OF OVULATION

Cows are spontaneous ovulators, ie. they do not require coitus for ovulation. Ovulation occurs 24 to 30 hours after the initial maximal gonadotrophin surge (Hafez,

1993b) or 10 to 12 hours after the end of standing oestrus (Jainudeen *et al.*, 1993). Normally, one follicle ovulates per oestrous cycle. Ovulation occurs in response to a combination of physiological, biochemical, and biophysical mechanisms (Fig. 2.6). These involve: neuroendocrine and endocrine mechanisms, GnRH, steroids, and prostaglandins; neurobiochemical and pharmarcologic mechanisms; neuromuscular and neurovascular mechanisms and enzymatic interactions. The relative importance of any of these factors is still equivocal. However, the main features have already been elucidated.

Ovulation is initiated by the gonadotrophin surge, resulting in major changes in the follicle destined to ovulate: cytoplasmic and nuclear maturation of the oocyte, disruption of muculus cell cohesiveness among the granulosa cells, and thinning and rupture of the external follicular wall (Lipner, 1988, Hafez., 1993a).

The gonadotrophin surge suppresses production of the granulosa cell miotic-inhibiting factor, leading to metabolic modification of that follicular layer and miotic resumption of the ovum (egg maturation) (Hafez *et al.*, 1980). Blood flow increases to all follicles; however, the one destined to ovulate not only receives the largest volume of blood, but also has capillaries that are more permeable (Moor *et al.*, 1975). The preovulatory follicle shortly becomes hyperaemic and subsequently oedematous. The andrenergic neurones in the follicle wall are activated either by the LH surge or neurogenically and secrete norepinephrine. Histamine released from mast cells and the α-andrenergic agonist effects may be to enhance the hyperaemia by affecting the contractility of the endothelial cells, the pericytes, and the post-capillary venules (Lipner, 1988).

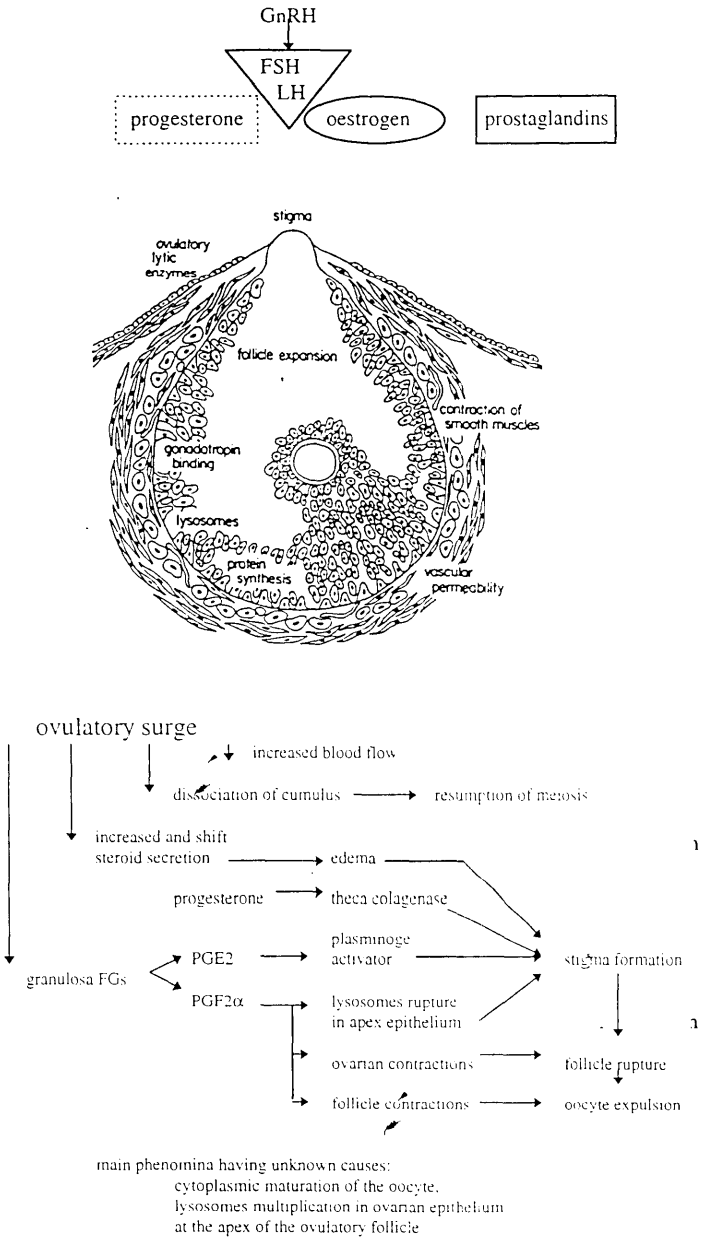

Figure 2.6: *Diagram of some of the mechanisms involved in ovulation* (top). *Tentative synthesis of biochemical processes in ovulation* (bottom) (from Hafez, 1993a)

According to Hafez (1993a), the preovulatory gonadotrophin surge first induces an immediate and temporary rise in steroid levels and then prostaglandins (PGF2α and PGE2α) from follicular tissues. LH initiates protein synthesis which is responsible for cellular differentiation of the membrane granulosa to lutein cells. LH also stimulates the theca interna to increase secretion of progesterone and androgens as well as prostaglandins. In addition, the β-andrenergic agonist effects may contribute to enhanced progesterone secretion. The prostaglandins and progesterone increase plasmalogen activator which converts plasmalogen in the follicular fluid and extracellular oedema fluid to plasmin. Plasmin activates latent collagenase attached to the collagen fibres. The induced collagenolysis and serine proteases then complete the proteolysis of the collagen, decreasing the tensible strength of the follicle wall.

Therefore, prostaglandins and progesterone contribute to the rupture of epithelial cell lysosomes at the follicular apex. Furthermore, PGF2α stimulates the neuro-muscular system in the ovarian stroma and the connective layer of the theca externa of the preovulatory follicle, causing follicular contractions.

Under the conditions described above, the ovarian follicle, having increased in size due primarily to the large amount of fluid produced, exerts pressure on the tunica albuginea of the ovary, resulting in a definite bulging and consequent thinning of the ovarian surface, and eventually it ruptures (Frandson, 1986). After follicular rupture, the thecal neuro-muscular system, stimulated by PGF2α, contributes to the extrusion of the oocyte and the viscous mass, which has been secreted by cumulus cells and spread at the ovarian surface, facilitates the "pick up" of the oocyte by the ovarian fimbriae. The follicular layers are then remodelled terminating in *corpus luteum* formation (Hafez, 1993a).

If pregnancy occurs the *corpus luteum* will exist until the end of gestation under particular conditions (not covered in this review); then, after parturition the cow undergoes a post-partum period to resume the above cyclic activity. The post-partum period has an important impact on reproductive efficiency.

2.3 THE POSTPARTUM PERIOD IN THE COW

2.3.1 THE POSTPARTUM PERIOD AND ITS VARIOUS PROCESSES

2.3.1.1 The Postpartum Period

Parturition is followed by a period of sexual quiescence before oestrous cycles recommence. The post-partum period, or the *puerperium*, is broadly defined as the period from delivery till the time the cow has returned to its non-pregnant state (Bazer *et al.*, 1993). During the post-partum period the cow makes a series of physiological

and anatomic readjustments both in the uterus and ovary for the restoration of her reproductive capacity (Fig. 2.7). These include the involution of the uterus and the resumption of ovarian activities leading to normal oestrous cycles.

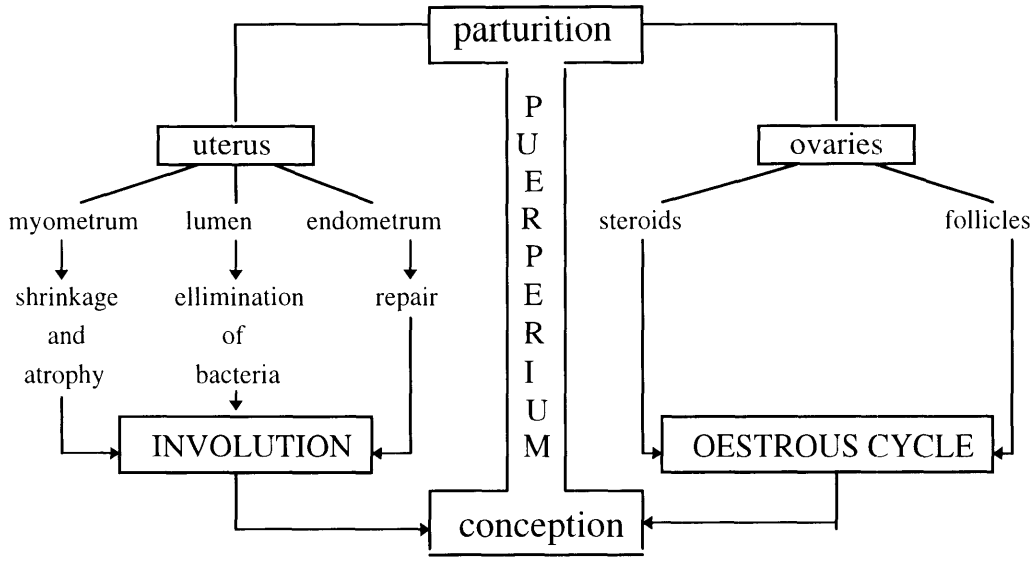

Figure 2.7: *Various processes in the post-partum period*
(from Bazer *et al.*, 1993)

Practically, the post-partum period is measured as the normal post-partum interval (PPI) between parturition and the occurrence of the first oestrus, although the incidence of first post-partum ovulation without oestrous behaviour is relatively high (Murphy *et al.*, 1990, Jainudeen *et al.*, 1993). The reported incidence of silent ovulation at the first post-partum ovulation ranges from 50 to 94% (Allrich, 1994). Murphy *et al.* (1990) reported a study showing that the interval from calving to first ovulation in beef suckler cows was 35.9 ± 3.3 days (range 20-61 days), while the post-partum interval to first oestrus was 54.2 ± 3.2 days (range 19-22 days). In other words, ovulation is not preceded by oestrous behaviour. High levels of oestradiol during late gestation apparently induce a refractory state such that the brain cannot respond to the oestrus-inducing actions of oestradiol at the first post-partum ovulation. Progesterone can "reset" the brain, thus allowing it to respond to subsequent oestradiol exposure; therefore, the *corpus luteum* formed after the first ovulation provides the progesterone

that resets the brain, inducing oestrous behaviour prior to the second post-partum ovulation (Allrich, 1994).

1.3.1.2 Uterine Involution

The restoration of the uterus is necessary for the cow to conceive again. However, Kiracofe (1980) concluded that uterine involution has no relationship to the length of the post-partum period. In practice, uterine involution is not a problem, especially for beef cattle, since it does not affect anoestrus as long as disease conditions do not prevent or delay normal involution. This may be because uterine involution is usually more or less complete before the first post-partum ovulation occurs (Peters *et al.*, 1987a). Nevertheless, uterine involution is a barrier to fertility during the early post-partum period (Short *et al.*, 1990).

Uterine involution is dependent on myometrial contractions, elimination of bacterial infection, and regeneration of the endometrium (Bazer *et al.*, 1993). After calving the uterine horn that carried the foetus is considerably larger than the opposite one. Both horns lack firm muscle tone. The uterine discharge (lochia), composed of mucus, blood, shreds of foetal membranes and maternal tissue and foetal fluids, ceases by the first week after parturition. The weight and size of the uterus soon decrease, the number and size of myometrial cells decrease, and the muscle tone of the uterus gradually improves (Jainudeen *et al.*, 1993). The expulsion of lochia and reduction of uterine size are caused by myometrial contractions. This is due to a sustained release of PGF2α after parturition. In cows that have a relatively long duration of post-partum release of PGF2α, the involution of the uterus is completed within a shorter time (Madej *et al.*, 1984). The sterile conditions of the uterus, which are disrupted after parturition, resume due to uterine defence mechanisms and the increased myometrial activity with the onset of oestrogen activity in the ovary. Together with these processes the endometrium is also regenerated between the fourth and fifth week post-partum (Bazer *et al.*, 1993.).

2.3.1.3 Resumption of Oestrous Cycles and Ovulation

Oestrus and ovulation are usually suspended during the post-partum period due possibly to inhibition at several levels of the hypothalamo-pituitary-ovarian axis (Peters *et al.*, 1990). Ovarian activity may be blocked by preventing the release of GnRH, FSH and LH or ovarian follicles from responding to gonadotrophin stimulation (Bazer *et al.*, 1993). Although many of the mechanisms controlling post-partum anoestrus are not fully understood, an endocrine model has been suggested (Fig.2.8) by Short *et al.* (1990). The PPI is characterised by a transitional period of acyclicity due mainly to impairment of endocrine mechanisms controlling oestrus and ovulation. The post-partum female is prevented from continuing through the normal steps (1 to 6) leading to oestrus and ovulation. There is already some evidence for how each of these steps is involved in the post-partum period.

The functional competence of the hypothalamus and pituitary is decreased for a period after parturition. The major limiting step in the restoration of normal cycles is the rate of LH secretion (McNatty, 1988). During this time the amount of LH in the pituitary is lower, less LH is released in response to either oestradiol or GnRH, and the bioactivity of LH is also lower (Short *et al.*, 1990). This is because during pregnancy high circulating concentrations of progesterone and oestradiol result in a prolonged negative feedback on the hypothalamic-pituitary axis, thus inhibiting the synthesis of LH. Since the synthesis of LH is inhibited for an extended period of time, pituitary stores of LH become depleted and the basal release of LH is diminished during gestation and the early post-partum period (Nett, 1987; Wise, 1990). In addition, the responsiveness of the anterior pituitary to GnRH is reduced, perhaps due to the reduction in the number and size of gonadotrophs during this time (Wise, 1990). Moreover, most evidence suggests that post-partum acyclicity is caused by a failure of GnRH release, resulting in a deficient secretion of gonadotrophins as reviewed by McNatty (1988) and Bazer *et al.* (1993).

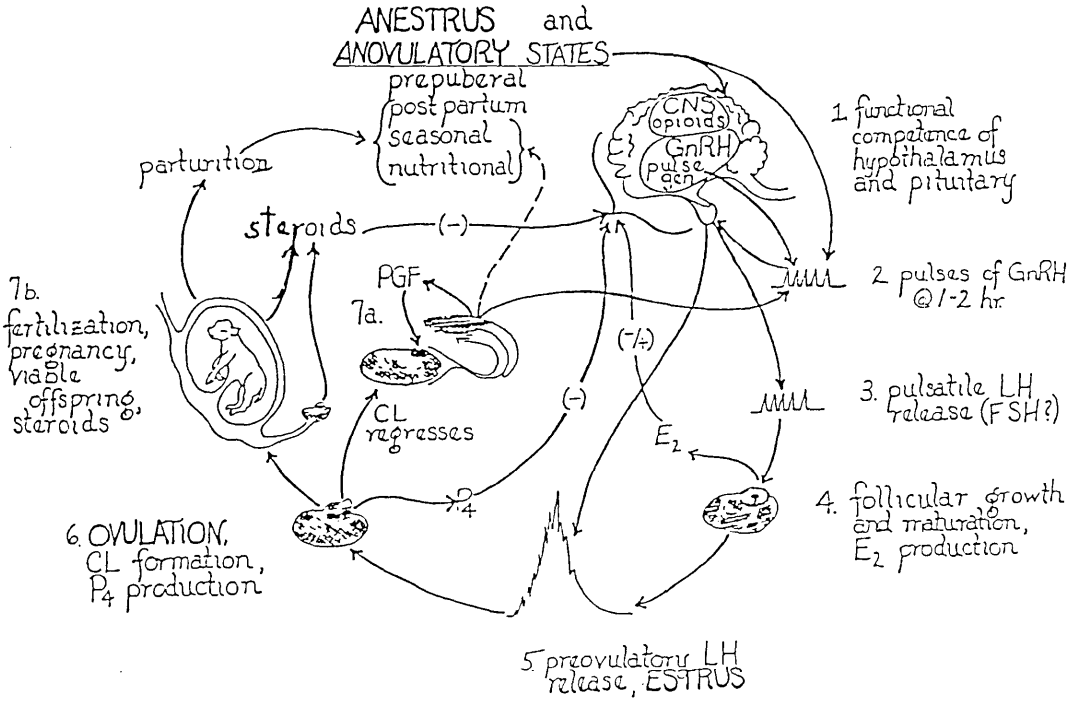

Figure 2.8: *A model depicting the hormonal control of oestrus and ovulation in post-partum cows* (from Short *et al.*, 1990)

As the post-partum time interval increases, the rate of LH synthesis is increased, coupled with a relatively slow rate of release, creating a situation in which pituitary stores of LH are replenished. Similarly, FSH concentrations are low for a period after calving and then rise again; it is presumed that this rise initiates follicular recruitment and selection of a dominant follicle (Roche *et al.*, 1992). The pulsatile secretion of LH is also gradually normalised stimulating increased secretion of oestradiol from the dominant follicle, which stimulates production of its own receptors in the hypothalamus and the pituitary. At this point, the frequency of GnRH discharges also increases (Nett, 1987, Roche *et al.*, 1992). In post-partum beef cows pituitary receptors for both oestradiol and GnRH are normally elevated on day 15 post-partum, possibly increasing the sensitivity of the anterior pituitary to these hormones and leading to an increased rate of LH synthesis which subsequently restores pituitary content to normal by day 30 post-partum (Nett *et al.*, 1988). These events lead to a gonadotrophin surge and therefore the final stages of follicular development which culminate in oestrus and ovulation.

The post-partum cow gradually progresses from 'deep' to 'shallow' anoestrus with time after parturition. The amount of time required to make the transition is variable and

influenced by many factors (section 2.3.2). Suckling and environmental factors appear to be tightly coupled with the later phase of recovery, ie. events leading to an increased frequency of LH discharges, while the early post-partum anoestrus is relatively independent (Nett, 1987).

The role of prolactin and oxytocin in the control of post-partum anoestrus has been examined, but the evidence for their involvement is contradictory and tends to have been dismissed as of minor significance (Webb *et al.*, 1981, Wheeler *et al.*, 1982, Peters *et al.*, 1987a, Stewart *et al.*, 1987, Short *et al.*, 1990).

2.3.1.4 Short Oestrous Cycles

In reality, the first post-partum oestrous cycle is usually short (Murphy *et al.*, 1990). In about 50% of cows the first progesterone cycle is shorter (<10 days) than that of the normal luteal phase (Peters *et al.*, 1987a). Short oestrous cycles contribute to post-partum infertility during the first 30-40 days after calving, because the *corpus luteum* regresses before the ovary receives a signal from the uterus that a pregnancy exists (Short *et al.*, 1990).

It has been shown that the functional capabilities of the early post-partum *corpus luteum* are normal, but the *corpus luteum* regresses prematurely. This is caused by abnormally high prostaglandin concentrations, presumably involved in uterine involution (Manns *et al.*, 1983, Short *et al.*, 1990). Active immunisation against PGF2α extends the lifespan and progesterone secretion of *corpora lutea* anticipated to be short-lived (Copelin *et al.*, 1989). In addition, it appears that the *corpus luteum* associated with the short cycle has a short life span as a result of lack of luteotropic support and the failure of the luteal tissue to recognise a luteotropin (Hafez, 1993d).

2.3.2 FACTORS CONTROLLING POSTPARTUM ANOESTRUS IN THE COW

Anoestrus is the major component of post-partum infertility besides general infertility, lack of uterine involution and short oestrous cycles. Postpartum anoestrus is, in turn, affected by two major factors, namely suckling and nutrition, and several minor factors such as season, genotype, parity, dystocia, sexual interplay (male effect), and carryover effects from the previous pregnancy (Short *et al.*, 1990). The nature of genetic, environmental and husbandry factors and their interactions that affect the duration of post-partum anoestrus in the cow is complex, but many of these factors appear to act via common hormonal mechanisms (Jolly *et al.*, 1995).

2.3.2.1 Major Factors

2.3.2.1.1 *Suckling and Lactation*

It has been reported that suckling delays post-partum ovarian activity and thus considered to be a main regulator of post-partum rebreeding in beef cattle (for review see Galina *et al.*, 1989, Williams, 1990). Cows that have their calves weaned at birth have shorter PPIs than do cows that are suckled, and if calves are weaned at some time after birth but before oestrous cycles begin cows will return to oestrus within a few days (Short *et al.*, 1990). This section only briefly describes possible mechanisms whereby suckling influences the post-partum period.

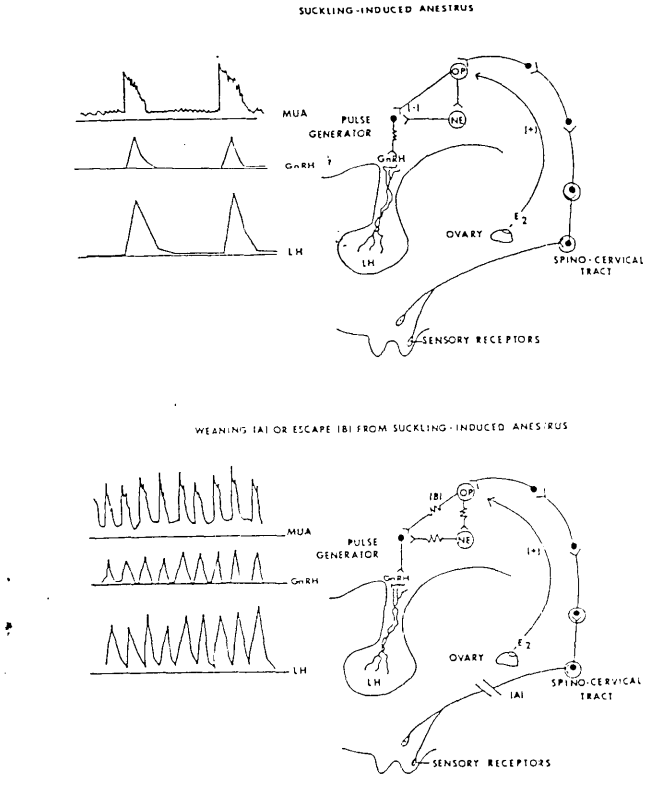

Figure 2.9: *Hypothetical model for neuroendocrine control of suckling-induced anoestrus. Abbreviations: multiunit electrical activity (MUA), opioid peptides (OP) norepiphedrine (NE), oestradiol (E2)* (from Williams, 1990).

Suckling is proposed to act via neuroendocrine pathways (Fig. 2.9.). Both suckling and the act of milking apparently inhibit the release of GnRH necessary for restoration of the pulsatile pattern of LH release (Bazer *et al.*, 1993). However, suckling rather than lactation suppresses concentrations of LH in the peripheral circulation (Nett, 1987, Williams, 1990), because suckling suppresses pulsatile LH secretion for a longer period after parturition than milking (Peters *et al.*, 1981, Schallenberger *et al.*, 1985). Recent data suggests that the presence of "own"calf can also cause anoestrous (Williams et al 1996). The suckling-induced inhibition of LH secretion is thought to act via neurotransmitters, especially endogenous opioid peptides (EOP). Evidence for this supposition came from administration of naloxone, an opioid antagonist, which stimulates the secretion of LH in suckling cows (Greeg *et al.*, 1985, Rund *et al.*, 1989). Morphine, an opioid agonist, can decrease the number of LH pulses in post-partum cows (Peck *et al.*, 1988). Therefore, it has been proposed that suckling induces secretion of EOPs, which in turn inhibit discharges of GnRH from the hypothalamus and consequently LH from the anterior pituitary.

2.4.2.1.2 *Nutrition*

Nutritional factors play a major role in determining the duration of the anoestrous period. Inadequate precalving and/or post calving energy or protein nutrition will lower PPI in suckled post-partum beef cows, yet excessive protein intake may depress post-partum rebreeding performance, especially in older dairy cows (see review by Randel, 1990). Nutritional effects are elicited via a complex interplay among many variables such as quantity and quality of feed intake, nutrients from other physiological functions besides reproduction (see review by Short *et al.*, 1990). A

schematic presentation of the allocation of nutrients to various body functions is shown in Figure 2.10.

It has been demonstrated that both higher body condition at calving and higher levels of feeding after calving can reduce PPI and improve reproductive performance (Houghton *et al.*, 1990, Wright *et al.*, 1992, DeRouen *et al.*, 1994, Spitzer *et al.*, 1995). However, Short *et al.* (1990) and Randel (1990) show that the nutritional differences at calving are more important than after calving in determining the length of post-partum anoestrus and the effects of post-partum feeding levels depend on the body condition at calving. Body condition of the cow at calving is of primary importance for the prompt restoration of ovarian activity (see review by Galina *et al.*, 1989).

The mechanisms by which nutritional status affects post-partum reproduction are unclear. However, it has been found that there is a good relationship between energy balance and first ovulation post-partum, and changes in LH pulsatility are related to changing energy balance status over a wide time frame (Perry *et al.*, 1991, Roche *et al.*, 1992), suggesting that it is energy balance that regulates LH secretion. Nutrition is probably involved in regulation of GnRH secretion and hence LH frequency. Wright *et al.* (1992) demonstrated that LH pulse frequency post-partum was positively correlated with body condition at calving. In addition, decreased utilisation of amino acids for the production of glucose was found to be associated with approaching ovulation and with increased LH secretion during post-partum anoestrus (Zurek *et al.*, 1995). Therefore, energy- or animo acid-related compounds or catabolites such as glucose, nonesterified fatty acids or EOPs, presumably act on the hypothalamic-hypophyseal-ovarian axis as the nutritional state of the animal is altered (Randel, 1990). Furthermore, Zurek *et al.* (1995) conclude that declining energy balance is the most powerful factor in inhibition of ovarian activity after calving. Complex dynamic changes during improvement, rather than absolute energy balance, provide the cow with information about its metabolic status and, as a response to those changes, cows may resume reproductive activity while they are still energically deficient.

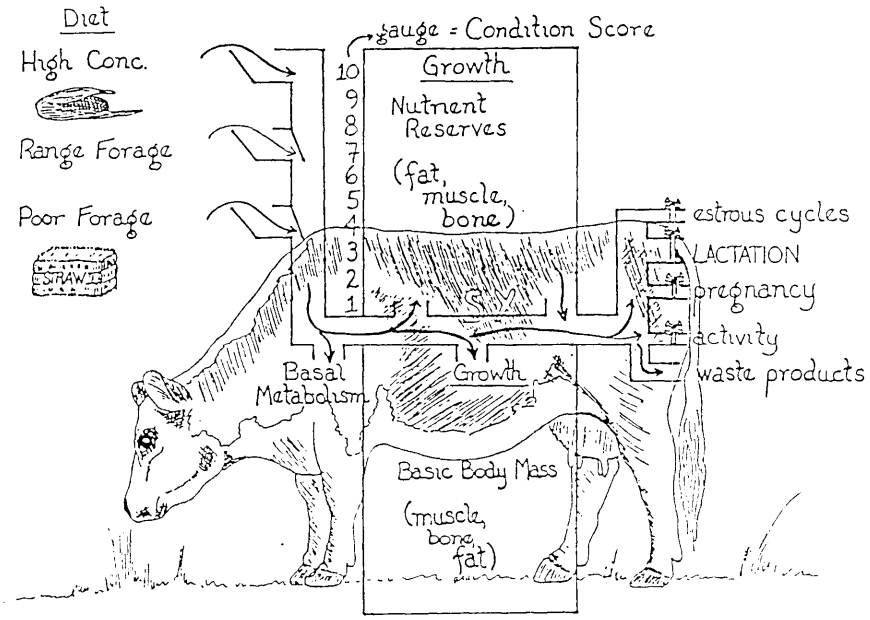

Figure 2.10: *Partitioning of nutrients in cow with nutrient intake varying in quantity and quality* (from Short and Adams, 1988)

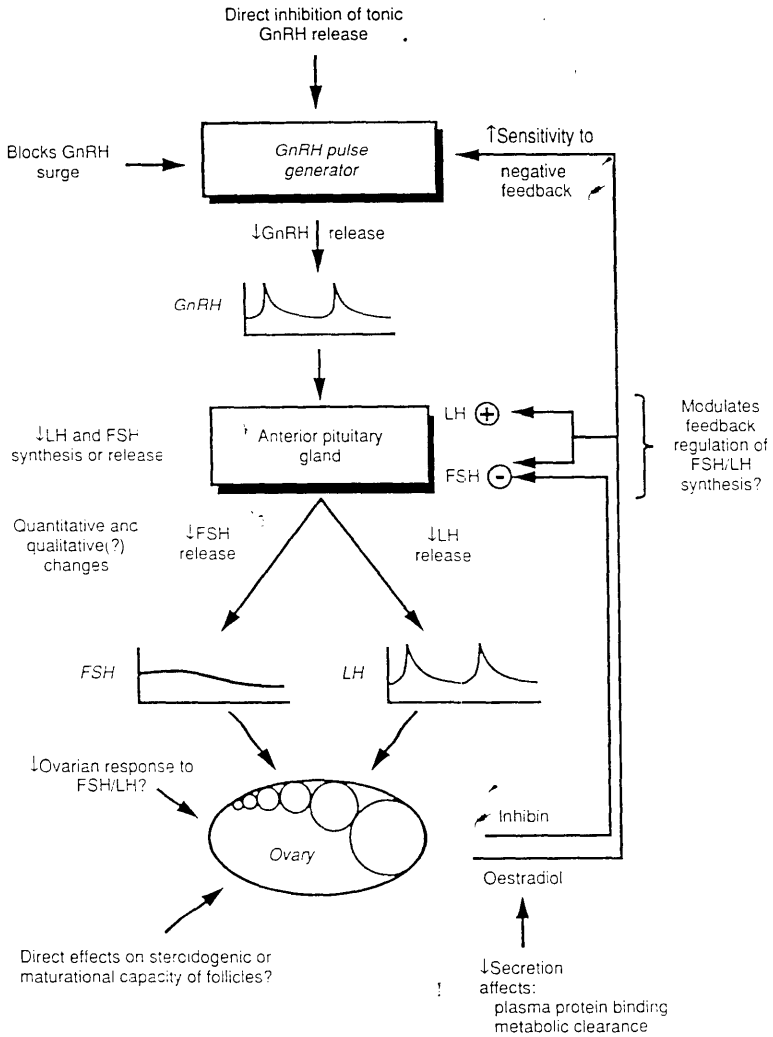

Figure 2.11: *Schematic summary of putaive mechanisms by which undernutrition may inhibit the reproductive axis in post-partum cows* (from Jolly *et al.*, 1995).

It has also been suggested that cows in poor condition after calving have small inactive hard ovaries; therefore, nutritional deficiency may cause either a delay in resumption of development of dominant follicles or dominant follicles which do develop are smaller and less oestrogen active (Prado *et al.*, 1990, Spicer *et al.*, 1991, Roche *et al.*, 1992). In the study of Murphy *et al.* (1991) long term energy deprivation was associated with reductions in both dominant follicle diameter and persistence. Lack of ovarian activity has been attributed to reduced gonadotrophin secretion.

A schematic summary of possible mechanisms by which undernutrition may inhibit the resumption of ovarian cycles in post-partum cows is present in Figure 2.11. According to Jolly *et al.* (1995), tonic or surge release of GnRH from the hypothalamus may be inhibited by mechanisms that regulate sensitivity to ovarian feedback as well as through mechanisms that are independent of ovarian control. Reduced GnRH input to the anterior pituitary results in reduced LH pulse frequency and reduced LH and FSH synthesis and FSH secretion if pronounced. Inhibition of dominant follicle growth and persistence may reflect effects of reduced FSH or LH secretion, or effects of other endocrine or metabolic signals acting directly within the

ovary. Undernutrition may also modulate feedback regulation of FSH and LH synthesis or secretion by effects on ovarian output of plasma protein binding or metabolic clearance of, or hypothalamic or pituitary sensitivity to, feedback hormones such as oestradiol and inhibin.

2.4.2.2 Minor Factors

2.4.2.2.1 *The Climate and Season*

Marrion *et al.* (1968) and Zain *et al.* (1995) report that the calving season, though not significant to ovarian resumption, plays an important role in uterine involution: involution is completed earlier in cows calving during spring-summer than in those calving during autumn-winter. Galina *et al.* (1989) show that the time of the year when the cow calves has an important effect on the onset of ovarian activity. King *et al.* (1983) and Smeaton *et al.*, (1986) also indicate that cows calving from late spring to early winter have shorter PPI than those calving from later winter to early spring. The effects of climate and season could be real effects associated with light and temperature differences, or the effects could be confounded with nutritional changes (quantity and quality of feedstuffs available to the animal) as the year progresses (Short *et al.*, 1990). The seasonal effects are also modified by other factors such as genotype and suckling (Vandaplassche, 1985, Hansen, 1985).

2.4.2.2.2 *The Animal Breed and Genotype*

Dairy breeds that are milked have shorter PPI than suckled beef breeds (Laming *et al.*, 1981), but when dairy cows are suckled they have longer PPI than beef cows (Short *et al.*, 1990). Hansen *et al.* (1985) found that dairy genotypes have longer PPI than beef genotypes when managed comparably, and these effects are pronounced at first parity and at lower levels of feed intake. Different PPIs are apparently found between *Bos taurus* and *Bos indicus* breeds, and between purebred, crossbred and inbred breeds, and between native and introduced breeds (Vandeplassche, 1985, Galina *et al.*, 1989). Effects may be due to physiological differences and/or confounding effects such as

differences in amount of milk produced or appetite and feed intake (Short *et al.*, 1990).

2.4.2.2.3 *The Age and Parity*

Reports agree that parity (Marion *et al.*, 1968, Izaike *et al.*, 1989, Zain *et al.*, 1995) and age (Morrow, 1969, Fonseca *et al.*, 1983, Galina *et al.*, 1989) influence uterine involution. The uterus takes longer to return to its normal size with increasing age and parity. Moreover, the regression is faster in younger cows. In contrast to the findings on uterine involution, primiparous cows usually exhibit the first post-partum oestrus later than multiparous cows (Laming *et al.*, 1981, Short *et al.*, 1990, Zain *et al.*, 1995). Dystocia is also associated with age and will increase PPI and delay rebreeding (Doorubos *et al.*, 1984). However, the role of age on the *puerperium* is equivocal in many other studies and further reseach is warranted to add more evidence and clarification.

2.4.2.2.4 *Male Effect*

Contact with bulls results in an increased incidence of oestrus and stimulation of ovarian activity, thus shortening PPI in the cow (Vandeplassche, 1985, Alberio *et al.*, 1987, Scott *et al.*, 1987, Custer *et al.*, 1990, Stump *et al.*, 1992, Hornbuckle *et al.*, 1995). This effect is thought to be provoked by way of pheromones from glands, urine, faeces, and tactile stimuli from body contact. However, the mechanisms whereby presence of bulls accelerates the physiological process that initiates the resumption of oestrous cycles has not been fully elaborated.

3.4.2.2.5 *Other Minor Factors*

Postpartum interval may also be affected by factors such as stress, management, disease, and drug treatments (Vandeplassche, 1985, Short *et al.*, 1990). Dystocia and retained placenta may result in a longer post-partum acyclic period. It is possible that these conditions may cause an inhibition of uterine involution.

So far, the reproductive phenomena of the bovine oestrous cycle and the post-partum period have been seen to occur in relation to the development of ovarian follicles under neuroendocrine control. More insights into follicular development should provide significant reasons for studying the physiological regulation of bovine reproductive activity and generate ideas for artificial manipulation of reproduction in female cattle.

2.4 FOLLICULAR GROWTH AND DEVELOPMENT IN CATTLE

Histological observations, follicle marking techniques, hormone measurements, and more recently, ultrasonographic examination of ovaries have elucidated patterns of follicular development and brought about a new understanding of the regulation of follicular growth, development and regression. Although the mechanisms controlling important ovarian events such as follicular recruitment, selection, and dominance are not fully understood, many previous controversies about the patterns of follicular development in cattle have been resolved.

2.4.1 FOLLICULOGENESIS

Folliculogenesis is unique not only because a primordial follicle may increase in diameter 400-600 fold before ovulation, but also because the growth of 500-1000 primordial follicles each oestrous cycle normally results in development of only a few ovulatory follicles. During the lifespan of an animal 99.9% of the primordial follicles fail to ovulate (Ireland, 1987) and this can be seen through the stages of follicular development and follicular dynamics during each oestrous cycle.

2.4.1.1 Stages of Follicular Development

Folliculogenesis in cattle as well as in primates is composed of the stages which are described by Greenspan (1991) as follows:

The basic reproductive unit of the ovary is the small **primordial follicle**, consisting of (1) a small oocyte arrested in the diplotene stage of meiotic prophase, (2) a few, or a complete ring, of poorly differentiated granulosa cells, and (3) a basement membrane that surrounds the granulosa cells, separating them from the adjacent ovarian stroma (Fig. 2.12). There are several million primordial follicles present in the ovary of the cow at birth (Lobb *et al.*, 1992). Primordial follicles are found principally in the outer cortex just beneath the fibrous capsule of the ovary. These primordial follicles constitute an inactive or resting pool from which all ovulatory follicles will eventually develop. Throughout the pre-pubertal period, a small percentage of these small, inactive follicles are continually initiating growth. However, at an early stage of development and prior to antrum formation, growth is arrested and the follicles undergo atresia.

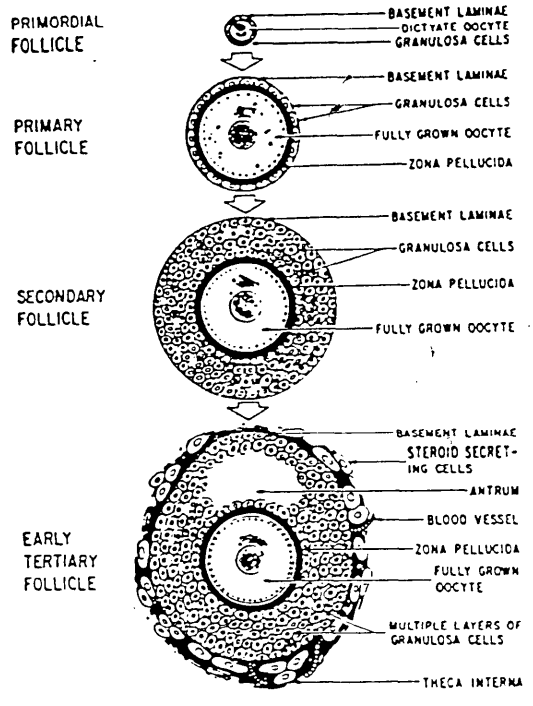

Figure 2.12: *Morphologic changes in ovarian follicles during growth and development* (Erickson, 1981).

The earliest morphologic change in the primordial follicle that has left the pool of resting follicles and begun the process of growth is an increase in size of the oocyte. As the oocyte enlarges, the zona pellucida, a membrane that will eventually surround the oocyte, begins to form. The flat, poorly differentiated granulosa cells, which form a single layer, assume a cuboid shape as the oocyte approaches its maximum size. At this stage , the follicular unit is known as a **primary follicle**.

Subsequently, the granulosa cells rapidly proliferate, forming a multilayered covering around the oocyte. Small patches of fluid form between the granulosa cells, and, as the follicle continues to enlarge, cells that are indistinguishable from mesenchymal fibroblasts align themselves concentrically outside the basement membrane. These cells form the thecal layer and complete the formation of the **secondary follicle** (**preantral follicle**).

As the granulosa cells continue to multiply, there is further production and accumulation of fluid within the granulosa cell layer, leading to the formation of a follicular cavity, or *antrum.* The oocyte and a portion of the surrounding granulosa cells (cumulus cells) are gradually displaced to one side of the follicular cavity, and a **tertiary follicle** (**antral follicle**) is formed. There is both a rapid accumulation of follicular fluid and additional growth of granulosa cells, causing further enlargement of the **preovulatory** (**Graafian**) **follicle.**

2.4.1.2 Follicular Dynamics during the Oestrous Cycle

Recent ultrasonographic studies have shown that folliculogenesis during the bovine oestrous cycle is characterised by the development of large antral follicles in waves at regular intervals (Fig. 2.13). Each follicular wave consists of 3 major phenomena: *recruitment* - an event during which a cohort of small follicles is recruited to grow and a group of about 3-6 large antral follicles, 5 mm or more in diameter develop; *selection* - a process whereby one of the recruited follicles is "selected" to become "dominant"; and *dominance* - the mechanism that the dominant (ovulatory) follicle uses to survive and suppress the subordinate follicles and new waves of follicular development (Goodman *et al.*, 1984, Fortune, 1993, Adams, 1994). The subsequent

follicular wave cannot be detected until after the start of the regression of the previous dominant follicle (Webb *et al.*, 1992).

Most oestrous cycles consist of 2 or 3 follicular waves. On average, wave emergence is detected on day 0-2 (day 0 is the day of ovulation) and on day 10-11 for 2-wave cycles, and on day 0-2, 9 and 16 for 3-wave cycles (Ginther *et al.*, 1989a, Savio *et al.*, 1990b, Fortune, 1993, Adams, 1994). A correlation between the length of the cycle or the luteal phase and the number of follicular waves has been observed. In animals with 2 waves both the luteal phase and the oestrous cycle were significantly shorter than those in heifers with 3 waves (Ginther *et al.*, 1989b, Lavoir *et al.*, 1990).

It is clear that there is subsequent growth and atresia of dominant follicles during the oestrous cycle. Usually, there are 2 or 3 dominant follicles present during an oestrous cycle as a result of the above mentioned follicular waves. Savio *et al.* (1988) reported that the first dominant follicle, detectable on or after the day of ovulation as one of a

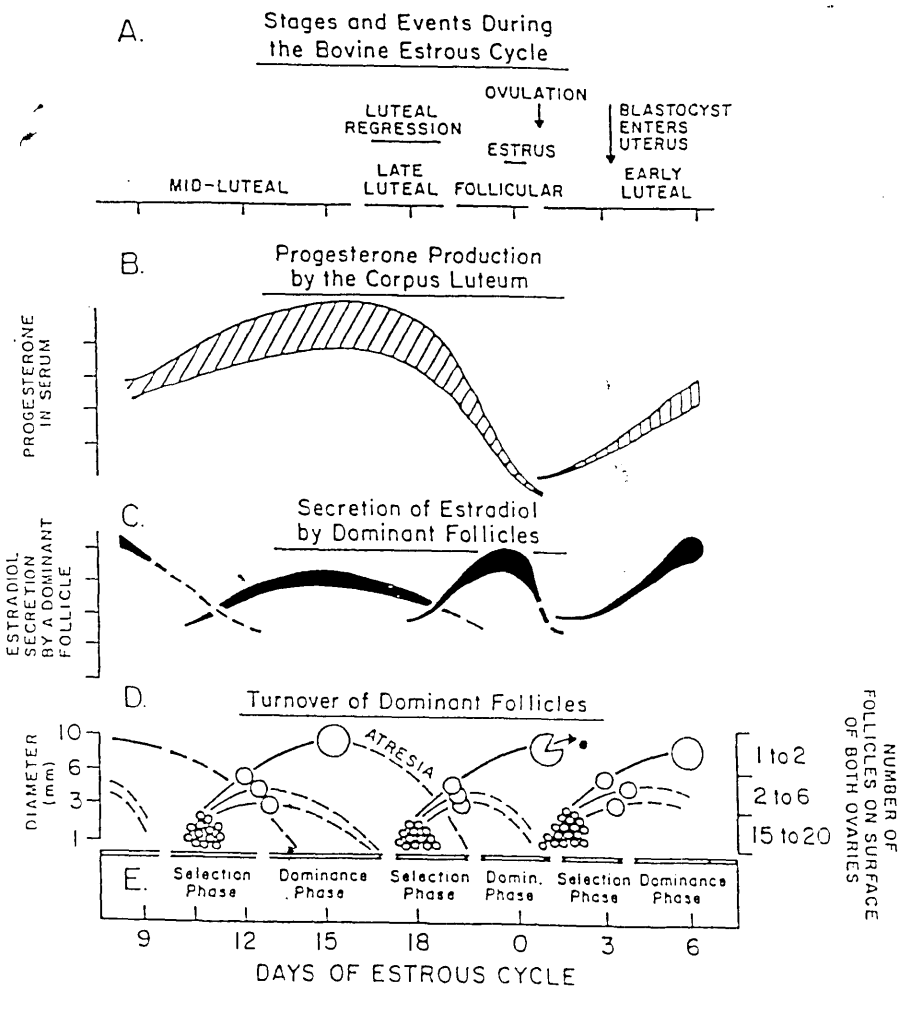

Figure 2.13: *Model for cycles of development of dominant follicles during a bovine oestrous cycle* (Ireland and Roche, 1987).

group of large antral follicles, was identified on day 4 and increased in size daily until maximum diameter was reached on day 6-7. It then went through a period of relative stability between day 6 and day 10, thereafter it decreased in size and was not identifiable on day 15. Medium follicles in the first wave ceased growth after the development of the dominant follicle. The second dominant follicle was the ovulatory follicle in cycles with 2 follicular waves, or it underwent atresia, generally on day 16-17 in cycles with 3 follicular waves where the third dominant follicle ovulated. Furthermore, Ginther *et al.* (1989a) found that the ovulatory dominant follicle in cycles with 3 follicular waves emerged later (day 16 vs 10), ovulated sooner after emergence (7 vs 11 days) and was smaller. Thus, the second dominant follicle will ovulate in cycles of shorter duration (18-21 days). In addition, Kastelic *et al.* (1991) reported an earlier emergence of the second dominant follicle in three-wave cycles compared with 2-wave cycles. These observations suggest that the time of regression of the corpus luteum, relative to the time of appearance of the second dominant follicle, may play a major role in determining whether there will be 2 or 3 dominant follicles (or follicular waves) during a cycle. In other words, during the oestrous cycle there are recurrent periods of turnover of dominant follicles culminating in the final maturation and ovulation of the dominant follicle that is present at the time of luteolysis (Roche *et al.*, 1991).

2.4.1.3 Follicular Dynamics during the Postpartum Period

Resumption of ovarian activity in the post-partum period involves a variable period of growth of small follicles, which may reach 8-10 mm in diameter. From this cohort one follicle continues to grow and become dominant. The dominant follicle may ovulate or undergo atresia (Roche, 1989). When LH pulses are of sufficient frequency, the

follicle ovulates, followed by an oestrous cycle, which is usually short (section 2.3.1.4).

The initiation of follicular growth in the early post-partum period is not well defined because ultrasound examination during this period is usually difficult due to the anatomy of the involuting uterus. Savio *et al.* (1990c) reported that in dairy cows medium-sized follicles (5-9 mm) were present by day 5 after calving and the detection of a single large dominant follicle (>10 mm) was, generally, closely related to the resumption of ovarian activity. Murphy *et al.* (1990) showed that in beef cows follicular development and formation of a dominant follicle occur early after parturition but, in contrast to post-partum dairy cows where the majority of cows ovulated the first dominant follicle, the first dominant follicle in post-partum beef cows was more likely to undergo atresia. Therefore, recurrent growth and regression may occur before first ovulation and the longer post-partum anoestrous period in beef cows is due to the failure of ovulation of the majority of first dominant follicles, rather than a delay in development of dominant follicles. This is depicted in Figure 2.14.

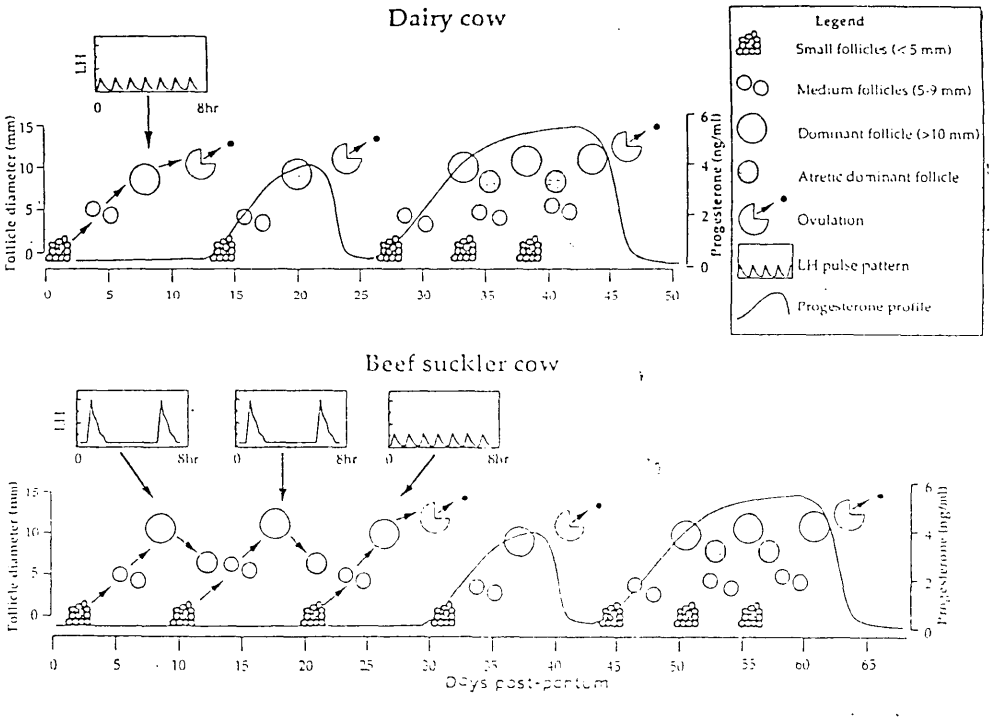

Figure 2.14: *Proposed scheme of resumption of dominant follicles and ovarian cycles during the post-partum period in dairy and beef suckler cows not nutritionally stressed* (from Roche *et al.*, 1992).

Figure 2.14: *Proposed scheme of resumption of dominant follicles and ovarian cycles during the post-partum period in dairy and beef suckler cows not nutritionally stressed* (from Roche *et al.*, 1992).

2.3.2 CONTROL OF FOLLICULAR GROWTH AND DEVELOPMENT

2.3.2.1 Regulation of Folliculogenesis

The development of the primordial follicle to the preovulatory stage can be divided into the gonadotrophin-independent stage and the gonadotrophin-dependent stage (Fig. 2.15). In the absence of gonadotrophins, follicles can be recruited from the primordial pool and grow to the preantral stage, but the preantral follicles cannot proceed further and undergo atresia (Richards, 1980).

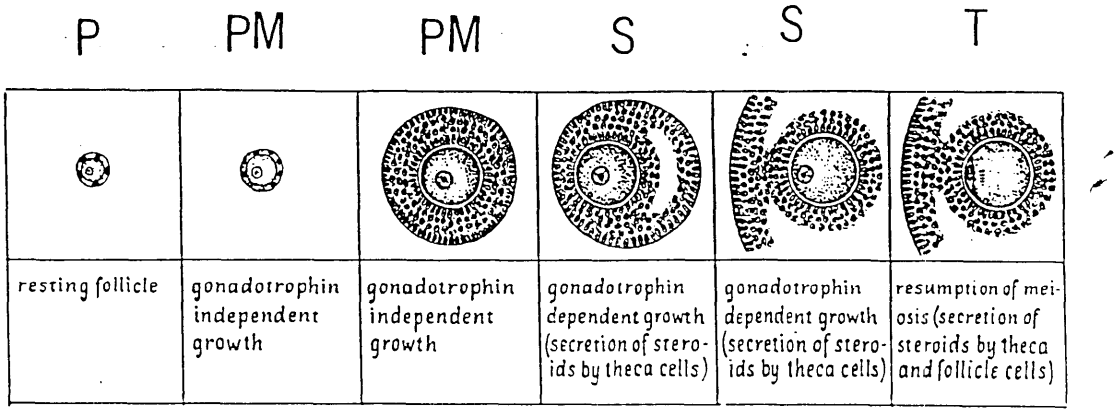

Figure 2.15: *Physiological stages of follicular development*

P: primordial follicle; PM: primary follicle; S: secondary follicle; T: tertiary follicle.

(from Dovrak and Tesarik, 1980)

After puberty and throughout the fertile life of the animal primordial follicles are continually stimulated to initiate progression through the gonadotrophin-independent stage, for possible development to the ovulatory stage in the presence of gonadotrophin and intraovarian regulators (Fig. 2.16). The growing follicle will undergo atresia unless it receives the appropriate signals from gonadotrophins to

complete its developmental program and form a preovulatory follicle that is competent to release an ovum at the time of ovulation (Lobb *et al.*, 1992).

In cattle, the development of antral follicles occurs in waves as has been mentioned. A wave is characterised by the synchronous growth of a number of small recruited follicles followed by selection of a dominant follicle and subsequent regression of subordinates. Evidence has shown that gonadotrophins are of utmost importance in the control of the follicular pattern (Driancourt, 1991). Ovarian follicles go through selection, dominance and atresia phases coincident with transient increases and decreases in FSH (Sunderland *et al.*, 1994). In the absence of gonadotrophins no preantral follicles are recruited (Dufour *et al.*, 1979, McNeilly *et al.*, 1986), but recruitment, selection and dominance can be induced in gonadotrophin-deprived animals by administration of exogenous gonadotrophins (Fry *et al.*, 1988). There is ample evidence indicating that follicular fluid contains compounds likely to supplement and modulate gonadotrophin actions (Lobb *et al.*, 1992). These compounds can act on the follicle that has been producing them (autocrine regulators) or on adjacent follicles (paracrine regulators). Further details on these processes will be provided in the following sections.

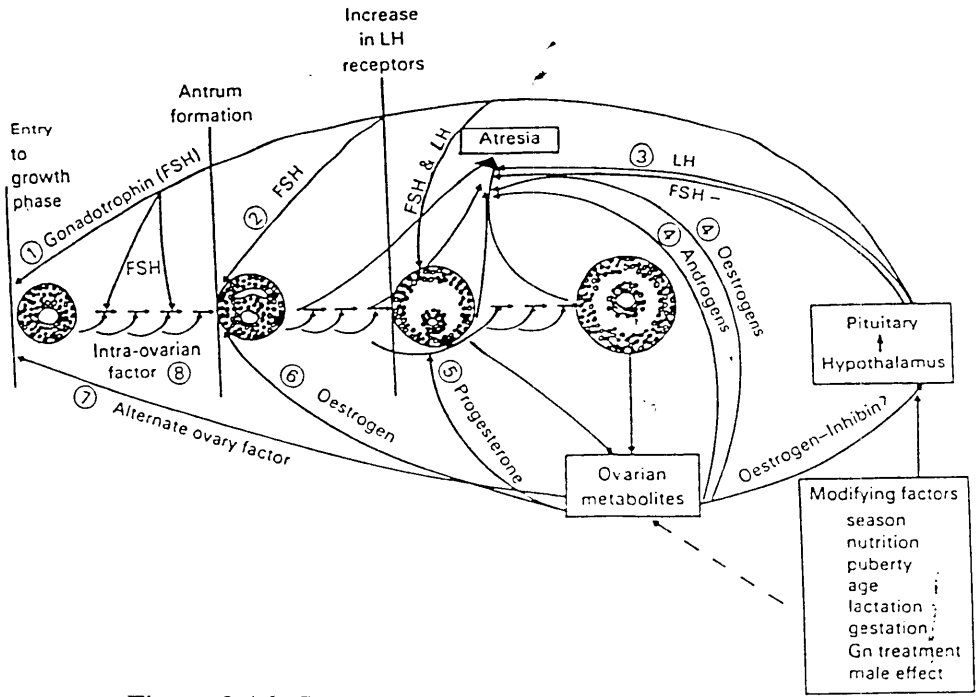

Figure 2.16: *Some regulating mechanisms controlling preantral and antral follicles* (from Hafez, 1993a).

Hormones Involved:	*Physiological Mechanisms:*
1 Gonadotrophin (probably FSH)	Initiation of follicle growth
FSH	Facilitation of growth of preantral follicles
2 FSH	Antrum formation
3 LH	Enhancement of atresia of antral follicles
FSH	Inhibition of atresia of antral follicles
4 Androgens	Enhancement of atresia of antral follicles
Oestrogens	Inhibition of atresia of antral follicles
5 Progesterone	Inhibition of the growth rate of large antral follicles
6 Oestrogens	Synergistic effect with FSH on follicles at antrum formation
7 Unknown	Enhancement of follicles to enter growth phase following hemicastration
8 Unknown	Intraovarian regulation resulting in relations between number and growth rates of follicles

2.3.2.2 Control of Cell Function during Tonic Folliculogenesis

The fate of a follicle is reflected in the multiplication and the death (atresia) of granulosa cells (Lobb *et al.*, 1992). As the follicle enlarges, there is a progressive decrease in the mitotic activity of both granulosa cells and thecal cells (Lussier *et al.*, 1987). This is the consequence of a shift from cell division to cell differentiation. As this shift proceeds, follicle growth is achieved by antral fluid accumulation. Endocrine, autocrine and paracrine factors have been shown to be involved in the control of cell division and differentiation (Fig. 2.17).

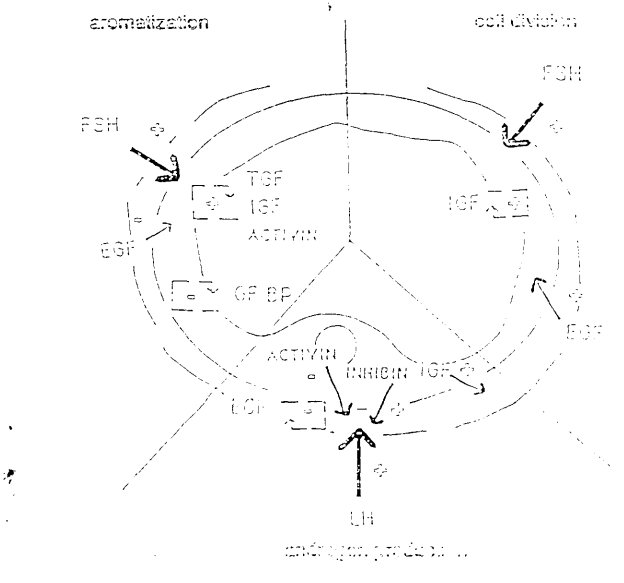

Figure 2.17: *Local modulation of endocrine signals to the follicle* (Driancourt, 1991)
(thick bars are endocrine, thin bars are paracrine and doted bars are autocrine regulations)

The pituitary gonadotrophins are required for normal follicular growth and development to the preovulatory stage. However, Lobb *et al.* (1992) showed that FSH and LH did not act directly as cell mitogens; androgens (produced by thecal cells under LH stimulation as precursors of oestrogens) and oestrogens (produced by granulosa cells under FSH influence) were not effective in stimulating the growth of bovine granulosa cells either. They demonstrated that polypeptide transforming growth factors (TGF-α and TGF-β) was produced by thecal cells and acted in concert with other local factors to regulate the proliferation of granulosa cells. Some of the other local regulators, such as insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF) and two important proteins, inhibin and acvitin, are produced by granulosa cells, while epidermal growth factor (EGF) is produced by thecal cells. Transforming growth factor TGF-β is produced by both granulosa and thecal cells (Driancourt, 1991).

A model for the role of transforming growth factors in ovarian physiology and follicular development proposed by Lobb *et al.* (1992) shows that in small follicles, TGF-α of thecal origin defuses into the granulosa compartment and positively stimulates granulosa cell mitosis. Granulosa cells of this size are not fully differentiated and do not synthesise oestrogens. While promoting granulosa cell growth TGF-α may also maintain the granulosa cells in an undifferentiated state. At a later stage of development (large antral follicle) granulosa cell mitosis declines and the cell begins to synthesise oestrogens. The other transforming growth factor (TGF-β) negatively affect granulosa cell growth while promoting gonadotrophin-induced steroidogenesis in follicles of this size (via FSH-induced aromatase activity). The

interplay of the two growth factors leads at first to increases in cell number and later to the augmentation of differentiated function, resulting in a preovulatory follicle.

IGF-1, EGF, and FGF are known to be potent mitogens for granulosa cells (Savion *et al.*, 1981). EGF was shown to extend the replicative lifespan of bovine granulosa cells and prevent terminal differentiation (Gospodarowicz *et al.*, 1977). Meanwhile, IGF-1 and activin, like TGF-β, clearly potentialise FSH action on aromatisation, but inhibin and follistatin are ineffective in granulosa cells (Adashi *et al.*, 1985a, Findley *et al.*, 1991).

Thecal cell differentiation is reflected by androgen production. LH action on thecal cells is also modulated by growth factors and proteins (Driancourt, 1991). IGF-1 increases while EGF decreases LH-stimulated androgen production by thecal cells, while inhibin augments the LH-stimulated production of androstenedione, activin suppresses this response to LH (Findley *et al.*, 1991).

2.3.2.3 Control of Follicular Recruitment

As mentioned earlier, in a wave of follicular development, a number of preantral follicles are recruited to develop further. A small surge in basal plasma FSH was detected 1-2 days before the emergence of each follicular wave (Adams *et al.*, 1992a). When the rise in FSH was prevented by administration of inhibin the emergence of the next wave was prevented for the duration of treatment, and occurred consistently 2 days after the first detectable increase in FSH levels following cessation of inhibin treatment (Turzillo *et al.*, 1990, Adams *et al.*, 1992a). These findings offer strong support for the suggestion that a surge in circulating concentrations of FSH is responsible for eliciting the emergence of a follicular wave. However, the magnitude of the FSH fluctuation required to induce recruitment is small in cattle and, therefore, a local signal (possibly produced by the large follicle of the previous wave when it becomes atretic) may potentiate the effects on the ovary of steady FSH levels. There is also evidence suggesting that in cattle the secondary (post-ovulatory) surge of FSH may play a role in initiating follicular recruitment following ovulation (Turzillo *et al.*, 1990).

To be recruited a follicle must have entered the gonadotrophin-dependent phase (Fortune *et al.*, 1991). Since preantral follicles possess FSH receptors, gonadotrophin responsiveness (recruitment) may be developed during this stage of folliculogenesis (Ireland, 1987).

2.3.2.4 Control of Follicular Selection

Selection of one of the recruited follicles to become dominant may be a process involving the ability of follicles to respond to gonadotrophins, secretion of inhibitory factors from an existing functionally dominant follicle and feedback between the dominant follicle and the pituitary gland.

Atresia has been observed in preantral follicles, therefore, selection may begin at the preantral stage (Ireland, 1987). Furthermore, at the time of recruitment, the different follicles of the cohort of recruited follicles are not all similar. The smallest of the cohort is the richest in EGF (Hsu *et al.*, 1987) and the most sensitive to the inhibitory effects of EGF on cell differentiation (Buck *et al.*, 1988). Hence, FSH stimulation of follicular aromatisation will be more marked on the largest and weaker on the smaller follicles of the cohort.

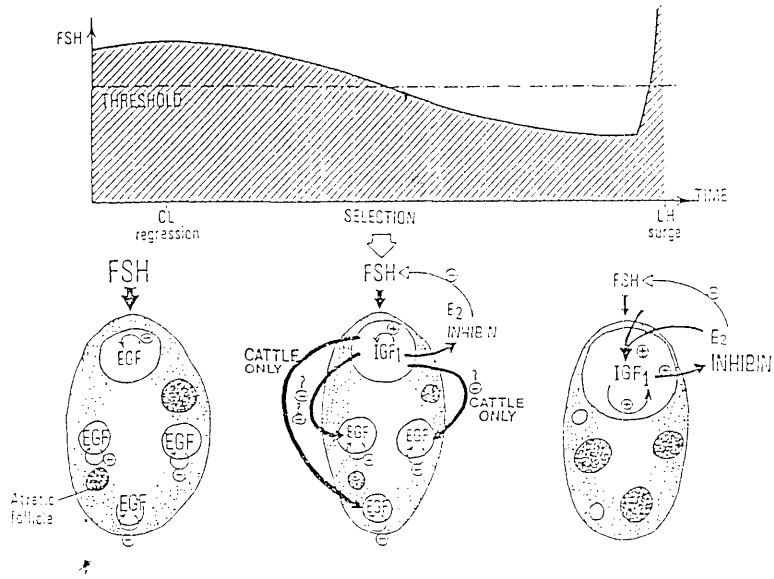

Figure 2.18: *Conceptual presentation of the mechanisms controlling differentiation of the ovulatory follicle in sheep and cattle* (Driancourt, 1991): The darker/thicker the arrows or symbols are, the higher the concentration of these compounds is. Recruitment is triggered by FSH. FSH action is locally modulated by EGF whose inhibitory effects are more marked in small follicles. Selection occurs in sheep as a consequence of the drop in circulating FSH concentrations which fall under the threshold required to support growth of small follicles. In cattle, an additional direct inhibitory loop between the largest and smaller follicles may exist. The largest follicle escapes selection and becomes dominant through its high production and sensitivity to IGF1 which stimulate aromatisation and increases in the number of LH receptors.

Selection may also be due to the interference of the largest, maturing follicle in the cohort with the ability of smaller, less mature follicles to receive enough gonadotrophin support. This can occur in two possible ways (Driancourt, 1991). In the "passive" way, the largest follicle indirectly inhibits the growth of less mature follicles by reducing FSH concentrations below the threshold necessary to maintain other follicles. In the "active" way, the maturing follicle directly inhibits the growth of less mature ones by secreting substances that reduce their sensitivity to FSH. The existing evidence suggests that a combination of active and passive mechanisms may be operative in cattle (Fig. 2.18).

Development of the largest follicle of the cohort produces increasing amounts of oestradiol and inhibin (Martin *et al.*, 1991, Guilbaut *et al.*, 1993). These compounds act through a negative feedback pathway to suppress FSH levels below the threshold necessary for the survival of the other recruited follicles. Therefore, a decline after the follicular wave-stimulating FSH surge is an integral component of the mechanism of selection of the dominant/ovulatory follicle (Adams *et al.*, 1993, Adams, 1994).

It is not clear what role autocrine and paracrine ovarian factors play in follicular selection in cattle. However, there is evidence for the active follicular selection mechanism. First, the presence of a large follicle decreases the superovulatory

response to PMSG or pFSH treatments (Rouillier *et al.*, 1990, Guilbaut *et al.*, 1991, Huhtinen *et al.*, 1992). Furthermore, bovine follicular fluid contains compounds other than inhibin which suppress follicular growth (Law *et al.*, 1990).

2.3.2.5 Control of Follicular Dominance

A functionally dominant follicle (still growing) appears to have two characteristics: (1) the ability to cause the demise of sister follicles in the same cohort and suppress a new wave of follicular development, but not itself, and (2) the ability to ovulate under appropriate hormonal conditions (Fortune, 1993). So, how can a dominant follicle, after selection, continue to mature in an hormonal milieu (especially low FSH) that is suppressive to the growth of other follicles, and, how can one of the several dominant follicles during each oestrous cycle survive and escape atresia to become ovulatory?

Two mechanisms have been suggested to contribute to the survival of a dominant follicle: a preferential delivery/storage of gonadotrophins to the dominant follicle, and an increased sensitivity of this follicle to gonadotrophins (Driancourt, 1991). However, evidence supporting the first hypothesis is very limited, while it is clear that the large dominant follicle is markedly more sensitive to FSH than smaller ones (Henderson, 1987) and this could be related to secretion of autocrine factors which amplify FSH action in cells of the dominant follicle. IGF-1 is such a positive autocrine factor which is present in high concentrations in large dominant follicles (Echternkamp *et al.*, 1990). The increased oestradiol levels in large dominant follicles have been known to interact with FSH to stimulate IGF-1 production (Hsu *et al.*, 1987). IGF-1, by amplifying FSH action on aromatisation (Adashi *et al.*, 1985a) and appearance/increase in the number of LH receptors (Adashi *et al.*, 1985b), plays a key role in the maintenance of dominance. As both FSH and LH act through cAMP, development of LH receptors (on granulosa cells) at a stage of the cycle when LH secretion is high could help maintain high intracellular levels of cAMP in the dominant follicle, thus compensating for the decreasing cAMP stimulus provided by the decreasing FSH concentrations (Driancourt, 1991).

Functional dominant follicles can ovulate while they are still growing, but lose functional dominance when their growth reaches a plateau (Fortune, 1993). In fact, in response to induced luteolysis even the dominant follicle of the first wave is almost always capable of ovulating while it is still growing (Savio *et al.*, 1990b). However, in the presence of an active *corpus luteum* the dominant follicle (first in cycles with 2 waves, and first and second in cycles with 3 waves) will eventually undergo atresia due to the absence of a proper LH pulsatile pattern to stimulate androgen and hence sufficient oestrogen production to induce an LH surge (Stock *et al.*, 1993). The negative feedback effect of progesterone on LH and subsequent reduction in oestradiol production by the dominant follicle form a mechanism by which the dominant follicle undergoes atresia (Taylor *et al.*, 1994). Only the growing dominant follicle, which is present at the time of regression of the *corpus luteum*, will become the ovulatory follicle. This is a consequence of an increase in follicular LH pulse frequency sufficient to stimulate the final maturation, pro-oestrous oestrogen and LH surge, and ovulation (Roche *et al.*, 1991).

2.5 CONCLUSION

Current and emerging concepts of the mechanisms controlling the bovine oestrous cycle and the post-partum period have been reviewed. Female cattle are polyoestrous animals and spontaneous ovulators. Before an oestrus commences, under the stimulation of FSH and LH, the follicles in the ovary develop quickly and produce increasing quantity of oestradiol which, when absorbed into the blood stream, stimulates increased vascularity and cell growth of tubular genitalia in preparation for mating and conception. When the circulating oestradiol level becomes high, it stimulates the nervous system, causing behavioural oestrus. Shortly after this time ovulation occurs. This is brought about by a preovulatory gonadotrophin surge under the stimulation of the increased oestradiol level to the hypothalamus, which secrets GnRH, and to the pituitary, which secrets FSH and LH. FSH increases follicular development during this time to the final stages. The LH surge is necessary for ovulation and luteinisation since it contributes to ovum maturation, stimulates

proteolytic enzymes which degrade the connective tissues in the follicular wall, increases follicular synthesis of prostaglandins which play an important role in follicular rupture and luteinisation.

After ovulation the cavity left by the follicular rupture begins to reorganise and becomes a new endocrine gland called the *corpus luteum* which secretes progesterone. Progesterone prevents increased gonadotrophin secretions through negative feedback effects, thus preventing the occurrence of further oestrous periods so long as an active *corpus luteum* is present (luteal phase). During the luteal phase gonadotrophins are still secreted at tonic levels under the stimulatory effects of GnRH and the negative feedback influence of steroids and inhibin from growing follicles. Tonic FSH stimulates the growth of ovarian follicles and their secretion of inhibin. Tonic LH, in combination with FSH, is necessary for the secretion of oestradiol from large follicles and progesterone from the *corpus luteum*.

During the oestrous cycle follicle growth occurs in waves of regular intervals. In cattle there are usually 2 or 3 waves during each cycle. Such a wave is characterised by the growth of a number of small antral follicles followed by selection of a dominant follicle which subsequently suppresses the further growth of subordinate follicles in the cohort. Endocrine, autocrine and paracrine signals are likely to be involved in the regulation of the follicular dynamics through controlling follicular recruitment, selection and dominance. In particular, a surge in plasma concentrations of FSH is thought to be responsible for eliciting the emergence of a new follicular wave; and the post-surge decline in FSH is an integral component of the mechanism of selection of the dominant follicle. The dominant follicle suppresses its subordinate follicles and the emergence of the next wave through a combination of inhibition of the FSH support, via negative feedback control of oestrogen and inhibin, and reduction of their sensitivity to FSH, via the secretion of a systemic paracrine factor. In the presence of a functional *corpus luteum*, the dominant follicle, along with the subordinate follicles, undergo atresia, and a new wave begins.

If pregnancy does not occur, after a certain length of exposure to oestradiol, progesterone and oxytocin the endometrium secretes PGF2α which results in

luteolysis and termination of the luteal phase. The growing dominant follicle which is present at the time of luteolysis, will become ovulatory due to appropriate endocrine conditions for final maturation and ovulation. The fall in progesterone levels causes an increase in the amplitude and frequency of GnRH release and, therefore, LH secretion increases, which stimulates oestradiol production from the preovulatory follicle triggering the subsequent proestrus (follicular phase).

If the ovum is fertilised after ovulation, the *corpus luteum* remains intact for the majority of the pregnancy period as the secretions from an active functional *corpus luteum* is necessary for gestation.

Following parturition, the uterus has to involute, both physically and physiologically, for normal maintenance of the next possible pregnancy, and the ovaries must recommence cyclical activity, ovulation and normal luteal function. These processes occur during the post-partum period and are influenced by a variety of factors, primarily suckling and nutrition, and other minor factors such as season, genotype, age, parity, and the male effect.

During the early post-partum period, oestrous cycles and ovulation do not occur; however, the reproductive system does not become inactive. Anoestrus is a transitional period of acyclicity due mainly to impairment of endocrine mechanisms controlling follicular development and thereby oestrus and ovulation. It is characterised by an LH episode frequency which is below that necessary for the induction of the final phases of follicle growth. The prolonged inhibition of LH synthesis from gestation to early post-partum period coupled with inhibitory effects of suckling results in reduced LH secretion in post-partum anoestrus. Suckling acts via neuroendocrine pathways in which some neurotransmitters such as melatonin and EOPs are thought to play an integral role in regulating the hypothalamic-pituitary axis. When the neuroendocrine system recovers due to internal and external changes, LH surges are resumed and subsequently the final stages of follicular development occur leading to oestrus and ovulation, followed by oestrous cycles or pregnancy.

Much is still to be learned about the intricate and complicated control processes of bovine reproduction. Further studies are needed to resolve many conflicting points in the literature, especially to clarify the role of neurotransmitters, metabolites, systemic and intra-ovarian factors in the regulation of reproductive activity and factors affecting the *puerperium*. Nevertheless, the current concepts of the bovine oestrous cycle and the post-partum period, and the elucidation of patterns of follicular development in the bovine ovary have provided experimental models for artificial intervention in the reproductive processes, and generated information that can help to explain variability in responses to oestrus control protocols as reviewed in the next chapter.

Chapter 3:

ARTIFICIAL MANIPULATION OF OESTRUS AND OVULATION IN CATTLE

(LITERATURE REVIEW)

3.1 INTRODUCTION

Artificial induction and synchronisation of oestrus and ovulation are based on manipulation of the hormonal processes that regulate the two physiological events. In the cyclic cow, the overriding event controlling the development of an ovarian follicle to the point of ovulation is the process of luteolysis or decrease in progesterone secretion (Chapter 2); in other words, the time of oestrus and ovulation in cyclic cows is controlled mainly by progesterone secreted from the *corpus luteum* (CL) through its negative feedback effects on gonadotrophin hormone secretion. By controlling the lifespan of the *corpus luteum*, the two events can be regulated or synchronised in a group of females. Therefore, in principle, there are two basic approaches for synchronisation of oestrus and ovulation in cyclic cows (Rayos *et al.*, 1990):

1) shortening the lifespan of the *corpus luteum* by means of premature luteolysis using luteolytic agents, leading to decreased progesterone levels, and subsequently to oestrus and ovulation.

2) prolonging the luteal phase of the oestrous cycle by exogenous progesterone or synthetic progestogens which continue to exert negative feedback on gonadotrophin hormone secretions after the *corpus luteum* has naturally regressed.

However, there is considerable variation in the interval from treatment to oestrus and ovulation subsequent to oestrus control treatments and much of the variability has been attributed to the status of the follicular wave at initiation of treatment (Adams, 1994). Therefore, synchronisation of follicular wave emergence should result in increased synchrony of response.

Oestrus and ovulation can also be induced and synchronised in acyclic females. From the discussions in section 2.3 it is postulated that to induce ovulation in anoestrous or acyclic females, follicles must be stimulated to develop to a state of maturity so that a gonadotrophin surge or a hormone with LH-like properties will cause ovulation. This may be accomplished by administering exogenous hormones or by manipulating the factors that suppress ovarian activity.

Based on these approaches various methods for inducing and synchronising oestrus and ovulation in cattle have been developed. This chapter examines these methods with particular attention to the drugs used, procedures applied and their effectiveness. Important factors that affect the efficacy of each method are also discussed.

3.2 SYNCHRONISATION OF OESTRUS AND OVULATION IN CYCLING CATTLE

3.2.1 OESTRUS SYNCHRONISATION BY LUTEOLYSIS

3.3.1.1 Manual Enucleation of the *Corpus luteum*

The suppression of the *corpus luteum* was an early technique for inducing oestrus in cattle. After the *corpus luteum* is squeezed from the ovary by manual palpation via the rectum, new follicles develop and oestrus occurs in about 4 days (Salisbury *et al.*,

1961). This technique is not advocated today because the risks involved are unacceptably high and it does not have better efficacy than safer alternatives.

3.2.1.2 Use of Prostaglandin F-2 alpha and its Analogues

Prostaglandin F-2 alpha (PGF2α) and its synthetic analogues are generally considered to be the most effective agents for synchronising oestrus in ruminants (Larson *et al.*, 1992). They cause regression of the *corpus luteum* and the subsequent rapid decline in the progesterone level leads to rapid follicular growth, oestrus and ovulation in the treated females (Britt *et al.*, 1980). Prostaglandins have no luteolytic effect if given to anoestrous cows (without a CL) and thus have limited use in suckler cows because a high percentage of such animals are still anoestrous 50 days after calving (Roche, 1989). The most common prostaglandin products used for oestrus synchronisation in cattle are listed in Table 3.1. They are used in a variety of regimes as summarised in Table 3.2.

Table 3.1: Common prostaglandin products for oestrus synchronisation

Commercial Product	Lutolytic agent	Dose
PGF2α	PGF2α	25 mg
Estrumate	cloprostenol	0.5 mg
Synchrocept-B or Bovilene	fenprostalene	1 mg
Lutalyse	dinoprost	25 mg
Prosolvin	luprostiol	15 mg
Alfaprostol	alfaprostol	5 mg

Table 3.2: Summary of methods using prostaglandins to control ovulation in cyclic cattle

Method	Treatment protocol	Time of AI
Single injection	1) Detect oestrus and AI for 5-6 days normally; inject remainder with prostaglandin on day 6-7.	At detected oestrus or fixed time
	2) Inject cows with progesterone levels > 1 ng/ml serum or > 5 ng/ml milk.	At detected oestrus
	3) Rectal palpation or ultrasound examination and inject cows having a CL.	At detected oestrus
Double injections	1) Two injections 11-12 days apart.	At detected oestrus or fixed time after second injection.
	2) Inject all cows and breed for 5 days by oestrus; reinject non-bred cows in 11-12 days	At detected oestrus or fixed time after second injection
Combination	1) Gonadotrophin/GnRH or oestrogens given 0-72 h after prostaglandin injection.	At detected oestrus or fixed time

3.2.1.2.1 *Single Prostaglandin Injection Regime*

PGF2α or its analogues can be injected into a group of animals to cause synchronised oestrus, coupled with double artificial insemination (AI) at either 48 and 72 or 72 and 96 hours after injection. Many studies, summarised by Peters (1986), have shown that fertility following double fixed-time AI is equivalent to that of control animals inseminated at observed oestrus. However, as reviewed by Odde (1990) and Larson *et al.* (1992), the stage of the oestrous cycle at prostaglandin treatment affects the response of the animals. In general, prostaglandin products are ineffective in causing luteolysis in the early stage of the cycle (first 5-6 days). The stage of the cycle when PGF2α is injected not only influences the degree of oestrous synchrony, but also influences the time of oestrous onset. There is increasing evidence that the functional status of the CL preceding induced ovulation may affect the onset of oestrus. This may be also due partly to the fact that the dominant follicle (DF), present at the time of luteolysis, becomes the ovulatory follicle. Ovulation occurred from the DF of wave 1 in heifers given PGF2α on day 5 and day 8 and from the DF of wave 2 in heifers treated on day 12 (Kastelic *et al.*, 1991). Therefore, the onset of oestrus is related to the state of follicular development at the time of prostaglandin injection (Roche, 1989).

To overcome the variability of response to a single prostaglandin injection (due to the lack of a constant, precise time of oestrus in relation to the appropriate time of prostaglandin injection rather than an inherent infertility) several strategies have been developed as outlined in the reviews of Britt *et al.* (1980), Hansel *et al.* (1983), Peters *et al.* (1987a), Roche (1989), Odde (1990) and Larson *et al.* (1992). The procedures and results can be summaried as follows:

(1) Animals are observed and inseminated at detected oestrus for 5-7 days after prostaglandin injection. This system tends to increase conception and pregnancy rates compared with controls inseminated at fixed time(s).

(2) Animals are detected for oestrus and inseminated for 4-7 days, then all of those not bred are given a single prostaglandin injection and bred once or twice at fixed times or following the observation of oestrus for the next 5 to 6 days. This system can increase pregnancy rate, but requires more labour for oestrus detection and AI. Laverdiere *et al.* (1995) indicated that the efficiency of PGF2α to synchronise oestrus was greater when the oestrus detection period increased from 4 to 7 days before prostaglandin injection.

(3) Cows with a functional *corpus luteum,* diagnosed by rectal examination or by milk/blood progesterone measurements, can be given a single prostaglandin injection and bred upon detection of oestrus. The efficacy of this method is dependent on the accuracy of *corpus luteum* diagnosis and it is a time consuming method with the added expense of progesterone examination and extensive labour involved.

(4) Milk samples are collected prior to intended insemination. If progesterone concentrations are greater than 2 ng/ml insemination is not carried out to avoid the possible risks of insemination of a cow that has not ovulated within the expected time. Conception rates are increased in these cows compared to cows inseminated without regard to progesterone concentrations.

(5) Combining prostaglandin treatment with natural service may also offer some benefits. However, Whittier *et al.* (1991) showed that treatment of cows and heifers with PGF2α 96 hours after bull "turn-in" was ineffective in synchronising behavioural oestrus and increasing the calving rate during the subsequent calving season. This may have been due to anoestrus in most of the cows.

Other attempts which have also been made to improve the efficacy of prostaglandin treatment on oestrus synchronisation include double prostaglandin injection regimes and prostaglandin administration in combination with other hormones.

3.2.1.2.2 *Double Prostaglandin Injection Regime*

Double injections of PGF2α or one of its analogues are usually given 10-12 days apart to cows at random stages of the oestrous cycle. All treated animals are inseminated once (80 hours) or twice (72 and 96 hours) after the second injection, or bred about 12 hours after oestrus begins. Theoretically, following the first injection only about 70 % of the cyclic cows should show oestrus; these cows and the remainder should be at a suitable stage of the cycle (day 8-15) to respond to the second injection (Odde, 1990). Lauderdale (1979) found that the 5 day pregnancy rate (conception within 5 days following treatment) was similar for lactating cows and beef heifers which were either bred according to oestrus or "time bred" at 80 hours after the second injection. Conception was greater for both treated groups than for controls. This scheme has also been evaluated in many other studies (Lauderdale *et al.*, 1980, DeSilva *et al.*, 1984, Greyling *et al.*, 1991, Kerr *et al.*, 1991, Morbeck *et al.*, 1991, Mutiga *et al.*, 1992) and all have found better oestrous response and similar fertility compared to controls which received a single prostaglandin injection.

However, there still exists the possibility of the lack of a consistent and precise timing of oestrus, and carry-over effects following this regime. Morbeck *et al.* (1991) and Cardenas *et al.* (1991) indicated that the oestrous cycle length in cows following synchronisation with two prostaglandin injections often averaged more than 21 days. In addition, Morrell *et al.* (1991) found an apparent decline in fertility in heifers after repeated oestrus synchronisation with cloprostenol (a prostaglandin analogue).

To reduce costs of prostaglandin products used and to overcome the lack of consistent and precise timing of oestrus, insemination after the first injection, assessment of the presence of a functional *corpus luteum* and oestrus detection have also been applied. Animals observed in oestrus after the first injection are inseminated and those not inseminated are given a second injection 11 or 12 days later. Insemination is either given at fixed times or upon observation of oestrus after the second injection. The main advantage of this method is the reduction of costs of treatment per cow. This method tends to give better results although it requires further effort for oestrus detection (Peters *et al.*, 1987).

The presence of a functional *corpus luteum,* susceptible to luteolysis, can also be used to determine the time of the first injection (Larson *et al.*, 1992). This can be diagnosed by means of ovarian palpation or by milk/blood progesterone concentrations (Archbald *et al.*, 1992). Slenning (1992) found no differences in reproductive performance of dairy cows with a palpable *corpus luteum* inseminated 72 and 96 hours after prostaglandin injection and cows inseminated following oestrus detection.

3.2.1.2.3 *Prostaglandins Combined with Other Hormones*

Gonadotrophin-releasing hormone (GnRH), oestrogens, testosterone, human chorionic hormone (HCH), pregnant mare serum gonadotrophins (PMSG) and follicle stimulating hormone (FSH) have all been tested in combination with prostaglandins in order to induce and control more precisely the time of ovulation. This is achieved by synchronising the luteinising hormone (LH) peak after prostaglandin treatment.

Dailey *et al.* (1983) found no effect of oestradiol benzoate (0.4 mg) injected 48 hours after prostaglandin treatment on either oestrous response or conception rate. Peters *et al.* (1987a) found an increased oestrous response when oestradiol was given 48 h after the second prostaglandin injection, but conception and pregnancy rates were not

improved. Ryan *et al.* (1995) concluded that 1 mg oestradiol benzoate administered 24 h after prostaglandin treatment on day 8 of the cycle reduced the mean interval to oestrus and ovulation in lactating dairy cows. Stevens *et al.* (1992) reported that 0.1 mg GnRH administered to dioestrous dairy cows simultaneously with a luteolytic dose (0.5 mg) of cloprostenol did not improve the synchrony of oestrus and ovulation compared with prostaglandin treatment alone. Although Archbald *et al.* (1992) found an improvement in conception rate of dairy cows which were not seen in oestrus when they were injected with 0.1 mg GnRH 72 hours after PGF2α, conception rate was no better than that in cows without PGF2α treatment but exhibiting oestrus and inseminated accordingly.

Twagiramungu *et al.* (1995a) used a GnRH-PGF2α-GnRH protocol as follows: PGF2 α was given 6 days after the first GnRH injection and AI was given 54 h after PGF2α was injected with a simultaneous injection of GnRH. They found that this protocol eliminated the need for oestrus detection without having a negative effect on pregnancy and calving rates.

The different prostaglandin treatments to synchronise oestrus and ovulation are based on the shortening of the CL lifespan. However, none of them has been shown to be technically and economically satisfactory. Alternatively, synchronisation of oestrus and ovulation can be done by means of exogenous progesterone/progestogens to prolong the luteal phase effect.

3.2.2 USE OF PROGESTOGENS

Since progesterone suppresses oestrus and ovulation, administering progesterone or its derivatives (synthetic progestogens) can be used to synchronise the two events in cattle. In this method, gonadotrophin release, and thus follicular maturation are

suppressed until simultaneous withdrawal of progesterone from the treated animals, which will theoretically synchronise ovulation in the group of animals. To ensure that all animals are in the follicular stage of the cycle at the end of treatment, it is necessary to treat them with progesterone for approximately the length of the natural luteal phase, ie. 12-16 days in cattle. This is because exogenous progesterone has little effect on the lifespan of the *corpus luteum* and in some cases the natural *corpus luteum* might outlive a short-term progesterone treatment, resulting in a failure of synchrony (Peters *et al.*, 1987a).

There are different ways to use progesterone and progestogens: in feed, by injection or implant (Table 3.3.). Treatment in feed requires that the compounds are orally active, ie. they are absorbed into the blood unchanged. The progestogens administered orally include melengestrol acetate (MGA), 6-methyl-17-acetoxy-progesterone (MAP), 6-chloro-6-dihydro-17-acetoxy-progesterone (CAP) flurogesterone acetate (FGA) and dihydroxy progesterone acetophenide (DHPA). Oral administration of these progestogens involves difficulty in controlling dosage and the possibility of tissue or product residues. Alternatively, progestogens can be injected, but this method has the disadvantages of the need for repeated treatment and an imprecise progestogen withdrawal. Consequently, intravaginal and subcutaneous implant techniques have been developed. These methods are suitable as the withdrawal of progestogens can be precisely controlled by implant removal. Intravaginal sponge pessaries, the progesterone releasing intravaginal device (PRID), the controlled internal drug releasing device - type B (CIDR-B) and Norgestomet (17α-acetoxy-11β-methyl-19-nor-pre-4-ene, 20-dione) are forms of progestogen implants which have been extensively used (see also sections 3.2.3.1.1. and 3.2.3.1.2.)

Long-term progestogen administration (14-21 days) has been widely tested in cows in numerous studies since early the 1960s (reviewed by Britt *et al.* (1980), Hansel *et al.* (1983), Peters *et al.* (1987a), Odde (1990), Larson *et al.* (1992) and Macmillan *et*

al. (1993)). Although this method is very effective in synchronising oestrus, poor pregnancy rates are common. Longer periods of progesterone treatment produce more precise synchrony of response possibly because the follicular wave pattern is not maintained (McMillan *et al.*, 1989), and a large follicle persists following spontaneous luteolysis (Sirois *et al.*, 1990). The mechanism contributing to reduced fertility following extended progestogen treatment is not well understood. This may be because of adverse changes in the intrauterine environment, which inhibit sperm transport (Peters *et al.*, 1987, Larson *et al.*, 1992), altered follicular growth and an increase in the number of atretic follicles (Guthrie *et al.*, 1970, Savio *et al.*, 1993b), retarded cleavage rates (Wishart *et al.*, 1974), or reduced fertilisation rate (Hill *et al.*, 1971, Reed *et al.*, 1972).

In particular, the stage of the oestrous cycle at initiation of treatment affects the duration of exposure to progestogens (endogenous progesterone plus exogenous progestogen) and therefore fertility. In the study of Gyawu *et al.* (1983) fertility was normal if progestogen treatment was initiated early in the cycle, but reduced if initiated late in the cycle. Likewise, Mihm *et al.* (1994) concluded that treatment with a synthetic progestogen toward the end of the luteal phase caused a variable extension of the period of dominance of the ovulatory follicle. The "persistent" follicle could ovulate and form a functional *corpus luteum,* but the pregnancy rate was sequentially decreased as the duration of dominance increased. Ovulation of abnormal ova from these aged follicles may be a main reason for low pregnancy rates in animals under progesterone influence for periods longer than the length of the normal luteal phase (Gyawu *et al.*, 1983, Macmillan *et al.*, 1990b, Savio *et al.*, 1993b).

Therefore, acute progesterone administration to induce turnover of persistent follicles maintained by MGA feeding or by a Nogestomet implant has been shown to increase fertility from ovulation of newly recruited follicles (Anderson *et al.*, 1994, Rajamahendran *et al.*, 1994); better fertility can also be achieved by reducing duration

of progesterone treatment but at the expense of synchrony (Patterson *et al.*, 1989, Macmillan *et al.*, 1990a).

As long-term progestogen administration suppresses fertility and short-term progestogen treatments, which result in better fertility, do not synchronise ovulation effectively, a possible solution is to incorporate a luteolytic agent with short-term progestogen treatments to ensure both efficient synchrony and normal fertility. Prostaglandins and oestrogens have been extensively used as such luteolytic agents in the following regimes.

3.2.3 PROGESTOGEN - LUTEOLYTIC AGENT COMBINATIONS

3.2.3.1 Progestogen - Prostaglandin Combinations

Luteolysis may be induced by administration of prostaglandins at or toward the end of a short term progesterone treatment. PGF2α and its synthetic analogues, when administered after day 5 of the oestrous cycle, block progesterone secretion by the CL within 24 h and cause irreversible luteolysis (Wenzel, 1991, Gyawu *et al.*, 1991). Therefore, at the end of progestogen treatment, cattle should either have a CL susceptible to regression by prostaglandin or have already undergone natural CL regression. Theoretically, these cattle should show oestrus soon after termination of the progestogen treatment and prostaglandin injection.

Treatments using short-term administration of progesterone in combination with an injection of a prostaglandin product have been shown to result in more satisfactory fertility than treatment with progesterone alone. For example, Heerche *et al.* (1979) reported that when beef heifers were treated with a 7 day Norgestomet ear implant and injected with PGF2α on day 6 or 7 after implantation, 93% showed oestrus within 5

days and 62% of these conceived, similar to a 60% conception rate for controls. Many other studies, as reviewed by Hansel *et al.* (1983), Odde (1990), and Macmillan *et al.* (1993), have also indicated that the use of prostaglandins at the end of 7-10 day progestogen treatments (oral consumption, subcutaneous implant, intravaginal devices) can result in normal fertility. However, this has not produced sufficient synchrony of oestrus/ovulation for fixed time AI (Bo *et al.*, 1995). Moreover, pregnancy rates were low when treatment was started during the late luteal phase (Beal *et al.*, 1986, Brink *et al.*, 1988). This is, again, because the interval from prostaglandin treatment to expression of oestrus is determined by the stage of development of the DF at the time of treatment (Kasletic *et al.*, 1990, Savio *et al.*, 1990a), and also because short term progestogen treatment initiated late in the oestrous cycle still prolongs the maintenance of the DF resulting in ovulation of an aged oocyte (Beal *et al.*, 1988, Savio *et al.*, 1993b). In addition, in 10% or more of cows treated with prostaglandin failure of complete luteolysis occurs and in up to 20% of cows injected with prostaglandin progesterone concentrations remain low for an unusually long period; this may be associated with a delay in the timing of oestrus and ovulation (Peters *et al.*, 1987a).

A tighter synchrony of response can be obtained by injecting prostaglandins 2 days prior to progestogen removal (Beal, 1983, Smith *et al.*, 1984, Odde *et al.*, 1984, Macmillan *et al.*, 1993). However, this requires an additional day of cattle handling. Alternatively, to overcome the potential problem of reduced fertility following a progestogen treatment and to take advantage of the greater effectiveness of PGF2α during the late luteal phase, another system has been developed. In this system, MGA was fed for 14 days and PGF2α was administered 16-18 days after the last day of MGA feeding. This system was designed to place cattle in a "good stage" of the cycle for PGF2α injection (Odde, 1990). Patterson *et al.* (1992) reported that heifers treated in this way had a greater proportion exhibiting oestrus compared with controls (79 % vs 32 %). Although the conception rate at first service did not differ (64 and 67 %,

respectively), synchronised pregnancy rates were higher for MGA - prostaglandin treated heifers than for controls (50 % vs 21 %). Similar results have also been reported by Brown *et al.* (1988) and Jaeger *et al.* (1992) in beef heifers, and by Yelich *et al.* (1995a) in suckled post-partum beef cows.

3.2.3.2 Progestogen - Oestrogen Combinations

The results from the use of prostaglandins point to the need to synchronise follicular development in order to ensure the presence of a viable growing DF at the end of treatment (Fortune, 1993). Short term progestogen treatments which rely on the concurrent use of a luteolytic agent still produce less precise synchrony unless follicle wave patterns are also synchronised (Macmillan *et al.*, 1993). It appears that treatment with progesterone and oestrogen in combination may be used to effectively control and synchronise follicular wave development (Bo *et al.*, 1994a, Adams, 1994, Bo *et al.*, 1995). This is because the combination is more effective in inducing follicle suppression than treatments with either hormone alone. Exogenous oestradiol causes regression of the progestogen-maintained DF by altering serum LH profile and the DF from the next wave ovulates (Rajamahendran *et al.*, 1994). Oestradiol administration can also cause premature luteolysis by increasing PGF2α release and the response of luteal tissue to PGF2α (Goodman, 1988).

There are some well-known progestogen-oestrogen combinations which are widely available today including the PRID/CIDR-B and Syncro-Mate B/Crestar treatment regimes.

The PRID (progesterone releasing intravaginal device) consists of a stainless steel coil covered by a layer of inert silicon with 1.55 g natural progesterone impregnated into it. The CIDR-B (controlled internal drug releasing device - type B) is a nylon spine coated with silicon-based elastomer containing 1.9 g of natural progesterone. In these

progesterone-oestrogen combination treatment regimes a gelatin capsule containing 10 mg of oestradiol benzoate (OB) is attached to the inner surface of the PRID or CIDR-B. The PRID or CIDR-B is inserted into and held within the cow's vagina for up to 12 days. The OB rapidly acts as a luteolytic agent after being absorbed through the vaginal wall, and the progesterone is gradually released from the elastomer maintaining the suppression of oestrus and ovulation until removal of the device. Cows should show oestrus 48-72 hours after the removal and AI may be performed during this period (Peters *et al.*, 1987a).

A high degree of synchrony has been shown when cows were treated with the CIDR-B regime (Bo *et al.*, 1995). Tjondronegoro *et al.* (1987) found better oestrous response and synchrony in cows treated with the PRID for 12 days compared to cows treated with the CIDR-B for either 9 or 12 days; conception and pregnancy rates per group for the first service were similar. In addition, data from Tribulo *et al.* (1995) suggest that oestradiol treatment 1 or 2 days after the insertion of CIDR-B can result in a more synchronous oestrus and ovulation than traditional approaches to synchronise ovulation for timed AI which use progesterone alone or with oestradiol from the beginning of progestogen treatment.

The Syncro-Mate B (SMB) and Crestar regimes have been used for oestrus control in dairy and beef heifers and post-partum beef cows. They consist of a 9 day ear implant containing 6 or 3 mg of a synthetic progesterone (Norgestomet) and a 2 ml i.m. injection of 3 mg of Norgestomet plus 5 mg of oestradiol valerate (OV) at the time of implant insertion. Whitter *et al.* (1986) showed that a combination of a Norgestomet implant with a Norgestomet plus oestradiol injection at implant injection in this way produced a more precise synchrony than that using an implant with PGF2α injected at implant removal. Mikeska *et al.* (1988) reported that 73% of heifers exhibited oestrus within 54 h following SMB removal with a conception rate of 59.3%. Odde (1990), summarising studies on the effectiveness of Synchro-Mate B, showed that this

treatment resulted in a high and early oestrous response (77-100 %), but still variable fertility (first conception rate ranged from 33 to 68 %).

Factors which influence the success of progestogen-oestrogen combination treatment regimes include proportion of animals cycling prior to treatment, post-partum interval from calving to treatment, body condition, stage of the oestrous cycle at initiation of treatment, effectiveness of the luteolytic agent and others (Larson *et al.*, 1992). More detailed discussion on most of these factors will be presented in chapter 4.

In general, better fertility with reduced synchrony will be obtained if the treatment is initiated early in the cycle or, vice versa, better synchrony with reduced fertility will be achieved if treatment is started late in the cycle (Brink *et al.*, 1988, Pratt *et al.*, 1991, Fanning, 1992, Burns *et al.*, 1993, Mihm *et al.*, 1994). The reduced synchrony may be due to ineffectiveness of the luteolytic agent. Evidence has shown that oestradiol is not a very effective luteolytic agent nor does it prevent formation of the CL when administered early in the cycle with the CL outliving the progestogen treatment (Peters, 1984). In addition, in some circumstances progesterone levels in the cow may fall before progestogen withdrawal (Roche *et al.*, 1981), resulting in oestrus and ovulation occurring before removal of the device. Such a premature fall is thought to be related to progesterone-induced changes in absorption across the vaginal wall rather than to exhaustion of progesterone in the device (Peters *et al.*, 1987a). On the other hand, the reduced fertility in animals treated late in the cycle may be, as with progestogen-prostaglandin combinations, because of an uninterrupted long term progesterone exposure (endogenous and exogenous) and maintenance of a persistent DF resulting in ovulation of an aged follicle (Odde, 1990, Larson *et al.*, 1992).

The progestogen-luteolytic agent combinations have been widely used in practice; Nevertheless, as discussed above, results are inconsistent. In an attempt to improve

success rate other hormones have also been tested in a number of different combinations.

3.2.4 OTHER COMBINATIONS

Administration of GnRH or its derivatives has long been used in combination with the above mentioned treatment regimes. However, the results obtained are still variable. For example, GnRH injected 30 hours after Norgestomet removal has been reported to increase timed breeding pregnancy rates in cyclic beef cows (Troxel, *et al.*, 1993). In another report (Roche, 1975), the use of GnRH 30 hours after progesterone withdrawal could synchronise the onset of ovulation in heifers. On the contrary, GnRH injected 40 hours after implant removal in a Syncro-Mate B program has been shown not to improve fertility in dairy heifers (Anderson *et al.*, 1982).

Treatment with GnRH given 6 days before induction of luteolysis with prostaglandin is a practical method for controlling ovarian follicular and luteal functions and increasing the precision of oestrous synchronisation in cyclic cows and heifers, as reviewed by Twagiramungu *et al.* (1995b). This method reduces considerably the period of time needed for oestrus detection, synchronises the oestrous cycle of 70-80% of the cyclic cows to within a 4 day interval without any detrimental effect on the fertility rate (65-85%). Also, a GnRH-PGF2α-GnRH protocol has been shown to be effective in synchronising ovulation in dairy cow, facilitating fixed time AI (Pursley *et al.*, 1995).

The use of PMSG with long term (12-16 day) progestogen treatments has been shown to be an important factor in increasing calving rates after treatment (Mauleon, 1974, Munro *et al.*, 1985). Beef cows treated with the PRID for 7 days and given 0.5 mg cloprostenol plus 750 i.u. PMSG i.m. injected 24 h before PRID removal had better

calving rates than those receiving the PRID plus only cloprostenol (Munro, 1987). In another experiment on beef cattle, where all animals received the PRID (with OB) for 7-14 days with 750 I.U.. PMSG i.m. injected 24 h before PRID removal and half of the animals also received 25 mg dinoprost at either 0 or 24 hours before PRID removal, Munro and Bertram (1988) found that the most appropriate regime for use in extensive environments would be based on a 14 day progestogen treatment plus OB and PMSG. This eliminates the need for a prostaglandin injection before PRID removal while still producing acceptable fertility.

Combinations of progesterone/progestogen with both oestradiol and prostaglandin have also been tested. In an experiment on beef heifers (Bo *et al.*, 1994a), treatment with CIDR-B for 7 days plus 100 mg progesterone and 5 mg oestradiol im at the time of CIDR-B insertion and 0.5 mg cloprostenol at the time of CIDR-B removal, resulted in 75% of the heifers ovulating between 72 and 84 h after CIDR-B removal; in contrast, only 40% of heifers treated with 2 injections of PGF2α 11 days apart and 33% of heifers treated with CIDR-B without oestradiol ovulated during the same period of time.

Variable results of the above synchronisation methods suggest the need for further research into more effective techniques of synchronisation of oestrus and ovulation in cycling cattle. However, in reality, most oestrus control programs are undertaken in the post-partum period or in herds of random reproductive status. Therefore, it would not be sufficient for such a program to direct attention to cycling animals only. In addition to synchronisation of oestrus and ovulation in cycling animals, induction of these two events in non-cycling cattle is necessary and will be discussed in the following section.

Table 3.3: Summary of methods of progestogen treatment for synchronising oestrus and ovulation in cyclic cattle

Method	Treatment protocol	Time of AI
Long-term Progestogen	Progestogen (oral consumption, daily injection, subcutaneous or intravaginal implant) for 14-21 days	At fixed time or detected oestrus
Progestogen Plus	1) Progestogen for 12-16 days followed by PMSG.	At detected oestrus or fixed time
	2) Progestogen (implant or oral) for 5-9 days; prostaglandin given at or toward the end of progestogen treatment; PMSG or GnRH given toward, at or following progestogen removal (optional).	At detected oestrus or fixed time
	3) Oestradiol plus progestogen injection on day 1 with progestogen implant for 9-10 days beginning on day 1 (SMB, Crestar); prostaglandin and/or PMSG given toward, at or following progestogen removal (optional).	At detected oestrus or fixed time
	4) Progesterone releasing intravaginal device plus oestradiol capsule held for 10-12 days (PRID, CIDR-B); PMSG or GnRH given toward, at or following progestogen removal (optional).	At detected oestrus or fixed time

3.3 INDUCTION OF OESTRUS AND OVULATION IN ANOESTROUS CATTLE

3.3.1 GONADOTROPHIN/GnRH TREATMENTS

Follicle growth and ovulation in noncycling cows can be stimulated by administering hormones that have gonadotrophic activity: either FSH, in the presence of LH, or PMSG can stimulate follicular growth. Follicular growth can be stimulated with a single dose of PMSG, whereas multiple doses of FSH are usually required to obtain the same response since it has a shorter biological half life. In some cases, it may be necessary to administer LH or HCG about 48-96h after gonadotrophin injection if developing follicles do not secret enough oestrogens to induce an endogenous LH surge (Britt *et al.*, 1980, Hafez, 1993c).

Kamomae *et al.* (1989) gave a single injection of between 750-6000 i.u. of HCG or 1000-2000 i.u. of PMSG to cows with inactive ovaries and a palpable follicle. HCG increased plasma concentrations of LH without a preovulatory surge and most treated cow (12/13) ovulated without showing behavioural oestrus. PMSG increased plasma LH levels and all of the treated animals ovulated with most of them (4/5) showing a preovulatory LH surge and oestrous signs. However, in other studies, there have been different responses following a given treatment with exogenous gonadotrophins, ranging from no ovulation to excessive ovulatory response. This is possibly due to genetic variations, physiological state of the animal when treated and the hormone doses used.

One of the most important causes of delayed ovulation in cows is the failure of frequent GnRH pulse secretion in the early post-partum period due to absence of the negative feedback effects of progesterone (Roche *et al.*, 1992). Therefore, in addition to exogenous gonadotrophin treatments, GnRH has been administered to stimulate

endogenous FSH and LH secretion and subsequent ovulation. However, stage post-partum, breed of the animal, age, season and nutrition all appear to influence the response to GnRH (Britt *et al.* 1980).

Ovulation can be induced in post-partum cows by a single injection (0.1-0.5 mg), two injections 10-14 days apart, or frequent low dose injections at 1-4 h intervals of GnRH or its analogues (Britt *et al.*, 1980, Peters *et al.*, 1987a, Roche *et al.*, 1992). GnRH was effective in cows with cystic ovaries or for the treatment of repeat breeder cows (Humblot *et al.*, 1993). However, these treatments have consistently failed to induce ovulation in over 90% of treated anoestrous cows when the follicular status was not known (Roche *et al.*, 1992).

The follicular status at the time of treatment has been a major factor suggested to affect the ovulatory response to GnRH. For cattle to respond to a single dose of GnRH, a mature follicle must be present at the time of treatment. GnRH causes a preovulatory LH surge, which then causes the mature follicle to ovulate (Hafez, 1993c). In beef suckler cows a single dose of GnRH analogue (0.02 mg i.m. buserelin) was successful in ovulating the first dominant follicle (Crowe *et al.*, 1993), but in the absence of an identified dominant follicle there were variable oestrous and ovulatory responses to GnRH treatments (Roche *et al.*, 1992). This may explain why the use of GnRH in the early post-partum period has not produced a clear improvement in subsequent reproductive efficiency (Thatcher *et al.*, 1993). This is also because the induction of an LH surge by GnRH depends on the endocrine state of animals at the time of treatment. The responsiveness of the anterior pituitary to GnRH stimulation increases during the early post-partum period (Peters *et al.* 1987a) and is governed by the follicular state prior to treatment (Peters *et al.*, 1987b). GnRH induced a peak of LH equivalent to the normal preovulatory surge after 20 days post-partum in dairy cows and after 30 days post-partum in beef cows (Britt *et al.*, 1980).

In cattle, oestrus is not usually exhibited in response to gonadotrophin injection alone; it normally occurs after previous exposure to elevated progesterone or synthetic progestogens (Hafez, 1993c). Thus an ovulation induction regime in the cow should include a period of progestogen treatment if insemination is to take place.

3.3.2 STEROID TREATMENTS

Progesterone suppresses the release of gonadotrophins from the anterior pituitary. Following withdrawal of this suppression, an endogenous surge of gonadotrophins ensues approximately 48 h later (Peters *et al.*, 1987a). A transient increase in progesterone and LH frequently follows the gonadotrophin surge. Oestrus does not occur before this initial transient increase in progesterone. When the transient increase in progesterone decreases oestrus and ovulation may occur within a few days (Hafez, 1993c).

However, the use of progesterone to induce ovulation post-partum has produced equivocal results. Some authors reported a delay in the onset of ovarian cycles, whilst others reported that the presence of progesterone advanced or did not influence the time of the first ovulation or conception. For example, Murphy *et al.* (1989) reported that in early post-partum anoestrous dairy cows the intervals to first ovulation were prolonged by 10 days in a PRID treatment compared with untreated controls. They suggested that this was because it delayed the ocurrence of first ovulation until the PRID removal; on the other hand, PRID treatment did significantly advance days to first detected post-partum oestrus. The incidence of short oestrous cycles was also significantly reduced. In contrast, Kyle *et al.* (1992) indicated that administration of progesterone alone during the early post-partum period did not increase the proportion of cows expressing oestrus at the first ovulation. The reason for these differences between studies is not apparent.

In general, fertility at oestrus induced by progesterone treatments is low. For example, Roche *et al.* (1981) showed that a 12 day treatment of beef cows with PRIDs (without OB) induced ovulation in about half of the treated cows. Similarly, Bulman *et al.* (1978) reported a 75% success with a 12 day PRID treatment in dairy cows but with only a 50% conception rate.

Recent data have indicated that exogenous oestrogens can also be used for oestrus induction. A dose of 0.6 mg OB was found to induce behavioural oestrus in 50% of non-cycling post-partum dairy cows (McDougall *et al.*, 1992). Although exogenous oestrogens are able to elicit a preovulatory type LH surge during the early post-partum period, ovulation and ovarian cycles do not consistently follow. Garcia-Winder *et al.* (1988) found no differences in the average days post-partum when cows first ovulated between anoestrous beef cows treated with an ear implant containing 24 mg oestradiol for 21 days and untreated controls. Therefore, the use of oestrogens alone to induce ovulation has not been particularly successful since anovulatory oestrus may occur.

Better results in inducing oestrus and ovulation have been obtained when progesterone is given for a few days followed by a single dose of oestrogen. Otherwise, an oestradiol conjugate can be administered at the start of progestogen treatment as an ear implant or as an intravaginal pessary (Hafez, 1993c). McDougall *et al.* (1992) reported that 81% of noncycling post-partum cows treated with CIDR-B for 5 days followed by 0.6 mg OB 48 h after CIDR-B removal were detected in oestrus within 60 h after OB injection, compared with 39% of control cows given OB only. Oestrogen enhances release of gonadotrophins which have accumulated in the anterior pituitary during the period of progestogen treatment (Britt *et al.*, 1980). Progesterone treatment was suggested to sensitise cows to oestradiol and consequent behavioural responses (McDougall *et al.*, 1992).

The response of post-partum cows to steroid treatments is thought to be affected by the stage of ovarian resumption post-partum. In dairy cows, not under nutritional stress, experiments were carried out to determine the effect of the post-partum interval on oestrous response to 9-12 day progesterone (PRID) treatment together with an injection of 5 mg OB and 50 mg progesterone at the start of treatment (Roche, 1976). Cows having calved for more than 30 days had a higher (95%) oestrous response than those having calved for less than 30 days (45%).

Post-partum cows under nutritional stress have small and inactive ovaries and low nutrient intake may cause either a delay in resumption of DF development or development of smaller and less oestrogen-active DFs (Prado *et al.*, 1990). This may be an important cause of the inconsistent responses following steroid treatments. Thus, an additional gonadotrophin stimulus may be required for nutritionally deprived cows.

3.3.3 STEROID-GONADOTROPHIN COMBINATIONS

Enhanced oestrous response has been obtained when progestogen treatments are given in combination with oestrogens and/or gonadotrophins given at progestogen removal. The procedures of most of these methods are somewhat similar to those applied in oestrous synchronisation.

PMSG has been the most widely used gonadotrophin in such a regime since a single injection of PMSG at the end of progestogen treatment is effective. Presumably, PMSG stimulates oestrogen synthesis in developing follicles and increasing levels of circulating oestrogens stimulate endogenous FSH and LH release (Britt *et al.*, 1980). The dose required depends on the season, breed, and level of nutrition. About 400-800

i.u. of PMSG appears to give good results; however, there is considerable variation in ovarian response to a single dose of PMSG.

Data reported by Macmillan and Day (1987) suggest that early post-partum anoestrous cows in poor body condition may benefit from the use of PMSG in conjunction with a 7-12 day progesterone treatment. However, the use of gonadotrophins may not be essential if the anoestrous condition is a consequence of suckling and is not confounded by undernutrition (Macmillan *et al.,* 1993).

Combinations of progesterone-oestrogen-gonadotrophins have recently been used extensively. PMSG has been used in combination with 7-14 days of the progestogen-oestrogen treatment regimes using the PRID (Munro, 1987, 1989), CIDR-B (Macmillan *et al.*, 1988, Pickering, 1988, Jub *et al.*, 1989, Macmillan *et al.*, 1993) and SMB (Odde, 1990). The majority of post-partum suckler cows exhibit oestrus within 5 days after treatment involving oestrogen given on day 1 followed by 7-12 days of progestogen and PMSG injected on the last day of progestogen treatment. There is of course the possibility of multiple ovulations resulting from PMSG use.

In addition, GnRH and HCG have also been used in combination with steroid treatments. For example, Troxel *et al.* (1993) observed increased pregnancy rates in acyclic beef cows when GnRH was injected 30 h following Syncro-Mate B implant removal. Rao *et al.* (1991) indicated that cows treated with oestradiol prior to GnRH increased the GnRH-induced serum LH level and showed normal luteal function compared to those treated with GnRH alone. Agarwal *et al.* (1988) reported that HCG appeared to be beneficial to pregnancy due to the induction of more large CLs in a treatment in which post-partum cows were treated with 0.5 mg MGA/day for 14 days and given an injection of 0.4 mg OB 48 h after the last day of MGA feeding with an injection of 1500 i.u. HCG at the time of ovulation.

All the above treatments can be applied to induce oestrus and ovulation not only during post-partum anoestrus but also in the prepubertal period. However, the main cause of post-partum anoestrus is frequent suckling. Uterine involution in early post-partum cows is another concern. Addressing these problems may bring about some benefits for control of oestrus and ovulation in post-partum cows.

3.3.4 PROSTAGLANDIN ADMINISTRATION

PGF2α is involved in uterine involution (Bazer *et al.*, 1993). Administration of exogenous prostaglandins can hasten uterine involution in the post-partum cow (Lindell *et al.*, 1983) and be effective in the treatment of metritis (Korenic, 1984). Intramuscular injections of prostaglandins have been shown to increase GnRH-induced LH release (Harrison *et al.*, 1984). Therefore, it is possible that administration of prostaglandins during post-partum anoestrus improves subsequent reproductive function. Recent research has tended to support this hypothesis, although results have been variable.

Variation in results following exogenous PGF2α administration appears to be influenced by the time of treatment after parturition and the genetic character (beef or dairy) of the cows. Randel *et al.* (1988) reported that administration of alfaprostol (PGF2α analogue) to beef cows 21 days after parturition was not effective in reducing post-partum anoestrus or increasing pregnancy rates, but administration 32 days after parturition resulted in a significantly shorter PPI to oestrus and an increased pregnancy rate. Jaeger *et al.* (1995) concluded from their experiments that administration of PGF2α during the early post-partum period improved subsequent reproductive function in beef cows by improving pregnancy rates, although the optimal time for PGF2α administration was not resolved.

In dairy cows, Armstrong *et al.* (1989) administered PGF2α on the day of parturition or between 14 and 21 days after calving but found no improvement in conception and PPI to first service. In contrast, McClary *et al.* (1989) showed that exogenous PGF2α given at 14-16 days post-partum improved first service conception rate and reduced the number of days open. Similar results have also been reported by Young *et al.* (1986) when PGF2α was given between 14 and 28 days post-partum and by Etherington *et al.* (1984) when PGF2α was given 24 days after parturition.

In another approach, PGF2α was given in combination with short term progestogen treatments. Beal *et al.* (1986) reported that an injection of PGF2α at the end of a short term feeding of MGA induced cyclicity successfully in some noncycling cows. Galloway *et al.* (1987) gave anoestrous post-partum dairy cows treated with the SMB regime for 10 days an injection of Prosolvin (containing a PGF2α analogue) on day 8 plus an injection of PMSG at implant removal. It was concluded that this treatment regime could be effective for induction of ovulation in anoestrous post-partum dairy cows.

3.3.5 ALTERED SUCKLING

Some management regimes have been tested to address the problem of suckling effect on post-partum anoestrus. These include early weaning, temporary weaning or restricted suckling, or a combination of early weaning or calf-removal with drug treatment.

Williams (1990), in a review, showed that early weaning alone or in combination with hormone treatment reduced PPI and increased the overall conception rate. Allowing calves to suckle their dams only once a day for 30 to 60 min. also increased conception rates and decreased PPI. In addition, Tegegne *et al.* (1992) demonstrated

Table 3.4: Summary of methods for inducing oestrus and ovulation in anoestrous cattle

Method	Treatment protocol	Time of AI
Gonadotrophin/ GnRH treatment	1) Single injection of PMSG or GnRH; HCG or LH given 48-96 h later (optional) 2) Multiple injections of FSH or GnRH 3) Two GnRH injections 10-14 day apart	At detected oestrus or fixed time
Steroid Treatment	1) Progestogen alone (implant or intravaginal devices) for 9-12 days. 2) One injection of oestrogen plus progesterone followed by 9-12 days of progestogen. 3) oestrogen on day 1 followed by 7-12 days of progestogen. 4) Progesterone for 5-7 days followed by a dose of oestrogen.	At detected oestrus or fixed time
Steroid-gonadotrophin combination	1) A dose of PMSG, GnRH or HCG at end of steroid treatment.	At detected oestrus or fixed time
PGF2α	1) A dose of PGF2α during post-partum anoestrus. 2) A dose of PGF2α at the end of progestogen treatment.	At detected oestrus or fixed time
Altered suckling	1) Early weaning. temporary weaning or restricted suckling. 2) Temporary calf removal following drug treatment.	At detected oestrus or fixed time

that restricted suckling increased the percentage of cows showing oestrus and reduced their PPI compared with continuous suckling. However, there is a high cost of intensive labour inputs associated with the handling of the young to allow altered suckling patterns.

Calf separation has been shown to temporarily remove the suckling-induced suppression of pituitary gonadotrophin secretion (Hafez, 1993c). Separation of calves from their dams from the time of implant removal until breeding is recommended to be used in combination with SMB/Crestar and other drug treatments. Calf removal has improved the oestrus response and pregnancy rate following drug treatments in many studies (Mares *et al.*, 1977, Dowling *et al.*, 1977, Kiser *et al.*, 1980, Brown *et al.*, 1986, Yelich *et al.*, 1995b).

However, Pace *et al.* (1980) reported no advantage in pregnancy rate for cows treated with SMB with calf removal compared with no calf removal if the cows were bred according to oestrus. The benefit of calf removal also appeared to be reduced in herds in adequate body condition (Kiser *et al.*, 1980). In addition, Rivera *et al.* (1994) indicated that temporary calf removal plus FSH supplementation resulted in increased ovulation and oestrus rates and shorter PPI, but subnormal CLs were found following the first ovulation in early post-partum beef cows.

3.4 CONCLUSION

Effective methods of controlling oestrus and ovulation have been sought to facilitate broader use of AI and ET, thereby allowing greater exploitation of genetically superior breeding animals. These techniques are also aimed at producing calves in favourable periods, eliminating the need for oestrus detection, and shortening the calving interval, thus improving the efficiency of production.

Basically, there are two ways to synchronise the oestrous cycle of a group of cyclic animals. The first method is to cause luteolysis in all animals with a luteolytic agent, after which all animals will come into oestrus and ovulate. The second method is to treat all animals with a progestational compound to prevent oestrus and ovulation for sufficient time to allow regression of the CL of all animals in the group. After withdrawal of the progestational compound all animals will theoretically show oestrus and ovulate. Based on these two principles a variety of methods have been developed, in which oestrus and ovulation are synchronised using prostaglandin F2α (and its analogues), progesterone (and synthetic progestogens), progesterone-luteolytic agent combinations, and their combination with other hormones.

Prostaglandins can be used in single or double injection programs. Although fertility of the oestrus-induced animals after single prostaglandin treatment is similar to that of controls, prostaglandin alone is ineffective in causing luteolysis and therefore synchronised oestrus in the early part of the luteal phase. Animals treated with prostaglandins in the late stages of the luteal phase have higher oestrous response and may be more fertile. Double prostaglandin injections given 10-14 days apart can result in good oestrous response and fertility. However, there still exist the possibilities of "carryover" effects and inconsistent response as well as imprecise timing of oestrus. To overcome these shortcomings, oestrus detection, *corpus luteum* palpation, progesterone measurements, natural service and the use of other hormones (GnRH, HCG, PMSG and oestrogens) have been incorporated in prostaglandin treatments. However, the results obtained are still equivocal and such procedures add to the cost and complications of the synchronisation protocols.

Progesterone administration by means of subcutaneous ear implant, i.m. injection or oral consumption for 14-21 days, can induce good oestrous synchrony but low fertility. For this reason, prostaglandins and oestrogens are incorporated as luteolytic

agents to reduce duration of progesterone treatment. The use of prostaglandin at the end of a short term progestogen treatment can result in normal fertility, but the onset of oestrus is more variable, especially when given at mid cycle. Therefore, this regime does not produce sufficient synchrony of oestrus/ovulation for timed AI. In addition, fertility may be reduced if treatment is initiated later in the cycle due to the ovulation of aged follicles. Injection of PGF2α two days before progesterone removal may lead to tighter synchrony. Alternatively, a 14 day progesterone treatment combined with a prostaglandin injection given 16-18 days after progesterone removal may result in greater fertility than that immediately following progesterone treatment. GnRH and gonadotrophins have also been used with either progestogens or progestogen-luteolytic agent combinations. PMSG administration appears to improve the effectiveness of these treatments.

For anoestrous cattle, oestrus and ovulation can be induced by stimulating resumption of follicular development and exposure of the DF to an LH pulse frequency pattern similar to that in the follicular phase of a normal oestrous cycle. Several hormones have been used for this purpose.

The growth of ovarian follicles in anoestrous females can be induced using FSH with or without LH, PMSG with or without HCG. In addition, a surge of LH may be stimulated by the administration of GnRH or an artificial LH-like surge can be caused by means of HCG. Normally, a large dose of GnRH will cause an existing mature follicle to ovulate through release of endogenous LH and FSH. However, in cattle oestrus does not normally occur following gonadotrophin injection alone. Therefore, progesterone or progestogen-gonadotrophin combination treatments have been developed as oestrus is usually exhibited only after previous exposure to elevated progesterone or synthetic progestogens.

Combinations of progestogen and oestrogen have been used for ovulation induction in anoestrous cattle. Oestrogen may be administered at initiation or end of short term progestogen treatments given as subcutaneous implant or intravaginal devices. Improved results from this combination of oestrogen and progestogen can be obtained when PMSG is given at the end of progestogen treatment.

Administration of PGF2α during post-partum anoestrus appears to be beneficial. Reduced suckling or separating calves from their dams for 2-3 days after termination of drug treatment has also resulted in an increased response in many cases.

Improvements have been made in enhancing the efficacy of oestrus control. However, it is obvious that the current methods are still complicated and rarely cost effective. No single method has produced consistently good results which can be applied with ensured success in field conditions. Among factors identified to affect the response, the stage of the oestrous cycle, state of follicular development, nutritional status and effectiveness of the drugs used appear to be most important. How these factors affect the efficacy of oestrus and ovulation control is still to be elucidated. Further research is needed before the widespread use of oestrus and ovulation control programs in cattle will become a fact of life. Identification of the factors influencing the efficacy of each method of oestrus and ovulation control under particular circumstances is scientifically and practically important. That is the objective of the following experimental chapter.