PHYSIOLOGY OF THE ESTROUS CYCLE

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Summary

Current and emerging concepts of follicle growth and ovulation, hypothalamic-pituitary-ovarian interactions, corpus luteum function and estrous cycle regulation in cattle, sheep and swine are reviewed. Follicles grow, regress and are replaced by other large follicles continuously throughout the cycle; intra-ovarian factors appear to play important roles in this process. Estradiol concentrations rise in preovulatory follicles until the preovulatory LH surge and then decline sharply. A two ceil-two gonadotropin model for control of follicular steroidogenesis has emerged and the idea has been advanced that follicular androgens are synthesized via the $\Delta^5$ pathway, with high concentrations of intrafollicular estradiol suppressing progesterone production prior to the LH surge. The production of nonsteroidal inhibitors of FSH secretion and hypothalamic hormones by ovarian tissues has been suggested. Our present concepts are probably only partial explanations of the events that occur during corpus luteum regression. Evidence is summarized to suggest that prostacyclin and PGF$_2\alpha$ have luteotropic roles during the growth phase of the corpora lutea of cows and ewes, respectively. Several recent studies suggest that oxytocin may have a physiological function in luteal regression in the cow and the ewe. Phospholipid methylation appears to be an important regulatory step in the mechanism by which LH stimulates luteal cell adenylylate cyclase and subsequent progesterone production. Gonadotropins are secreted in distinct episodic patterns that vary with stage of cycle and reproductive status. Data cited are consistent with the view that consecutive exposures to pulsatile releases of GnRH, under estrogen dominance, progressively increase the magnitude of pulses of LH release a seriatim, thereby creating the preovulatory LH surge. Efficient methods for regulating the estrous cycles of cows and sheep have been developed so that groups of animals may be inseminated at pre-set times without checking for estrus. The most efficient of these methods are based on combining single PGF$_2\alpha$ injections with short-term progesterone treatments. The most promising method for controlling estrus and ovulation in swine is by the use of the orally active synthetic progesterational compound, L, allyl trenbolone. It may be possible to develop even simpler methods for synchronization of cycles in cattle, based on single injections of long-acting luteotropic agents to prolong the functional life of the corpora lutea, followed six days later by luteolytic doses of PGF$_2\alpha$.

(Key Words: Estrous Cycle, Follicle Growth, Ovulation, Corpus Luteum, Hypothalamic-Pituitary-Ovarian Interrelationships.)

Introduction

Although the early investigators made some surprisingly accurate inferences regarding control of the estrous cycles of farm animals (Asdell, 1955), most of the remarkable recent advances in the field of reproductive biology have depended on the development of techniques for rapid, accurate and sensitive measurements of concentrations of pituitary, ovarian and adrenal hormones in blood, tissues and urine. The decade of the 1960's was characterized by the rapid evolution of radioimmunoassay and competitive protein binding techniques for steroid and peptide hormones (Midgley, 1969; Murphy, 1970). These techniques made it possible to carry out large numbers of analyses at relatively low costs. As a result, it became possible to present in 1972,
profiles of the plasma concentrations of pituitary and ovarian hormones during the estrous cycles of the cow, ewe and sow (Hansel and Echternkamp, 1972).

Indeed, there are few areas of biological research where advances have been more closely tied to technological developments and these developments have not been limited to procedures for measuring hormone concentrations. Methods for studying ovarian tissues in vitro, including incubation and culture methods (Hansel, 1971), cell separation techniques and methods for examining the functions of cell organelles as they relate to hormone secretion rates in specific cell types, have played important roles.

Development of knowledge of mechanisms controlling the estrous cycle in domestic animals has been influenced by a number of major fundamental discoveries made since 1950. Two of the most important of these were the discovery and development of the hormone-receptor concept and the discovery that the hypothalamus and brain regulate secretion of anterior pituitary hormones through a number of small peptides of neurosecretory origin. Several of the hypothalamic peptides, including the gonadotropin releasing hormone (GnRH) that causes release of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, have been isolated and synthesized in recent years.

**Follicular Growth and Development**

**General Concepts.** The primordial follicles are established in the ovary during embryonic development. During the lifetime of the female, primary follicles enter a pool of growing follicles in response to a stimulus that remains undefined. But, follicular development to the antral stage probably is independent of gonadotropin support. It is now clear that development of large follicles on the ovary is a dynamic process. For example, large follicles appear on the surface of the ovary, regress and are replaced by other larger follicles. Why some follicles regress and others go on to ovulate is not understood. Follicles destined to ovulate probably arise from a pool of growing follicles less than 48 h before ovulation.

Synthesis of ovarian steroids requires the coordinated activities of several ovarian cell types and two gonadotropins. A classic experiment by Falck (1959) provided the first evidence that different ovarian cell types interact to produce estrogens. Recent evidence suggests that androgens are produced in the theca under the influence of LH and aromatized to estradiol in the granulosa cells of the follicle under the influence of FSH. Most of the follicles present in the ovaries at birth are destined to undergo atresia. Yet, very little is known about this important process.

**Morphology.** Primordial follicles, with their single oocyte surrounded by a squamous follicular epithelium, are established in the ovary during fetal life. These constitute the largest class of follicles in the ovary throughout life. After birth most oocytes are arrested in the dictyate stage of the first meiotic prophase. Primordial follicles enter a growing phase when the squamous follicular cells proliferate and form several layers of granulosa cells. The stimulus that induces primary follicles to enter the growth phase remains a mystery. As the follicle grows it is displaced toward the center of the ovary, the theca layer differentiates from surrounding connective tissue into two layers, externa and interna, and the oocyte acquires a distinct zona pellucida. Growing follicles form fluid filled antra by the coalescence of small fluid filled cavities between follicular cells. The diameter of the oocyte has approximately doubled by the time the antrum appears. The micromorphology of ovarian follicles of cows (Rajakoski, 1960), ewes (Turnbull et al., 1977) and sows (Bjersing, 1967) has been reviewed.

**Follicle Growth.** Factors controlling growth of ovarian follicles and selection of those that will ovulate is not completely understood. Follicular development to the antral stage may occur independent of gonadotropin action (Dempsey, 1937), but the rate of pre-antral follicle growth is accelerated by gonadotropins. Number and size of follicles on ovaries collected at various days of the estrous cycle have been reported for cows (Rajakoski, 1960), ewes (Turnbull et al., 1977) and sows (Robinson and Nalbandov, 1951). One fact emerges from these studies, i.e., follicles of all sizes, including large follicles, exist on each day of the estrous cycle.

Further, it is clear that a sequence of follicles grow, regress and are replaced by other large follicles during the cycle (Smeaton and Robertson, 1971; Matton et al., 1981). For example, Matton et al. (1981) marked large follicles with India ink in heifers, and reported that most of the largest and second largest follicles present.
on the ovaries on d 3 regressed and were replaced by other follicles by d 8. In addition, half of all the largest follicles present on d 8 were no longer the largest by d 13 and all large follicles present on d 13 were replaced by d 18. The large follicle that eventually ovulates is identifiable on the ovary only 48 h before estrus (Dufour et al., 1972).

Analyses of gonadotropin secretions during the cycle reveal no obvious changes that might explain control of growth and subsequent regression of follicles during the estrous cycle. Changes in the pattern of episodic fluctuations in gonadotropin secretion (see below) may be related to follicular dynamics but that relationship cannot be examined without additional data. Possibly, intraovarian factors play a role in development and turnover of follicles. For example in sheep, the ovary bearing the corpus luteum has more follicles than the contralateral ovary (Dufour et al., 1971). This effect may be a local one, due to progesterone or other luteal products, or perhaps to greater blood flow in that ovary. In addition, Bherer et al. (1976) hypothesized that larger follicles exert an inhibitory influence on growth of smaller follicles. In support of this view, Matton et al. (1981) demonstrated that large follicles are quickly replaced following cautery of all follicles >5mm diameter and this occurs at times during the cycle when small follicles would not normally grow to >5mm diameter.

The dynamic functional status of ovarian follicles cannot be adequately assessed by gross or micromorphological evaluation. Thus, biochemical changes in ovine (England et al., 1981) and bovine follicles (Ireland and Roche, 1983a) during the proestrus period have received increased attention. Follicles were classified as estrogen active (nonatretic) and estrogen inactive (atretic) based upon histological assessment of the granulosa and steroid hormone content of the follicular fluid (Moor et al., 1978; Carson et al., 1981). Following luteal regression, both estrogen active and estrogen inactive follicles are present on the ovaries. By the time of the LH surge, only estrogen active follicles are present. Ovulatory follicles grow in size and the numbers of LH receptors in the theca and granulosa increase. As a result, these follicles become more responsive to LH and acquire an increased ability to secrete estradiol. In contrast, numbers of FSH receptors decrease in ovulatory follicles relative to those present earlier.

Apparently, the preovulatory gonadotropin surge converts estrogen active follicles to estrogen inactive ones, i.e., following the surge but before ovulation, estradiol content and numbers of LH receptors in the theca and granulosa decrease. Estradiol concentrations are consistently high (>1 µg/ml) in preovulatory follicles prior to the LH surge but have decreased markedly after the surge in sows (Eiler and Nalbandov, 1977), cows (Fortune and Hansel, 1983) and ewes (Murdoch and Dunn, 1982). During the period leading up to the time of ovulation and subsequent to the time estradiol has decreased, progesterone contents of thecal and granulosa tissue and follicular fluid of ewes decrease (Murdoch and Dunn, 1982).

During the postovulatory period (d 3 to 7) in heifers a single estrogen active follicle develops and all other estrogen active follicles regress (Ireland and Roche, 1983b). This follicle is probably the source of increased estradiol in serum at this time (Glencross et al., 1973; Hansel et al., 1973). Between d 7 and 13, the large postestrous follicle regresses and is replaced by another estrogen active follicle.

Selection of Ovulatory Follicles. The mechanism by which preovulatory follicles are selected for ovulation in farm species is not known. Removal of the corpus luteum of cows (Hammond and Bhattacharya, 1944) or ewes (Smeaton and Robertson, 1971) results in ovulation within 48 to 72 h. But, large follicles present on the ovary at the time of corpus luteum removal (Smeaton and Robertson, 1971) or prostaglandin F₂α (PGF₂α) induced luteal regression (Ireland and Roche, 1983a), do not ovulate. Rather, they regress and new follicles, recruited from the pool of growing follicles, develop into ovulatory follicles within this narrow time frame. Whether follicular growth following removal of the corpus luteum is due to elimination of a local inhibitor, or is the consequence of increased gonadotropin stimulation, or both, has not been determined. Final growth and maturation of preovulatory follicles may be due to the fact that they develop coincident with luteal regression and a preovulatory gonadotropin surge. This view is given credence by the observation that large follicles present on the ovaries of ewes on d 10 will ovulate in response to exogenous gonadotropin (Robinson, 1967).

Steroidogenesis. The two-cell systems of estrogen biosynthesis is operative in sheep, cattle and swine. A model of some current concepts is
Figure 1. A model of the current concepts of control of steroidogenesis in bovine preovulatory follicles (courtesy of J. E. Fortune).

shown in figure 1. Thus, granulosa cells of ewes (Moor, 1977) and cows (Hansel and Fortune, 1978) are capable of producing estradiol only when provided with an aromatizable substrate or co-cultured with thecal tissue. The thecal tissues are the site of androgen synthesis in the follicles and androgen secretion is increased by LH, but not FSH, in sheep (England et al., 1981), cows (Hansel and Fortune, 1978) and pigs (Evans et al., 1981). The porcine system differs slightly in that porcine granulosa cells will produce estradiol in the absence of added androgen. It is thought that this is made possible by a store of androgen, of thecal origin, in the granulosa cells (Evans et al., 1981). Additionally, porcine thecal cells, as well as granulosa cells, can aromatize androgen to estradiol.

Follicular Atresia. Of the approximately 150,000 primordial follicles present at birth in heifers (Erickson, 1966), less than 100 will mature and ovulate during the lifetime of an average animal. The vast majority of follicles present at birth degenerate by a process known as atresia. Follicular atresia has been described for several mammalian species including cows (Rajakoski, 1960; Ireland and Roche, 1983b) and ewes (Hay et al., 1976; Moor et al., 1978). Why atresia occurs and why some follicles escape atresia and ovulate are questions that remain to be answered. Histological, biochemical and endocrine changes associated with atresia have been recently reviewed (Richards, 1980).

Emerging Concepts and Future Research Directions. Technology is currently available to study the biochemical regulation of follicular growth, ovulation and steroidogenesis in farm animals. Although progress has been encouraging, there is a need to investigate control mechanisms by following individual intact follicles throughout their life cycle in vivo and to relate ovarian changes with changes in tropic hormones in the blood. Before this can be
done, methods of identifying and repeatedly sampling individual follicles need to be developed; this will be a major challenge to workers in this area in the next decade.

The concept that intraovarian factors initiate growth of primordial follicles and control, in part, growth and turnover of follicles is emerging. How dominant follicles, or corpora lutea suppress follicular growth remains an unanswered question. In addition, the nature of stimuli that initiate growth of primordial follicles is unknown. But, a new attack on these problems has already been launched by investigators willing to look beyond the limits of traditional endocrine concepts. Early reports demonstrate: 1) the presence of unique peptides in the ovary capable of binding gonadotropin receptors; 2) synthesis of hypothalamic hormones in ovarian tissue; 3) ovarian receptors for hypothalamic hormones and 4) existence of nonsteroidal factors that inhibit FSH secretion.

Several new ideas concerning control of steroidogenesis in bovine preovulatory follicles have recently been expressed (Fortune, 1981; Fortune and Hansel, 1983). Pregnenolone concentrations are higher than progesterone concentrations in proestrous follicles. In addition, high concentrations of estradiol found in proestrous follicular fluid support the idea that, prior to the LH surge, the estrogen-rich follicular microenvironment inhibits pregnenolone production but stimulates androgen and estradiol production by increasing the supply of pregnenolone available to the theca for conversion to androgen, via the Δ², rather than the Δ⁴, pathway. The decrease in intrafollicular estrogen concentrations after the LH surge probably enhances the conversion of pregnenolone to progesterone.

The basic causes of atresia of ovarian follicles are poorly understood. A great deal of work on this problem is needed if successful and highly repeatable procedures for superovulation are to be developed.

**Corpus Luteum Function**

_**General Concepts.** LH is the major luteotropic hormone in the cow and ewe; its exact role in the sow is less well established, but it has the ability to stimulate progesterone synthesis by porcine luteal tissues, both in vitro and in vivo. Most evidence suggests that LH exerts its steroidogenic effects on luteal tissues of all three species by increasing adenylate cyclase and cyclic AMP (cAMP), as it does in other species (Marsh et al., 1966). Niswender et al. (1981) proposed that the sequence of events involved is as follows: 1) LH binds to its receptor in the plasma membrane; 2) adenylate cyclase is activated and cAMP production occurs; 3) protein kinase activation occurs; 4) phosphorylation of steroidogenic enzymes and enhanced protein synthesis follow; 5) a portion of the LH bound to the receptor is internalized and degraded and 6) LH receptors are recycled via secretory granules and incorporated into the plasma membrane by exocytosis, thus maintaining the full complement of LH receptors.

GnRH, by virtue of its ability to cause release of LH can also serve as a luteotropic hormone. Indeed, a long-acting GnRH analog given frequently during the luteal phase of the bovine cycle produced prolonged increases in plasma progesterone concentrations and resulted in prolongation of the estrous cycle (R. A. Milvae and W. Hansel, unpublished data).

There is no evidence that prolactin has a luteotropic function in cattle (Hoffman et al., 1974) and, although it may play a permissive role, it is not primarily involved as a luteotropin in the ewe (Hansel et al., 1973). Catecholamines increase cAMP and progesterone synthesis by luteal tissues in vitro and in vivo (Black, 1979), but there is no evidence that these compounds play luteotropic roles, except, perhaps in stressed animals.

The concept that PGF₂α of uterine origin is transferred from the uterine vein, by counter-current exchange, into the ipsilateral ovarian artery and reaches the corpus luteum where it causes luteolysis in the ewe was suggested by McCracken et al. (1971). The anatomical basis for this transfer was established by Del Campo and Ginther (1973). Ginther (1974) reviewed a series of experiments involving surgical anastomoses of uterine veins or arteries that indicated that veins draining the uterine horn serve as the proximal component and that the ovarian artery serves as the distal component of the veno-arterial pathway. Hansel et al. (1973) summarized a series of experiments suggesting that this mechanism may exist in cattle and Moeljono et al. (1976) proposed that PGF₂α of uterine origin is the luteolysin in swine, as well.

Blood flow studies (Nett et al., 1976; Niswender et al., 1976) show that blood flow to the luteal ovary is correlated with peripheral blood levels of progesterone during luteal
regression and that PGF$_2$α causes reduced blood flow to the ovary containing the corpus luteum and reduced circulating levels of progesterone. Morphological studies indicated that PGF$_2$α affects the vascular component of the corpus luteum, causing swelling of endothelial cells and a decrease in perfusion rate.

However, it is becoming increasingly evident that our present concepts are, at best, only partial explanations of the physiological events that occur during luteal regression. A number of observations cited below are at variance with the concept that a local luteolytic action of PGF$_2$α of uterine origin is solely responsible for luteal regression under physiological conditions.

Cattle. A series of in vitro and in vivo experiments were conducted to show that LH is the major luteotropic hormone in the cow (Hansel, 1967). Purified bovine LH and other LH-containing preparations overcame the inhibitory effects of concurrently injected oxytocin on luteal tissue weights, progesterone concentrations and content. Single injections of purified bovine LH given at mid-cycle prolonged the functional life span of the corpus luteum and lengthened the estrous cycle. Purified bovine LH stimulated progesterone synthesis in vitro by bovine luteal tissue; prolactin was without effect. A potent anti-bovine LH serum caused regression of corpora lutea in normal and hysterectomized heifers and inhibited LH stimulation of progesterone synthesis by luteal tissues in vitro.

A key early observation in development of the concept that a substance of uterine origin acts locally to cause regression of the bovine corpus luteum was that the administration of oxytocin during d 2 to 6 of the estrous cycle inhibited development of the corpus luteum and caused premature estrus and ovulation (Armstrong and Hansel, 1959). This effect did not occur in completely hysterectomized animals or in animals in which the uterine horn ipsilateral to the corpus luteum was removed (Ginther et al., 1967; Brunner et al., 1969). A number of uterine irritants placed into the horn of the uterus ipsilateral to the corpus luteum caused shortening of the estrous cycle (Hansel and Wagner, 1960). Hansel et al. (1973, 1975) found surprisingly large amounts of arachidonic acid (up to 1 mg/g of dried tissue) in bovine endometrial tissue and suggested that this immediate precursor of PGF$_2$α plays a luteolytic role.

Hixon and Hansel (1974) found that sectioning the broad ligament ipsilateral to the corpus luteum resulted in prolongation of the functional life of the corpus luteum. These authors also measured PGF$_2$α in plasma samples collected simultaneously from the ovarian artery and the carotid following infusions of PGF$_2$α (6 mg) directly into the lumen of the uterus. Concentrations of PGF$_2$α were higher in the ovarian artery than in the carotid during a period of 40 to 120 min after PGF$_2$α treatments.

However, final proof for the existence of a physiologically important veno-arterial transfer mechanism requires a demonstration of simultaneous, or sequential, increases in PGF$_2$α concentrations in both uterine venous and ovarian arterial blood, prior to a decline in progesterone output from the ovary during estrous cycles of normal animals. This has not been achieved for the cow; indeed, evidence to the contrary has been reported. Shemesh and Hansel (1975a) found that levels of PGF$_2$α in the endometrium, and in uterine vein blood rose between d 15 and 21 of the cycle, but PGF$_2$α in the ovarian arterial blood did not increase at any time, even when uterine vein levels were greatly elevated. More convincingly, Milvae and Hansel (1980b) measured PGF$_2$α concentrations in blood samples collected frequently and simultaneously from the uterine vein and the ovarian artery following oxytocin injections during d 4 to 6 of the cycle in unanesthetized animals. Each oxytocin injection was followed by a large and prolonged elevation in uterine vein PGF$_2$α concentration and a marked decline in jugular plasma progesterone. However, no corresponding elevations were observed in ovarian arterial PGF concentrations; ovarian arterial PGF$_2$α concentrations remained low and were not different from jugular vein concentrations.

A number of additional observations are difficult to reconcile with our current concepts. These are as follows: 1) simultaneous removal of the uterine horn ipsilateral to the corpus luteum and the contralateral ovary is followed by corpus luteum regression and recurrent estrous cycles in 30 to 80% of the surgically treated animals (Ward et al., 1976; W. Hansel, J. Lukaszewska and A. J. Sherman, unpublished data); 2) bovine luteal tissues, per se, produce PGF$_2$α and other prostaglandins, both in vitro and in vivo (Shemesh and Hansel, 1975b; Milvae and Hansel, 1983a); thus the corpus luteum is not entirely dependent on prostaglandins of uterine origin; 3) PGF$_2$α is
luteotropic, not luteolytic, when added to bovine luteal cells in vitro (Hixon and Hansel, 1979) and 4) hysterectomized cows appear to be more, rather than less, sensitive to exogenous PGF than uterine-intact cows (Lavoie et al., 1975; Hansel and Fortune, 1978) suggesting that the uterus may produce a luteotropic substance, as well as a luteolysin.

Furthermore, numerous experiments show that estradiol is a potent luteolytic factor in the cow (Hansel et al., 1973). To some extent, this effect is independent of the uterus (Brunner et al., 1969). Destruction of follicles by x-irradiation has been shown to prolong the functional life of the corpus luteum in the cow (Villa-Godoy et al., 1981), suggesting a physiological role for estrogens. The luteolytic effects of estradiol and PGF$_2$α are additive, and it has recently been shown (Hixon et al., 1983) that the luteolytic effect of estradiol is not due to inhibition of the steroidogenic effect of LH on the luteal cells.

A number of other substances (GnRH, PGF$_2$α analogs and metabolites and catecholamine antagonists) have been shown to have luteolytic effects in vitro or in vivo in various experimental situations but physiological roles for these compounds have yet to be established.

**Sheep.** LH appears to be the major luteotropin in the ewe (Hansel et al., 1973; Niswender et al., 1981). Key observations in development of this concept were made by Kaltenbach et al. (1968) and Karsch et al. (1971) who showed respectively that LH is necessary for luteal function following hypophysectomy and that constant infusions of LH, but not prolactin, extend the life span of the corpus luteum. LH was found to stimulate progesterone synthesis by luteal tissues in vitro (Kaltenbach et al., 1967) and treatment of ewes with LH antiserum caused decreased corpus luteum weights and progesterone concentrations and a delay in return to estrus (Fuller and Hansel, 1970).

The concept of transfer of PGF$_2$α of uterine origin from the utero-ovarian vein to the ovarian artery and the ipsilateral ovary and corpus luteum by way of a countercurrent mechanism, as outlined by McCracken et al. (1971), resulted from a number of experiments carried out with ewes bearing ovarian autotransplants. The evidence for this concept is more extensive and convincing for ewes than it is for cows (Land et al., 1976; Baird, 1978). However, several authors have found evidence to the contrary. Coudert et al. (1974) studied veno-arterial transfer of PGF$_2$α in ewes with intact ovaries and uteri and concluded that transfer did not occur, except in two cases in which blood flow in the utero-ovarian vein was impeded. Lamond and Drost (1973) found that sectioning the ovarian artery distal to the region where counter-current transfer is thought to occur did not prevent luteal regression or prolong the estrous cycle. Corteel (1975) reported that ultrastructural changes in luteal cells following PGF$_2$α infusions into the uterine vein did not mimic those that occur during natural luteolysis; a number of the changes observed were related to the reduced blood supply caused by PGF$_2$α. The increase in rough endoplasmic reticulum characteristic of normal regression did not occur. However, Land et al. (1976) compared the concentrations of PGF$_2$α in ovarian arterial blood and aortic and carotid blood samples collected at different stages of the estrous cycle in anesthetized ewes and found higher concentrations in the ovarian artery than in the aorta at all stages.

As in the cow, estradiol plays an important luteolytic role. Administration of estradiol-17β at mid-cycle causes premature luteolysis (Hawk and Bolt, 1970). Selective destruction of follicles, the primary source of estradiol, by x-irradiation retards the rate of corpus luteum regression (Karsch et al., 1970). Estradiol appears to potentiate the luteolytic effects of PGF$_2$α, since doses of estradiol and PGF$_2$α, each insufficient to induce luteolysis when administered alone to ewes with x-irradiated ovaries, did so when combined (Hixon et al., 1975). Further experiments with hysterectomized ewes, in which the ovaries were x-irradiated, indicated that this potentiating effect of estradiol on PGF$_2$α may be independent of the uterus (Gengenbach et al., 1977).

**Swine.** In contrast to the cow and ewe, continued LH secretion does not appear to be necessary for development and limited function of porcine corpora lutea. Anderson and Melampy (1967) reviewed experiments indicating that corpora lutea continue to function for 12 d in hypophysectomized gilts. However, as in ruminants, LH has a stimulatory effect on the production of progesterone by porcine luteal tissue in vitro (Watson and Wrigglesworth, 1975). The stimulatory effects of LH on progesterone production were mimicked by dibutyryl cyclic AMP. Furthermore, exogenous human chorionic gonadotropin (HCG) delays...
luteal regression and prolongs the functional life span of the corpora lutea (Guthrie and Rexroad, 1981).

A major difference between ruminant and porcine corpora lutea is that estrogens are luteotropic in the pig, while they are usually luteolytic in the cow and ewe. Treatment with estrogens results in elevated plasma progesterone concentrations and a prolongation in the life span of the corpora lutea (Ford et al., 1982). Estradiol was without effect on in vitro production of progesterone by the corpora lutea of cycling gilts (Cook et al., 1968), but stimulated progesterone production by porcine granulosa cells (Goldenburg et al., 1972). A reduction in PGF$_2$α concentrations in utero-ovarian venous plasma (Ford et al., 1982) and endometrial tissue (Guthrie and Rexroad, 1981) follows estradiol administration.

The uterus exerts control over the functional life span of the porcine corpus luteum. Complete hysterectomy results in an extension of the life span of the corpus luteum and a local utero-ovarian relationship has been proposed, on the basis of experiments using partially hysterectomized sows (Anderson and Melampy, 1967). However, exogenous PGF$_2$α induces luteolysis only when administered after d 12 of the estrous cycle (Diehl and Day, 1974; Moeljono et al., 1976). PGF$_2$α of uterine origin was the luteolysin in swine (Moeljono et al., 1976, 1977). Increases in utero-ovarian vein concentrations of PGF$_2$α are coincident with luteal regression (Gleeson et al., 1974); Moeljono et al., 1976; Ford et al., 1982) and do not occur in pregnant gilts (Moeljono et al., 1977) or in gilts previously treated with exogenous estrogens (Ford et al., 1982). Exogenous PGF$_2$α was luteolytic in hysterectomized gilts (Moeljono et al., 1976) and in gilts in which the corpora lutea were maintained beyond their normal life span by estradiol benzoate treatment (Kraeling et al., 1975). Guthrie and Rexroad (1981) reported that pretreatment of gilts with estradiol resulted in reduced in vitro synthesis of PGF$_2$α by endometrial tissue. However, no studies to determine ovarian arterial PGF concentrations have been reported and it is unknown if changes in utero-ovarian vein PGF concentrations play a physiological role, or whether these changes are merely coincident with changes in luteal function.

**Emerging Concepts and Future Research Directions.** A number of new concepts concerning the control of corpus luteum functions are beginning to emerge, giving rise to the hope that a unifying concept that will accommodate all of these diverse observations may be evolved.

Our understanding of the biochemistry and physiological effects of products of the arachidonic acid cascade has expanded greatly since the original observations of the luteolytic effects of exogenous PGF$_2$α in the cow and ewe were made. Several of these compounds have been shown to have luteotropic effects in the cow and the ewe. Recent evidence for a luteotropic role of prostacyclin (PGI$_2$) during the early development of the bovine corpus luteum may be summarized as follows: 1) injection of PGI$_2$ directly into the corpus luteum at mid-cycle produced a prolonged increase in peripheral plasma progesterone concentrations (Milvae and Hansel, 1980a); 2) PGI$_2$ also stimulated progesterone synthesis by dispersed luteal cells in vitro; 3) administration of indomethacin, a blocker of prostaglandin synthesis by the cyclooxygenase pathway twice daily on d 4 to 6 of the estrous cycle inhibited corpus luteum development and caused a reduction in estrous cycle length, suggesting the existence of a luteotropic prostaglandin during this stage of the cycle (Milvae and Hansel, 1983b) and 4) PGI$_2$ synthesis by luteal cells was greatest during the period of early corpus luteum development (d 5 to 10 of the estrous cycle), reached a very low level by d 15 and remained low during the remainder of the cycle (Milvae and Hansel, 1983a). Thus, the possibility exists that those factors that interfere with corpus luteum development during the early part of the bovine estrous cycle (oxytocin administration, uterine irritants and certain viral and bacterial uterine infections) may act by inhibiting either endometrial or luteal production of PGI$_2$.

Work in the ewe has focused on prostaglandins of the E series, rather than on PGI$_2$; this difference in emphasis may be more apparent than real, since it has been shown that PGE$_1$ binds to PGI$_2$ receptors (Lands, 1979). Magness et al. (1981) and Huie et al. (1981) concluded, on the basis of experiments in which PGE$_1$ or PGE$_2$ was infused into either the ipsilateral or contralateral uterine horn of unilaterally ovariectomized ewes, that both compounds had antiluteolytic effects. PGE$_1$ and PGE$_2$ have each been shown to inhibit the luteolytic effects of estradiol (Colcord et al.,
1978; Hoyer et al., 1978). Henderson et al. (1977) found that infusions of PGE$_2$ directly into ovaries transplanted to the neck inhibited the luteolytic effects of concurrently infused PGF$_2$α. Multiple intrauterine infusions of PGE$_2$ also resulted in an increase in peripheral progesterone concentrations and a delay of two days in the time at which plasma progesterone concentrations declined in the sow; estrous cycle lengths were unaffected (Schneider et al., 1982). Thus, the idea that corpus luteum development, maintenance and regression are regulated by a balance of luteotropic and luteolytic prostaglandins appears to be growing.

Our concepts of how LH exerts its luteotropic effect are also being modified. In recent studies Milvae et al. (1983) linked phospholipid methylation to LH action in the dispersed bovine luteal cell system. Addition of the methylation inhibitors, s-adenosyl-homocysteine (SAH) and 3-deazoadenosine (DZA), to the incubation medium abolished the LH stimulatory effect on progesterone synthesis. Addition of a stimulator of phospholipid methylation, s-adenosyl methionine (SAM), increased the synthesis of progesterone in response to added LH. Addition of these compounds affected only LH-stimulated progesterone synthesis. These studies suggest that phospholipid methylation is an important regulatory step in the mechanism by which LH stimulates luteal cell adenylate cyclase and subsequent progesterone production.

New concepts are also emerging concerning the luteolytic mechanisms. Although the inhibitory effects of oxytocin on bovine corpus luteum development and progesterone secretion have been known since 1959, these effects have been considered pharmacological in nature. However, several recent studies suggest that oxytocin may have a physiological function in luteal regression in both the cow and the ewe. Wathes and Swann (1982) discovered large amounts of oxytocin within ovine and bovine corpora lutea and suggest that it may be produced there. Flint and Sheldrick (1982) found increased concentrations of oxytocin in ovarian venous blood after administration of a PGF$_2$α analog. Although a number of earlier studies showed that progesterone synthesis by bovine luteal cell preparations in vitro was not inhibited by oxytocin, more recent studies (Tan et al., 1982; R. A. Milvae, P. Burg and W. Hansel, unpublished data) indicate that addition of larger amounts (10 to 100 ng) of oxytocin to the incubation medium inhibits progesterone synthesis. Although oxytocin injections in vivo cause large and prolonged increases in uterine vein PGF$_2$α concentrations, veno-arterial transfer of this PGF$_2$α to the ovarian artery does not appear to be the mechanism by which oxytocin inhibits corpus luteum development and function in the cow (Milvae and Hansel, 1980b).

Administration of oxytocin into a uterine artery of the ewe also increases concentrations of PGF$_2$α in uterine venous blood on d 3 to 14 of the cycle (Roberts and McCracken, 1976); this enhanced production of PGF$_2$α arises mainly from endometrial tissues (Roberts et al., 1976). Clearly, the interrelationships among oxytocin, prostaglandins and estradiol in controlling corpus luteum function in the cow and ewe will be subjects for intensive research in the near future.

Another emerging concept is that membrane changes are important, perhaps even initiating events in corpus luteum regression. X-ray diffraction studies of microsomal lipids from regressing bovine corpora lutea reveal that, coincident with a decline in luteal function induced by the PGF$_2$α treatment, a portion of the lipid bilayer is in the gel phase at body temperature. In contrast, all microsomal membrane lipid from normal functional mid-cycle corpora lutea is in the liquid-crystalline phase at body temperature (Carlson et al., 1982). Availability of several new methods for probing membrane structure and function make this an attractive area for future research.

Yet another emerging concept is that luteal function during one estrous cycle can have a marked influence on function of the corpus luteum of the following cycle. For example, R. A. Milvae and W. Hansel (unpublished data), found that repeated injections of a potent GnRH analog during mid-cycle resulted in significant elevations in plasma progesterone concentrations over control animals and prolonged the length of the estrous cycle. However, plasma progesterone concentrations during the following estrous cycle in the same animals were significantly lower than in controls and the cycles were of normal length. The converse may also be true, i.e., low levels of progesterone secretion in one cycle may lead to elevated levels in the subsequent cycle.
Hypothalamic-Pituitary-Ovarian Interactions During the Estrous Cycle

**General Concepts.** Changes in hormone concentrations and follicular growth in farm animals are shown schematically in figure 2. The major release of LH and FSH occurs at estrus. This surge release of gonadotropins is triggered by a positive feedback effect of estradiol from the preovulatory follicle. During the immediate postestrous period, when ovarian steroid concentrations in blood are relatively low, FSH increases in the absence of any increase in LH. This rise in FSH may be due to removal of the negative feedback effect of inhibin when its source is destroyed by ovulation. The increase in FSH may play a role in recruitment of pre-antral follicles. At approximately 3 to 4 d postestrous, a large follicle appears on the ovary. Estrogen from this follicle, plus progesterone from the newly formed corpus luteum feedback negatively on LH. During the luteal phase of the estrous cycle, gonadotropin secretion is under the negative feedback influence of estradiol and progesterone. Following luteal regression, a slight but significant increase in LH occurs, which may be important in inducing follicular maturation and the proestrous rise in estradiol.

For purposes of describing the interactive changes in hormone secretion and follicular growth, it is convenient to partition the estrous cycle into: 1) a pre-surge period—from luteal regression until the gonadotropin surge; 2) a post-surge period—from the gonadotropin surge until resumption of luteal function and 3) the luteal period—during the life span of the corpus
luteum. Inasmuch as the basic mechanisms controlling hypothalamic-pituitary-ovarian relationships are similar in pigs, sheep and cows, the supporting references have been integrated into a single scheme with important deviations acknowledged.

The Pre-Surge Period. Estradiol increases in the ovarian venous blood (Bjersing et al., 1972; Baird and Scaramuzzi, 1976) and in peripheral blood (McCracken et al., 1971; Hansel et al., 1973) during the preovulatory period, reaching a peak at estrus. LH concentrations increase in peripheral blood of cows and ewes following natural (Chenault et al., 1975) or PGF₂α-induced (Baird and Scaramuzzi, 1976) luteal regression, or withdrawal of progesterone treatment (Hauger et al., 1977). This increase probably results from withdrawal of the negative feedback effect of progesterone. Increased LH concentrations during this period are characterized by increased frequency and decreased amplitude of pulsatile LH release in cows (Rahe et al., 1980), ewes (Baird, 1978) and sows (Van de Wiel et al., 1981). These pulses of LH may stimulate estradiol secretion from the preovulatory follicles, since Baird et al. (1976) observed that each pulse of LH was followed by an increase in estradiol concentration in ovarian venous blood of ewes. In addition, pulsed injections of LH induce ovulation (McNeilly et al., 1980) in ewes and exogenous LH increases estradiol output from autotransplanted ovaries (McCracken et al., 1971). Evidence contrary to this view has been published for cattle. Fogwell et al. (1977) blocked the increase in LH that normally follows PGF₂α-induced luteal regression in heifers and noted that estradiol increased unabated.

LH and FSH are released coincidently at or near the onset of estrus in cows (Akbar et al., 1974) and ewes (Pant et al., 1977). Based on data currently available, it would appear that FSH is not released at estrus in sows or, if released, the increase in peripheral blood is slight (Van de Wiel et al., 1981). The increase in estradiol that occurs during the preovulatory period is clearly the stimulus that triggers the gonadotropin surge. Thus, chemical or immunological inhibition of estradiol at proestrus inhibits occurrence of the LH surge in cattle (Martin et al., 1978), and ewes (Fairclough et al., 1976). In addition, exogenous estradiol induces a preovulatory-like surge of LH in cows (Beck and Convey, 1977), ewes (Howland et al., 1971) and gilts (Elsaesser and Parvizi, 1979).

Apparently, a decrease in progesterone is prerequisite to estradiol causing the gonadotropin surge. Estradiol does not exert a positive feedback effect in females bearing a functional corpus luteum (Bolt et al., 1971; Short et al., 1973) and exogenous progesterone blocks the estradiol-induced gonadotropin surge in ewes (Scaramuzzi et al., 1971), gilts (Lantz and Zimmerman, 1974) and heifers (Kesner et al., 1981). A species difference that should be acknowledged is that progesterone remains at less than .5 ng/ml for approximately 4 to 5 d prior to the next estrus in sows, a considerably longer time than the comparable interval for sheep and cattle. This difference may reflect a fundamental difference in rate of development of preovulatory follicles.

The mechanism by which estradiol induces the gonadotropin surge is not completely understood, but there is sufficient evidence to suggest that estradiol acts to: 1) increase the capacity of pituitary gonadotrophs to release LH and FSH in response to GnRH; 2) increase GnRH self-priming, i.e., the process by which GnRH increases the capacity of the pituitary to respond to subsequent exposure to GnRH and 3) set a timed mechanism in the hypothalamus which culminates in a surge release of GnRH that induces the gonadotropin surge.

The capacity of the pituitary gland to release LH in response to GnRH is greatest during estrus and least during the luteal phase of the estrous cycle (Convey, 1973). In addition, exogenous estradiol increases the capacity of the pituitary gland to release LH and FSH in response to GnRH in ewes and cows (Reeves et al., 1971; Kesner et al., 1981). GnRH also has the ability to prime the anterior pituitary gland, thereby increasing the quantity of LH and FSH released by a standard dose (Crighton and Foster, 1977). This priming effect is responsible, at least in part, for the marked increase in pituitary sensitivity that occurs during the proestrus period. Estradiol markedly increases the ability of GnRH to prime bovine pituitary cells in vitro (Padmanabhan et al., 1982) and progesterone inhibits this effect.

The data are consistent with the view that consecutive exposures to pulsatile releases of GnRH, under estrogen dominance, progressively increase the magnitude of pulses of LH release a seriatim, thereby creating the preovulatory LH surge. Both increased GnRH secretion and increased pituitary responsiveness are necessary
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for the preovulatory LH and FSH surges (Kesner and Convey, 1982). Termination of the preovulatory LH and FSH surges results from refractoriness of the pituitary gland to GnRH (Chakraborty et al., 1974; Kesner and Convey, 1982) and not to depletion of gonadotropin content (Convey et al., 1981).

The Post-Surge Period. During the period immediately following the preovulatory gonadotropin surge, there is a marked decrease in estradiol concentration in the ovarian venous drainage (Baird et al., 1976) and peripheral blood (Henricks et al., 1972a; Glencross et al., 1973; Pant et al., 1977). Estradiol content in the follicular fluid and LH binding to theca and granulosa cells decrease while progesterone content of the follicular fluid increases in ewes (England et al., 1981) and cows (Ireland and Roche, 1983a). Progesterone, LH and FSH concentrations in serum remain at basal concentrations during this time.

Approximately 24 h after the preovulatory gonadotropin surge, but before ovulation, serum concentration of FSH increases in ewes (Dobson and Ward, 1977). A lesser but significant increase has been reported in cows (Dobson, 1978; Ireland and Roche, 1983a). A slight increase may occur in sows (Van de Weil et al., 1981). This increase in FSH may play a role in recruitment of pre-antral follicles, since Cahill et al., (1981) found a high correlation between the magnitude of this FSH peak and number of antral follicles present 17 d later.

The releasable pool of pituitary LH is depleted during the postovulatory period and, as a consequence, LH remains low, even though the principal feedback hormones, estradiol and progesterone are also low. In fact, LH will not increase even if animals are ovariectomized at this time (Convey et al., 1977).

The Luteal Phase. Weight of the corpus luteum increases during the first postestrous week and reaches mature size by d 7 (Duncan et al., 1960; Donaldson and Hansel, 1965; Deane et al., 1966). Progesterone and 20-β-hydroxyprogesterone content of the bovine corpus luteum (Hafs and Armstrong, 1968) and progesterone concentrations in peripheral blood (Hansel et al., 1973) increase, peaking at approximately d 10. During the early postovulatory period, estradiol also increases in ewes (Cox et al., 1971), cows (Glencross et al., 1973) and gilts (Henricks et al., 1972b). The large estrogen active follicles that appear on the ovary at this time (Holst et al., 1972; Ireland and Roche, 1983a) are likely sources of this estrogen.

Clearly, LH is secreted in a pulsatile fashion during the luteal phase of the cycle in cows (Rahe et al., 1980), ewes (Hauger et al., 1977) and sows (Van de Wiel et al., 1981). Frequency and magnitude of LH pulses change with changes in steroid hormone secretion, i.e., low frequency-high amplitude when progesterone is present and high frequency-low amplitude under estrogen dominance (Rahe et al., 1980). Both estradiol and progesterone play a role in negative feedback control of LH in cows and ewes. In these species, concentrations of LH increase after ovariectomy (Hobson and Hansel, 1972; Foster et al., 1975; Foxcroft, 1977). Replacement of estradiol or progesterone will not return LH to precastration concentrations but a combination of these hormones will (Beck et al., 1976; Karsch et al., 1980).

Knowledge of negative feedback control of FSH in farm animals is scant. Estradiol, given as a large, single (1 mg) injection, will reduce FSH concentrations to precastration values in ovariectomized heifers (Kesner and Convey, 1982). Bovine follicular fluid from which endogenous steroids have been removed with charcoal will decrease serum FSH in heifers without affecting LH concentrations (Curato et al., 1982). The inhibitory effect of estradiol on FSH secretion is mediated, at least in part, directly on the pituitary, since estradiol decreases FSH secretion by ovine pituitary cells in primary culture (Miller et al., 1977).

Emerging Concepts and Future Research Directions. The observation that gonadotropins are secreted in distinct episodic patterns that vary with reproductive status of the animal has raised a number of questions. Are these patterns of gonadotropin secretion merely a consequence of ovarian hormone feedback mechanisms? Is the pattern of presentation of gonadotropins to the ovary of any biological significance?

The concept that seasonal effects control reproductive processes in sheep is an old one, but the idea that these also affect bovine and porcine reproduction is now emerging. In addition, nutritional factors and stress clearly affect pituitary and ovarian functions in ewes, cows and gilts, and interest in the exact mechanism(s) by which this happens is expanding.

Neuroendocrinology, as it applies to animal science, has not been an active area of research, but development of knowledge regarding con-
trol of gonadotropin secretion has now developed to the point where the difficult questions regarding involvement of the nervous system must be addressed. Improved techniques for control of reproductive processes may well come from research in this area.

The concept of releasable and nonreleasable pools of gonadotropin in the pituitary gland has been advanced. This concept is supported by the observation that only a portion of the hormone present in the hypophysis can be released at any one time and the releasable portion varies with stage of the estrous cycle. The biochemical basis for these hormone pools is not known. But, research is underway to improve our understanding of the relationships between pool size and biochemical events, such as releasing hormone-receptor binding, post-receptor amplification, and calcium and potassium channel availability.

Estrous Cycle and Ovulation Regulation Techniques

General Concepts. It has long been the aim of reproductive physiologists to develop techniques for controlling the estrous cycles of cattle, sheep and swine so that animals can be brought into estrus and inseminated once at a pre-set time without the necessity of checking for estrus. In cattle, the major reason for developing cycle regulation techniques is that they offer opportunities for widespread use of artificial insemination and for more rapid genetic progress in improving meat and milk production. Currently, only about 5% of the beef cattle in the US are artificially inseminated. In the sheep industry, cycle control methods have an added potential, in that synchronized ewes may be bred at times other than the normal reproductive season. In the swine industry, cycle control methods can be used to induce and control estrus in prepuberal gilts and, possibly to induce estrus and ovulation during lactation. Cycle regulation and ovulation control techniques for use in the rapidly expanding embryo transfer industry are also receiving increased attention.

Basically, there are two ways to synchronize the estrous cycles of groups of animals. The first is to treat all animals with a progestational compound to prevent estrus and ovulation for sufficient time to allow regression of the corpora lutea of all animals in the group. Theoretically, all animals will come into estrus and ovulate after withdrawal of the progestational compound. Shorter treatment periods have generally resulted in higher conception rates than longer treatment periods. The second method is to lyse the corpora lutea of all animals with injections of PGF2α, or one of its potent analogs, after which all animals will come into estrus and ovulate.

Cattle. Hansel and Beal (1979) divided the developments in regulation of ovulation in cattle into four distinct phases. During the first phase, beginning about 1960, numerous progestational compounds administered by a variety of methods, including feeding, additions to the drinking water, subcutaneous implants, topical applications and vaginal pessaries, were tested for their abilities to synchronize estrous cycles. Estrus occurred in a high percentage of the treated animals (80 to 90%) over a 4 d period beginning 2 d after withdrawal of the progestational agents. By 1967, it was clear that conception rates were lower (10 to 15%) after cycle regulation by progestational treatments than in control animals. The second phase was characterized by attempts to combine progestational treatments with either estrogen or gonadotropins, in order to gain better control of the times of estrus and ovulation. Although these attempts resulted in improved conception rates and better synchronization of estrus in some cases, they were not generally successful.

The third phase began about 1972, following demonstrations of the luteolytic effects of prostaglandin PGF2α in cows (Rowson et al., 1972). Two workable methods for cycle synchronization by the use of PGF2α or its analogs have been developed. Both methods accommodate the fact that PGF2α, given during the first 5 d of the estrous cycle, fails to cause luteal regression. In the first method, animals are inseminated as they come into a normal estrus during a 5 d period, after which all remaining animals are treated with PGF2α on d 5 and inseminated at the ensuing estrus. This method has given good conception rates (65 to 75%). The second method, in which all animals are given two treatments of PGF2α 11 d apart and inseminated at the estrus following the second treatment, has resulted in conception rates equal to controls in some experiments, but not in others.

In the fourth and most recent phase, attempts have been made to combine progesterone and PGF2α treatments. A treatment consisting of progesterone administered by way of a pro-
gesterone releasing intra-vaginal device (PRID) for 7 d, combined with PGF$_2$$\alpha$ administered once on d 6 has resulted in conception rates equal to controls in artificially inseminated Holstein heifers (Hansel and Beal, 1979; Roche et al., 1981). The combined progesterone-PGF$_2$$\alpha$ methods have received most attention in recent years because of their simplicity, the short treatment periods required, and the improved synchronization of estrus that results.

In an experiment representative of others now being carried out, Smith et al. (1983) compared the effects of treatment with PGF$_2$$\alpha$ given twice at an 11 d interval with the PRID-PGF$_2$$\alpha$ method (PRIDS in place for 7 d and 25 mg PGF$_2$$\alpha$ given on d 6). All animals were inseminated once at a pre-set time. There was no difference between the pregnancy rates of the control animals inseminated in the usual way during a normal estrus and the PRID-PGF$_2$$\alpha$-treated heifers. However, the pregnancy rate in the animals treated twice with PGF$_2$$\alpha$ was lower than found in the control animals. Thus, the PRID-PGF$_2$$\alpha$ treatment provides the necessary precision in estrus and ovulation control for successful insemination at a single pre-set time. The potential usefulness of this scheme for synchronizing estrus and ovulation in both cycling and noncycling lactating beef cows during the postpartum period has been demonstrated.

Sheep. Dutt and Casida (1948) were the first to demonstrate the use of progesterone to inhibit estrus and ovulation in ewes. By 1962, several groups (Evans et al., 1962; Hogue et al., 1962) had reported successful control of estrus and ovulation in ewes by long-acting, orally effective progestational compounds. Studies on the control of estrus by progestational compounds were summarized by Robinson (1967), Gordon (1975) and Haresign (1978), among others.

A major advance was reported by Robinson in 1964, when he showed that progestational compounds can be administered in physiologically active doses over the required period of time (12 to 14 d) by the intravaginal route. These results led to the development of two highly effective intravaginal sponges for control of estrus and ovulation in the ewe. These two sponges [one impregnated with 60 mg medroxyprogesterone acetate (MAP) and one with 30 mg fluorogestone acetate (FGA)] were equally effective (Gordon, 1975) and have been widely and successfully used in synchronizing the estrous cycles of ewes during the breeding season (Robinson, 1975).

However, if ewes in late anestrus are to be successfully treated, a gonadotropin (usually 500 IU of PMS given at the time of sponge removal) must be used in addition to the intravaginal progestagen treatment. Gordon (1975) obtained a conception rate of 67% in late anestrous ewes artificially inseminated at the estrus following the combined vaginal sponge-PMSG treatment. Colas (1975) showed that use of PMSG, in addition to intravaginal progestagen treatment, during the normal breeding season resulted in more precise synchronization and enabled pre-set insemination without estrous detection. This method reduces the time from sponge removal to the onset of estrus and is claimed to partially overcome the lowered fertility resulting from impaired sperm transport in ewes treated with progestational compounds (Robinson, 1967; Hawk and Conley, 1971). These developments resulted in several systems of controlled sheep breeding for year-round lamb production in Ireland, France and Scotland (Robinson, 1975).

Despite the voluminous literature on the luteolytic effects of PGF$_2$$\alpha$ in the ewe, relatively few studies appear to have been carried out on the use of PGF$_2$$\alpha$ to synchronize estrus and control ovulation. PGF$_2$$\alpha$ injections are not luteolytic when given during the first four days of the cycle. Haresign (1978) demonstrated that two injections of PGF$_2$$\alpha$ given 8 or 9 d apart resulted in good synchrony of estrus and normal conception rates following the second injection of PGF$_2$$\alpha$.

Few attempts have been made to combine progesterone and PGF$_2$$\alpha$ treatments as a method of estrous cycle regulation in the ewe. Hackett et al. (1981) reported on experiments in which ewes were first treated with a sponge impregnated with 40 mg FGA and intramuscular injections of PMSG (500 IU) at the time of sponge removal and then with PGF$_2$$\alpha$ (15 mg) and PMSG (500 IU) at d 10 or 12 of the ensuing estrous cycle. In one experiment, 24 of 29 ewes (83%) had viable fetuses 9 wk after the PGF$_2$$\alpha$-PMSG treatment.

Swine. The most promising method for controlling estrus and ovulation in sows is by use of the orally active synthetic progestin, allyl trenbolone (17$$\alpha$-allyl-estratriene-4,9,11,17$$\beta$$-ol-3-one). Low daily doses of allyl trenbolone (AT) and other progestins given for 10 to 18 d effectively suppress estrus but have frequently
resulted in cystic follicles (Webel, 1978). Higher doses of AT dependably result in effective control of synchronization of estrus (Webel, 1978) without causing a reduction in fertility or a high incidence of adverse side effects. Webel (1978) reported a shorter time interval between AT withdrawal and return to estrus when AT was given for 18 d than when it was given for 10, 12, 14 or 16 d. Stevenson and Davis (1982) compared 14 and 18 d feeding periods of AT to gilts and found that 14 d feeding effectively synchronized fertile estrus in gilts, regardless of stage of the cycle at the onset of treatment. Artificial insemination at the posttreatment estrus resulted in farrowing rates of 73% in both treatment groups.

PGF$_2$$\alpha$ is not luteolytic in the pig until d 12 of the estrous cycle (Diehl and Day, 1974) and therefore does not offer a practical means of synchronizing estrous cycles when used alone. Estrogen injections result in maintenance of porcine corpora lutea and these corpora are sensitive to exogenous PGF$_2$$\alpha$ (Kraeling et al., 1975). Guthrie (1975) administered estrogen on d 10 to 14 of the estrous cycle to maintain corpora lutea, which were regressed 5 to 20 d later with PGF$_2$$\alpha$. Estrus occurred 4 to 6 d after PGF$_2$$\alpha$ injections and fertility of sows bred at this estrus was normal.

Exogenous gonadotropins induce the formation of accessory corpora lutea at any stage of the estrous cycle in the pig (Neill and Day, 1964; Day et al., 1965; Caldwell et al., 1969), and corpora lutea formed on d 8, 10 or 16 were shown to delay estrus for at least 15 d (Caldwell et al., 1969). Guthrie and Polge (1976) induced accessory corpora lutea with PMSG, followed by human chorionic gonadotropin (HCG), and subsequently regressed these accessory corpora 12 d later by administration of PGF$_2$$\alpha$ analogs. Luteal regression occurred in 31 of 35 PGF$_2$$\alpha$-treated gilts, and 30 gilts were in estrus 4 to 7 d after PGF$_2$$\alpha$ treatment. Synchronized gilts were inseminated once on the second day of estrus and the resultant conception rate was 80%.

**Emerging Concepts and Future Research Directions.** The concept of combining PGF$_2$$\alpha$ injections with short-term progestagen treatments seems established for the cow, but not for the ewe or sow. Additional work is needed to determine the simplest and most effective methods for delivering the progestagen during the required 6 to 7 d period. These methods then need to be incorporated into integrated programs for reproduction management and these programs should be carried out by responsible agencies, such as large artificial insemination units.

It should now be possible to develop even simpler methods for synchronization of cattle cycles, based on single injections of luteotropic agents (LH, or long acting analogs of GnRH or PGF$_2$$\alpha$) to prolong the functional life of the corpora lutea, followed 6 d later by a luteolytic dose of PGF$_2$$\alpha$. Such a method might be particularly useful in large commercial beef cattle operations where minimal handling of animals is important.

In the ewe, deranged sperm transport following synchronization by either progestagen-impregnated sponges or PGF$_2$$\alpha$ is a major problem. Further work is also needed on induction and synchronization of estrus and ovulation in lactating ewes. Combined progestagen-PGF$_2$$\alpha$ treatments should be studied with the objective of developing a method that will allow insemination at a pre-set time in sows.

Finally, as Robinson (1975) has pointed out, care and attention to detail are necessary in order that systems of controlled breeding be successfully introduced into the animal industry. These systems must form a part of the husbandry and be integrated into the whole production pattern.

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