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Title: RESPONSE OF THE BOVINE CORPUS LUTEUM TO EXOGENOUS GONADOTROPIN-RELEASING HORMONE DURING THE ESTROUS CYCLE

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Two experiments were conducted to investigate the effects of gonadotropin-releasing hormone (GnRH), administered twice during the same estrous cycle, on bovine luteal function. Eight mature beef cows, each cow serving as her own control, were injected intravenously (i.v.) with saline on days 2 and 8 of the cycle (day of estrus=day 0 of the cycle), then with 100ug GnRH on days 2 and 8 of the subsequent cycle. Jugular blood samples were taken immediately prior to an injection and at 15, 30, 45, 60, 120 and 240 min postinjection to characterize changes in serum luteinizing hormone (LH). Blood was also collected on alternate days after an injection until day 16 of the cycle, to characterize serum progesterone levels. Treatment with GnRH did not significantly alter serum progesterone concentrations. Although GnRH caused release of LH on days 2 and 8, the quantity of LH released was significantly greater on day 8 ($P<0.0025$). Length of the estrous

cycle was not altered by treatment (control 20.4 ± .5 vs. treated 20.4 ± .4 days).

In Experiment 2, five mature beef cows, each serving as her own control were injected i.v. with 100ug GnRH on day 2 of the cycle, with saline on days 2 and 8 of the succeeding cycle and then with 100ug GnRH on days 2 and 8 of the third cycle. Corpora lutea (CL) were obtained per vaginam on day 10 of each cycle. Luteal tissue was sliced and incubated for 2 hr with saline (control) or LH (10ng/ml). Treatment of cows with GnRH on day 2 or days 2 and 8 caused a marked increase in *in vitro* basal progesterone production (ug/g) by luteal slices (control, 37.45 ± 2.93 vs. GnRH day 2, 43.97 ± 5.03 vs. GnRH days 2 and 8, 53.18 ± 3.03) but suppressed the ability of luteal tissue to respond to LH ($P<0.07$). Treatment did not affect the initial luteal progesterone concentration as determined by measurement of the steroid in unincubated samples (control, 23.99 ± 1.60 vs. GnRH day 2, 24.08 ± 2.15 vs. GnRH days 2 and 8, 29.80 ± 3.65 ug/g).

Results of this study demonstrate that LH, released in response to GnRH, can increase basal progesterone production and yet desensitize luteal cells to further stimulation by the homologous hormone .

Response of the Bovine Corpus Luteum to Exogenous Gonadotropin-Releasing Hormone During the Estrous Cycle

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I dedicate this thesis to my grandparents,

Richard and Vera Cousins

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REVIEW OF LITERATURE

INTRODUCTION

Elucidation of the factors that influence the function of the corpus luteum (CL) in mammals has been the aim of numerous investigations during the past 35 years. Domestic ruminants have proven to be excellent experimental models for studies on the development, maintenance and regression of the CL. Large quantities of luteal tissue from animals at known stages of the estrous cycle can be collected for *in vitro* experiments, blood can be frequently and easily sampled to quantify changes in hormone concentrations and in cows, rectal palpation can be used to follow CL development. This thesis consists of a review of the literature on the regulatory mechanisms controlling luteal function and presents the results of two experiments conducted to determine the response of the bovine CL to exogenous GnRH during the estrous cycle.

CHARACTERISTICS OF THE MAMMALIAN CORPUS LUTEUM

In domestic ruminants there are primarily four physiological systems that are involved in the regulation of luteal function. These four systems consist of the anterior pituitary, which secretes luteinizing hormone (LH), the primary luteotropin in the cow and ewe; the uterus, which produces prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) a luteolysin; the conceptus, which synthesizes antiluteolytic factors and the CL itself, which produces compounds that modulate its function in a paracrine or autocrine fashion. This review will encompass information concerning the morphology of the CL and general aspects of luteal formation, function, maintenance and regression. Emphasis will be placed on the role of the neuroendocrine system in regulating luteal function, especially gonadotropin-releasing hormone (GnRH), the hypothalamic peptide hormone responsible for regulating the secretion of LH.

FORMATION AND MORPHOLOGY OF THE CORPUS LUTEUM

The CL forms after ovulation, from cellular components of the emptied follicle that are transformed under the influence of LH into this transitory endocrine gland. In many species both follicular granulosa and theca cells undergo luteinization contributing to the formation of the CL as first noted by Corner (1919) in the sow. The CL of the cow (Donaldson and Hansel, 1965), ewe (Fitz et al., 1982), woman (Gillim et al., 1969) and rhesus macaque (Gulyas et al., 1979) contain at least two cell types that are classified on the basis of size (O'Shea et al., 1979). Ultrastructural analysis of enzymatically dissociated cells have shown that large luteal cells, of granulosal origin, are at least 22um in diameter, contain round nuclei with dispersed chromatin, a distinct nucleolus, numerous mitochrondria, smooth and rough endoplasmic reticulum (ER), electron dense granules and are characterized by a highly convoluted cell surface. Small luteal cells, of thecal origin, are 10-20um in diameter, contain irregularly shaped nuclei with heterochromatin lining the nuclear envelope, extensive smooth ER, mitochrondria with tubular cristae and abundant lipid droplets (Koos and Hansel, 1981). Specific monoclonal antibodies to granulosal and thecal cell surface antigens have confirmed that large luteal cells are of granulosal origin and that thecal cells give rise to small luteal cells (Alilia and Hansel, 1984). Both cell types are capable of synthesizing progesterone. However, large cells are less responsive to LH stimulation, acting independently of LH and cyclic adenosine 3', 5'-

monophosphate (cAMP) to secrete most of the progesterone produced by the CL, whereas small cells show a greater response to LH (Koos and Hansel, 1981; Rodgers and O'Shea, 1982). Furthermore, the majority of the LH receptors in the CL are found on small luteal cells (Fitz et al., 1982). The CL also consists of connective tissue and vascular elements (Niswender and Nett, 1988).

The CL undergoes pronounced changes in cellular composition throughout the cycle. The composition of the ovine CL, as determined by morphometric analysis, revealed that luteal weight and the number of steroidogenic cells increase throughout the luteal phase of the cycle (Farin et al., 1986). Donaldson and Hansel (1965) proposed that small luteal cells differentiate into large cells, under the influence of LH, as the CL matures. Alilia and Hansel (1984), using monoclonal antibodies against bovine granulosal and thecal specific antigens, showed that thecal cell antigen increased and granulosal cell antigen decreased as the CL matured, suggesting that many large cells of the mature CL are derived from transformed small cells. Cran (1983) reported that thecal (small cells) obtained from ovine cystic follicles exhibited characteristics of large luteal cells after treatment with pregnant mare serum gonadotropin (PMSG). Gamboni et al. (1984) found that in the ovine CL there was a decrease in the number of small cells after treatment of ewes with human chorionic gonadotropin (hCG) on day 5 of the cycle. More recently, Farin et al. (1988) reported that injection of ewes with hCG on days 5 and 7.5, or pharmacological dosages of LH every 6 hr from day 5 until day 10

of the cycle, caused a decrease in the number of small luteal cells with a concomitant increase in the number of large luteal cells. There was no change in serum concentrations of progesterone due to treatment with LH, however, there was an increase in progesterone beginning on day 10 after treatment with hCG. These reports support the hypothesis that LH promotes the conversion of small luteal cells into large luteal cells.

MAINTENANCE OF THE CORPUS LUTEUM

The CL is the major ovarian source of progesterone in the mature, nonpregnant animal. Functions of progesterone and thus, of the CL, are to prepare the uterus for embryo attachment or implantation, to decrease both uterine tone and contractions, to promote alveolar development of the mammary gland and to maintain pregnancy (Niswender et al., 1985). The CL is necessary for maintenance of pregnancy for varying periods of time in different species. The cow relies on the CL for progesterone for most of gestation (Estergreen et al., 1967), the ewe until about day 45 (Casida and Warwick, 1945) the rat until day 17 (Csapo and Wiest, 1966) and the guinea pig until day 30 of gestation (Csapo et al., 1981). If fertilization does not occur the CL regresses and the ovulatory cycle resumes, the duration of which varies from species to species of animal. Thus, for successful establishment of pregnancy, the conceptus must signal its presence to prevent luteolysis. The critical period for maternal recognition of pregnancy in the ewe is about day 13 (Moor and Rowson, 1966a) and in the cow about day 16 of gestation (Northey and French, 1980). The substance produced by the conceptus of the ewe that prevents luteal regression is ovine trophoblast protein-1 (oTP-1; Hansen et al., 1985) and in the cow bovine trophoblast protein-1 (bTP-1). Both proteins share considerable homology with each other (Martal et al., 1984) and with a class of molecules called the alpha-interferons, which are involved in immune responses (Imaka et al., 1987; Helmer et al., 1987). Administration of oTP-1 to

nonpregnant ewes late in the luteal phase of the estrous cycle increases the life span of the CL (Godkin et al., 1984) and reciprocal interspecies transfer of bovine and ovine trophoblastic vesicles into the uterus also extends luteal function (Helmer et al., 1987). Recently, administration of recombinant bovine alpha-interferon to late luteal phase cows has been shown to prolong the length of the estrous cycle (Plante et al., 1988). These reports suggest alpha-interferon-like molecules may represent antiluteolytic signals produced by the the conceptus to regulate the functional life span of the CL.

In addition to trophoblastic proteins, prostaglandins of the E series (PGE_1 and PGE_2) may play a role in preventing luteolysis. It has been observed that uterine secretion of PGE_2 is higher in pregnant than in nonpregnant ewes (Silvia et al., 1984), that bovine conceptuses secrete PGE_2 (Shemesh et al., 1979a) and that infusion of PGE_2 into the uterus can extend the life span of the bovine CL (Chenault, 1983). Additionally, chronic infusion of PGE_1 or PGE_2 was able to block intrauterine device (IUD) induced, estrogen-induced, or natural luteolysis in ewes (Reynolds et al., 1981). Huie at el. (1981) also found that constant infusion of PGE_1 into the uterine horn, either ipsilateral or contralateral to the ovary bearing the CL, prolonged the life span of the CL in the ewe. Furthermore, PGE_2 stimulates progesterone secretion by bovine luteal tissue in vitro (Speroff and Ramwell, 1970). The actions of PGE_1 and PGE_2, like $PGF_{2\alpha}$, are local (Huie et al., 1981). The foregoing reports support the hypothesis that prostaglandins of the

E series play a role in preventing luteolysis.

REGRESSION OF THE CORPUS LUTEUM

Average length of the estrous cycle for the cow is 21 days and for the ewe is 16 days, while the average duration of the menstrual cycle of the rhesus macaque is 28 days. The CL of the nonpregnant cow begins to regress about day 17 of the estrous cycle, with a concomitant decrease in progesterone secretion (Hansel and Snook, 1970). This decrease in progesterone secretion is required for estrogens, from the preovulatory follicle, to stimulate the ovulatory surge of LH (Hansel and Convey, 1983).

In the cow and ewe the uterus influences ovarian function by inducing luteal regression. Total hysterectomy of ewes and cows prolongs the life span of the CL (Wiltbank and Casida, 1956). Partial hysterectomy results in unilateral regression of corpora lutea on the ovary ipsilateral to the remaining uterine horn in cows (Ginther et al., 1967) and ewes (Inskeep and Butcher, 1966; Moor and Rowson, 1966b). Thus, removal of the ovary from the local influence of the uterus prolongs the life span of the ovine and bovine CL and maintains high serum levels of progesterone (McCracken et al., 1971; Hixon and Hansel, 1974). In pseudopregnant rats (Bradbury et al., 1950), mice (Critser et al., 1980) and hamsters (Caldwell et al., 1967), hysterectomy prolongs the life span of to CL, however, hysterectomy does not affect the normal duration of the estrous cycle in rats (Durrant, 1972), mice (Dewar, 1973) or hamsters (Caldwell et al., 1967). In women and nonhuman primates, hysterectomy does not alter the duration of the menstrual cycle, and it has been postulated that a

luteolysin is produce locally by the ovary in these species (Auletta et al., 1984).

ACTIONS OF PROSTAGLANDIN $F_{2\alpha}$

Babcock (1966) originally suggested that prostaglandins may be luteolytic in the cow. Pharriss and Wyngarden (1969) demonstrated that $PGF_{2\alpha}$ was luteolytic in the pseudopregnant rat, which prompted investigations into the actions of this hormone in other species. There is good evidence that in ewes and cows $PGF_{2\alpha}$ is a luteolysin. In cows, insertion of an IUD into the horn adjacent to the ovary bearing the CL on day 3 of the cycle promoted premature luteal regression (Ginther et al., 1966). Presence of the IUD presumably irritated the endometrium of the uterus causing increased prostaglandin synthesis and release. Infusion of $PGF_{2\alpha}$ into the ovarian artery of cows resulted in a rapid decline in plasma progesterone (Hansel and Snook, 1970). Furthermore, natural luteolysis can be prevented by immunization of ewes and cows with antisera against $PGF_{2\alpha}$ (Fairclough et al., 1981).

The mechanism by which $PGF_{2\alpha}$ is transferred from the bovine uterus to the ovary appears to occur by countercurrent exchange of $PGF_{2\alpha}$ through the wall of the utero-ovarian vein into the ovarian artery (Ginther, 1974; Hansel, 1975). Concentrations of $PGF_{2\alpha}$ in ovarian arterial blood are greater then in carotid or jugular blood,

suggesting that there is indeed a preferential local transfer of $PGF_{2\alpha}$ from the uterus to the ovary (Ginther, 1974; Hixon and Hansel, 1974).

When the fatty acid precusor of $PGF_{2\alpha}$, arachidonic acid, was injected directly into the corpora lutea of heifers on day 12 or 13 of the cycle there occurred an increase in $PGF_{2\alpha}$ concentrations in ovarian venous blood. Concomitantly increased $PGF_{2\alpha}$ levels were accompanied with increased estrogen and decreased progesterone concentrations in the jugular plasma (Shemesh and Hansel, 1975b). Shemesh and Hansel (1975a) also observed that on days 1-14 of the bovine estrous cycle $PGF_{2\alpha}$ concentrations were low in endometrial tissue and in uterine venous blood, however, there was a dramatic increase in $PGF_{2\alpha}$ beginning on day 15 and continuing until estrus. In women and other primates there is some evidence that $PGF_{2\alpha}$, possibly produced by the ovary, is luteolytic because infusion or injection of $PGF_{2\alpha}$ into the rhesus or human CL results in decreased plasma progesterone concentrations (Korda et al., 1975; Sotrel et al., 1981).

The precise mechanism by which $PGF_{2\alpha}$ promotes luteal regression is not clear. It has been suggested that $PGF_{2\alpha}$, a potent vasoconstrictor, may cause luteal regression by reducing blood flow to the CL (Pharriss et al., 1970). In the ewe both serum progesterone concentrations and blood flow to the CL decrease during luteolysis (Ford et al., 1979). Induction of luteal regression, by injecting $PGF_{2\alpha}$, is accompanied by decreased blood

flow to the ovary bearing the CL (Nett et al., 1976). However, at the cellular level the mechanism by which $PGF_{2\alpha}$ promotes luteolysis is more complex than can simply be explained by changes related to its vasoactive properties. Furthermore, it has not unequivocally been demonstrated that the effect of $PGF_{2\alpha}$ on ovarian blood flow is the cause and not the effect of luteal regression. Large luteal cells contain the majority of the $PGF_{2\alpha}$ receptors found in the CL and *in vitro* administration of $PGF_{2\alpha}$ decreases progesterone secretion by these cells (Silva et al., 1984). Treatment of small luteal cells with $PGF_{2\alpha}$ has no effect on basal progesterone production, however, $PGF_{2\alpha}$ inhibits LH-stimulated progesterone production (Silva et al., 1984). Thus, it appears that $PGF_{2\alpha}$ promotes luteal regression by acting directly on large luteal cells by inhibiting steroidogenesis and may act indirectly on small luteal cells by inhibiting the luteotropic effects of LH. It seems likely that both the vasoactive properties of $PGF_{2\alpha}$ and its effects on luteal cells are important in regulating the life span of the CL.

The exact mechanism regulating the synthesis and release of $PGF_{2\alpha}$ is not known, however, there is evidence to suggest a role for estrogens in luteal regression. If ovarian follicles, the major source of estrogens, are destroyed by irradiation luteal function is maintained in ewes and cows (Hixon et al., 1975; Villa-Godoy et al., 1981). Furthermore, estradiol treatment causes the release of uterine $PGF_{2\alpha}$, followed by decreased progesterone levels and

luteal regression in cows and ewes (Hansel et al., 1973). These reports suggest that the interactions between estrogens and $PGF_{2\alpha}$ play a major role in luteolysis.

THE ROLE OF OXYTOCIN

In ruminants there is clear evidence that $PGF_{2\alpha}$ causes luteal regression, however, the signal for the initiation of $PGF_{2\alpha}$ secretion at the end of the cycle is not clear. In 1959, Armstrong and Hansel (1959) reported that cows injected with the posterior pituitary nonapeptide, oxytocin (OT), on days 1 to 7 of the cycle had shortened estrous cycles. These inhibitory actions of OT on the bovine CL were not observed in hysterectomized cows. Ginther et al. (1967) reported that in unilaterally hysterectomized heifers, exogenous OT shortened the duration of the cycle if the remaining uterine horn was ipsilateral to the ovary bearing the CL. In another experiment, cows treated with OT early in the cycle showed decreased serum progesterone concentrations starting on day 5 of the cycle with no alterations in serum LH levels (Harms et al., 1969). Wilks and Hansel (1971) also observed that OT injections early in the cycle did not alter LH secretion. In early studies endogenous OT was presumed to be of neural origin only. However, it was subsequently discovered that large cells of the ovine (Wathes and Swann, 1982) and bovine CL (Fields et al., 1983) produce this peptide.

Currently, the regression of the bovine CL is thought to result

from the interactions of estrogens, OT and $PGF_{2\alpha}$. Vane and Williams (1973) speculated that the primary action of OT is to stimulate the synthesis and release of uterine $PGF_{2\alpha}$. Several investigators have shown that OT administered *in vivo* causes the release of uterine prostaglandins (Sharma and Fitzpatrick, 1974; Mitchell et al., 1975). Incubation of ovine endometrial tissue with OT caused increased synthesis of $PGF_{2\alpha}$, which was maximal on day 15 of the cycle (Roberts et al., 1976). McCracken (1980) proposed a hypothesis concerning the role of OT in ovine luteal function in which estradiol induces uterine OT receptors. Progesterone normally suppresses estrogen receptor formation (Koligian and Stormshak, 1977), but McCracken postulated that low levels of systemic estrogens could induce synthesis of its own receptor in the face of high levels of systemic progesterone. Meyer et al. (1988) found that the endometrium OT receptor population was high on days 17-18 of the cycle, reaching a maximum on the day of estrus and that OT receptors were not detectable during the luteal phase. They observed that cytosolic estrogen and progesterone receptor concentrations in bovine endometrium were maximal on days 1-8, with the nuclear estrogen receptor levels highest on days 19-21 of the cycle. Hence, the OT receptor is present at detectable levels only when progesterone levels are low and estrogen levels are high. When uterine concentrations of the OT receptor are increased, oxytocin is able to interact with its receptor causing the synthesis and release of $PGF_{2\alpha}$ (McCracken, 1980).

Flint and Sheldrick (1982) showed that the $PGF_{2\alpha}$ analog, Estrumate, injected into the ewe caused the release of ovarian OT. They suggested that ovarian secretion of OT in response to $PGF_{2\alpha}$ is involved in normal luteolysis (Flint and Sheldrick, 1983). In another investigation, the prostaglandin synthetase inhibitor indomethacin was able to partially overcome the inhibitory actions of exogenous OT *in vivo*, providing further evidence that ovarian OT, via its stimulatory effect on $PGF_{2\alpha}$ secretion, is involved in regulating luteal function (Milvae and Hansel, 1985).

Peripheral concentrations of OT vary during the ovine estrous cycle (Sheldrick and Flint, 1981; Schams et al., 1982). Wathes and Swann (1982) suggested that OT measured in systemic blood may be of luteal origin and that the fluctuations observed in blood sampled during the estrous cycle may represent changes in luteal concentrations of OT. Factors regulating the synthesis and release of OT are not known, however, it was recently observed that ovine luteal OT synthesis could be stimulated *in vitro* by arachidonic acid, phospholipase A_2 and phospholipase C, but in this study neither $PGF_{2\alpha}$ nor PGE_2 had an effect on OT synthesis (Hirst et al., 1988).

In the CL, as in the hypothalamus, the same messenger ribonucleic acid (mRNA) produces both OT and neurophysin (Jones and Flint, 1986). Concentrations of OT in the bovine CL during the estrous cycle have been quantitated (Abdelgadir et al., 1987), with the highest concentrations of OT observed in luteal tissue collected on day 8 of the cycle. Because mRNA for OT is highest on

day 3 of the cycle (Ivell et al., 1985) the high levels of OT observed on day 8 may represent stored hormone.

HORMONES INFLUENCING BOVINE LUTEAL FUNCTION

GONADOTROPIN-RELEASING HORMONE (GnRH)

BIOCHEMISTRY AND BIOSYNTHESIS

Gonadotropin-releasing hormone is the hypothalamic decapeptide that acts on the pituitary gonadotropes stimulating the secretion of LH. The primary structure of GnRH, pyroGlu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-Gly^{6}-Leu^{7}-Arg^{8}-Pro^{9}-Gly^{10}NH_2, was announced by Schally (Schally et al., 1971) at the annual meeting of the Endocrine Society in 1971. The amino acid structure was published that year (Matsuo et al., 1971) and GnRH was synthesized the next year (Burgus et al., 1972). Since then, over 2000 analogs of this peptide have been produced providing biomedical research with an array of hormones that can stimulate or inhibit reproductive functions (Karten and Rivier, 1986). The secondary structure of GnRH, as demonstrated by least energy configuration experiments, suggest a ß turn at Ser^{4}-Tyr^{5}-Gly^{6}-Leu^{7} (Nestor, 1984). Estimated biological half-life of GnRH in humans is 4 min, with the Gly^{6} peptide bonds considered to be the major site of proteolytic cleavage (Redding et al., 1973).

Biosynthesis of GnRH has been determined using recombinant DNA techniques to isolate the GnRH gene (Seeburg and Adelman, 1984). The GnRH gene is organized into four exons; the first exon codes for the untranslated 5' region, the second exon codes for the

signal sequence and GnRH, the third exon encodes residues 12-43 of GnRH-associated protein (GAP) and the final exon encodes the C-terminal end of GAP and the untranslated 3' end of the mRNA (Seeburg et al., 1987). The preprohormone contains the signal peptide, GnRH and GAP. Biosynthesis of GnRH involves cleavage of the signal sequence to expose the N-terminal Gln residue of GnRH, which spontaneously cyclizes to form pyroGlu^{1}. Cleavage at the Lys^{11}-Arg^{12} bond of the prohormone, followed by enzymatic amidation of the C-terminal Gly^{10} residue, yields the biologically active decapeptide.

PHYSIOLOGICAL EFFECTS

Administration of GnRH or its agonists results in increased serum concentrations of gonadotropins. A single injection of GnRH has been shown to promote ovulation in women (Grimes et al., 1975; Casper and Yen, 1979), hamsters (Airmura et al., 1971; Humphrey et al., 1973), rats and rabbits (Humphrey et al., 1973) presumably by inducing a preovulatory-like LH surge. In the cow, adminstration of GnRH at various stages of the estrous cycle has been reported to increase serum LH concentrations, without inducing ovulation or altering the duration of the estrous cycle (Kittok et al., 1973; Britt, 1975; Seguin, et al., 1977; Ford and Stormshak, 1978; Milvae et al., 1984; Lucy and Stevenson, 1986; Rodger and Stormshak, 1986). Phase of the estrous cycle appears to be an important factor governing response of the cow to GnRH.

Injection of GnRH during the midluteal phase has been shown to increase serum progesterone concentrations (Kittok et al., 1973; Milvae, et al., 1984). In contrast to these reports a midluteal phase injection of GnRH decreased serum progesterone levels (Rodger and Stormshak, 1986). In other investigations serum progesterone concentrations were not altered by treatment with GnRH in the midluteal phase of the estrous cycle (Britt, 1975; Seguin et al., 1977; Milvae et al., 1984). Reports on the administration of GnRH to cows during the the early luteal phase of the cycle have been more consistent. Ford and Stormshak (1978) found that injection of GnRH 55 hr after detection of estrus caused a decrease in serum progesterone levels later in that same cycle. Lucy and Stevenson (1986) reported that an injection of GnRH shortly after estrus also resulted in attenuated progesterone concentrations and Rodger and Stormshak (1986) found that GnRH injected into cows on day 2 of the cycle caused a decrease in serum progesterone concentrations beginning on day 8 of the cycle.

In domestic ruminants secretion of LH from the anterior pituitary is pulsatile due to the pulsatile release of GnRH from the hypothalamus (Rahe et al., 1980). Release of gonadotropins in response to GnRH stimulation differs with the reproductive status of the animal and appears to be mediated by changes in circulating gonadal steroids (Pelletier and Thimonier, 1975; Haresign and Lamming, 1978). During the follicular phase of the cycle when estradiol is the predominant ovarian steroid being secreted, LH pulses of low amplitude and high frequency have been found to occur in the ewe (Karsch et al., 1979). During the luteal phase

under the influence progesterone, LH pulses are of high amplitude and low frequency (Baird and Scaramuzzi, 1976). These changes appear to be caused by a direct effect of the ovarian steroids on the GnRH pulse generator (Karsch et al., 1979).

There is an increase in pituitary GnRH receptors in rats (Marian et al., 1981) on the day of proestrus and in ewes (Crowder and Nett, 1984) prior to the ovulatory surge of LH. Administration of estradiol augments GnRH-stimulated LH release in cycling and anestrous ewes (Reeves et al, 1971a, b), ovariectomized heifers (Beck and Convey, 1977), prepubertal heifers (Swanson and McCarthy, 1986) and rats (Greeley et al., 1975); however, chronic administration of estradiol to ovariectomized ewes resulted in decreased LH release in response to GnRH (Goodman and Karsch, 1980). In ewes, continuous circulating levels of administered estradiol or progesterone exerts long-term negative feedback on the pulsatile secretion of GnRH and LH. In rats and women, progesterone enhanced GnRH-induced LH release (Martin et al., 1974; Lasley et al., 1975). Progestins had no effect on GnRH-stimulated LH release in anestrous ewes (Chakraborty et al., 1974) or in ovariectomized ewes (Goodman and Karsch, 1980); however, chronic progesterone treatment alone or in combination with estradiol, decreased GnRH-stimulated LH release in ovariectomized ewes (Moss et al., 1981) and in prepubertal heifers (Swanson and McCarthy, 1986). Furthermore, it was observed that when luteal phase levels of progesterone were administered to ovariectomized ewes, the GnRH pulse frequency was slowed (Goodman and Karsch, 1980; Goodman et al., 1981). In ovariectomized ewes treatment

with estradiol or progesterone via subcutaneous silastic implants decreased or abolished GnRH and LH pulses (Karsch et al., 1987). In contrast, it was observed that estrogen treatment via push-pull perfusion of the median eminence of ewes resulted in increased GnRH and LH pulse frequency in ewes (Clarke and Cummings, 1985). Karsch et al. (1987) explained these contrasting results by hypothesizing that long term exposure to estradiol or progesterone results in negative feedback at the level of the hypothalamus, whereas the response to a single large dose of steroid may represent an initial effect at the level of the pituitary. Thus, the dosage and(or) duration of treatment with ovarian steroids may regulate the type of feedback on GnRH-stimulated LH release.

MECHANISM OF ACTION AT THE PITUITARY

Stimulation of LH release by GnRH *in vitro* is blocked by Ca^{2+} chelators (Hopkins and Walker, 1978; Marian and Conn, 1979), Ca^{2+} channel blockers (Conn et al., 1983) and calmodulin antagonists (Conn et al., 1981). These observations demonstrated a role for Ca^{2+} and calmodulin activation in the mediation of GnRH-stimulated LH release. Like other hormones that use Ca^{2+} as a second messenger, GnRH has been shown to cause hydrolysis of polyphosphoinositides by a phospholipase C-type reaction (Schrey, 1985; Andrews and Conn, 1986; Huckle and Conn, 1987). The metabolites of this hydrolysis are inositol trisphosphate, which in other systems has been shown to mobilize Ca^{2+} from intracellular

sources (Streb et al., 1983) and diacylglycerol, which is an endogenous activator of protein kinase C (Conn et al., 1985). Participation of protein kinase C in GnRH action has been demonstrated by the use of protein kinase C-activating phorbol esters, which in part, structurally resemble diacylglycerol. Phorbol esters have been shown to stimulate LH release in the absence of GnRH (McArdle et al., 1987). Furthermore, GnRH causes a redistribution of protein kinase C from the cytosol to the plasma membrane (McArdle et al., 1987). Although protein kinase C is activated by receptor occupancy by GnRH, it is questionable whether this enzyme has a physiological role in promoting the release of gonadotropins. Pituitary cells depleted of protein kinase C were found to respond to GnRH with increased LH secretion in the absence of this enzyme (Turgeon and Waring, 1986).

REGULATION OF GONADOTROPE RESPONSIVENESS

Use of high-affinity, metabolically stable, iodinated GnRH agonists has revealed a single class of specific GnRH binding sites in the plasma membranes of rat anterior pituitary cells (Clayton et al., 1979). Based on target size analysis, the molecular weight of this receptor protein has been estimated to be 136,000 daltons (Conn and Venter, 1985). The GnRH receptor-hormone complex shows lateral mobility in the plasm membrane and internalization, although internalization is not required for GnRH to stimulate LH

release (Conn and Hazum, 1981). In addition, microaggregation of receptors in the absence of GnRH has been reported to promote LH release in the rat (Conn, 1983).

Number of pituitary GnRH receptors and cellular responsiveness are regulated by GnRH (homologous regulation) and other molecules, including gonadal steroids (heterologous regulation). Chronic exposure of the anterior pituitary of the ewe to GnRH causes it to become refractory to further challenge by the homologous hormone (Chakraborty et al., 1974). Chakraborty et al. (1974) suggested that decreased responsiveness of the pituitary to GnRH stimulation may be due to depletion of stored LH. Nett et al. (1981) investigated whether down-regulation of GnRH receptors after chronic exposure to GnRH occurred, possibly explaining pituitary refractoriness. These researchers found that continuous infusion of GnRH into ovariectomized ewes caused an increase in GnRH receptors after 1 to 4 hr of infusion, however; after 12 to 24 hr of infusion the population of GnRH receptors was decreased. Because pituitary stores of LH were not depleted, it was concluded that down-regulation of the GnRH receptor, after continuous exposure to GnRH, caused the pituitary to become refractory to further stimulation by this decapeptide. Furthermore, pulsatile administration of GnRH does not promote down-regulation, or cause pituitary densensitization in ewes (Adams et al., 1975), rats (Badger et al., 1983) and monkeys (Knobil, 1981).

EXTRAPITUITARY ACTIONS

Chronic administration of GnRH to various species alters many reproductive functions including termination of pregnancy in rats (Beattie et al., 1977; Bex and Corbin, 1981) delayed implantation in rats (Humphrey et al., 1976), inhibition of ovarian steroidogenesis in ewes and rats (Foxcroft et al., 1975; Haresign et al, 1975; Jones, 1980) decreased gonadotropin receptor populations in women and rats (Kledzick et al., 1978; Cusan et al., 1979) and inhibition of ovulation in women and rabbits (Bergquist et al., 1979; Cusan et al., 1979). Initially, these antigonadal actions were attributed to gonadotropin receptor down-regulation and cellular desensitization due to exposure of the ovary to high systemic levels of LH induced by GnRH stimulation (Conti et al., 1976). Subsequent studies have demonstrated a direct action of GnRH on the ovary in the rat (Hsueh and Jones, 1981). Treatment of rat granulosa cells with LH resulted in increased estrogen and progesterone production, while concomitant treatment with GnRH or its agonists inhibited LH-stimulated steroidogenesis (Jones and Hseuh, 1981). Treatment of rat luteal cells with GnRH also inhibited LH-stimulated progesterone production and cAMP accumulation (Clayton et al, 1979; Massicotte et al., 1981). This inhibition of ovarian steroidogenesis by GnRH was also observed in hypophysectomized rats (Jones and Hseuh, 1980; Harwood et al., 1980). Receptors that bind GnRH with high affinity have been found in the ovary of the rat (Harwood et al, 1980a; Jones et al., 1980; Pieper et al., 1981). However, ovarian GnRH receptors have

not been detected in the cow, sow, ewe (Brown and Reeves, 1983) rabbit (Thorson et al., 1985) or woman (Clayton and Huhtaniemi, 1982).

Systemic concentrations of GnRH are nearly undetectable (Nett et al., 1974), thus, it is unlikely that hypothalamic GnRH is the endogenous ligand for ovarian GnRH receptors detected in the rat gonad (Hsueh and Jones, 1981). A larger protein with GnRH activity has been identified in the ovary of the rat (Birnbaumer et al., 1985; Aten et al., 1986), woman (Aten et al., 1987), cow and ewe (Aten et al., 1987). This protein is not immunoreactive to GnRH antisera and in contrast to GnRH, is heat sensitive (Aten et al., 1987). The apparent molecular weight of bovine GnRH-like protein as determined by gel electrophoresis is 17,500 daltons (Aten and Behrman, 1988). Luteal content of this protein during the estrous cycle of the cow has been reported to decrease as the CL matures (Ireland et al., 1988a). Partially purified bovine GnRH-like protein binds to rat ovarian GnRH receptors and inhibits LH-stimulated progesterone production (Aten et al., 1987). More recently, Ireland et al. (1988b) reported that bovine GnRH-like protein inhibits LH-stimulated progesterone production in vitro in dispersed bovine luteal cells. The role of this protein in ovarian function, especially in those species that lack ovarian GnRH receptors, awaits its further purification and characterization.

To date, GnRH appears to be exerting its action on the bovine ovary by the release of LH. In the cow LH is luteotropic (Simmons and Hansel, 1964; Donaldson et al., 1965); however, in the rabbit and rat brief exposure of the CL to high concentrations of LH

results in desensitization of the tissue to further stimulation by the homologous hormone (Hunzicker-Dunn and Birnbaumer, 1976; Lamprecht et al., 1977). Down-regulation of the LH receptor in the rat (Conti et al., 1976, 1977) and cow (Rodger and Stormshak, 1986) also occurs as a result of exposure of the ovary to high systemic concentrations of this gonadotropin. A decrease in the LH receptor population has been reported to decrease cAMP production and reduce progesterone synthesis (Marsh, 1975); however, in LH-desensitized Leydig cells the basal production of steroids was not altered, but LH-stimulated steroid production was inhibited (Hsueh et al., 1977).

LUTEINIZING HORMONE

LUTEOTROPIC EFFECTS

The hormone responsible for maintaining the structure and function of the rat CL is prolactin (Melampy et al., 1964) although a luteotropic complex of prolactin and LH has been shown to be optimal for pregnancy maintenance in this species (Ahmad et al, 1969). In the mouse and hamster prolactin and low quantities of LH are required to maintain the morphological and biochemical integrity of the CL, however, administration of high doses of LH causes luteolysis in pregnant, pseudopregnant and cyclic hamsters (Greenwald and Rothchild, 1968). Guinea pigs, unlike the other laboratory rodents discussed, do not display pseudopregnancy and have estrous cycles of longer duration (16 days vs. 4 days for mice and 4 or 5 days for rats) which are characertized by short follicular phases (3-4 days) and long luteal phases (12-13 days; Greenwald and Rothchild, 1968). The primary luteotropin in the guinea pig is LH and the pituitary is required for normal luteal formation until approximately day 4 of the cycle (Nalbandov, 1970).

Although prolactin is luteotropic in many laboratory rodents, it does not appear to act as a luteotropin in domestic ruminants (Kaltenbach et al., 1968; Karsch et al., 1971; Hoffman et al., 1974). It was first reported by Mason et al. (1962) that LH stimulated progesterone synthesis in bovine CL *in vitro* . Kaltenbach et al. (1968) reported that hypophysectomy of ewes on day 1 of the

estrous cycle resulted in failure of the CL to form and hypophysectomy on day 5 resulted in partial regression of the existing CL. According to Denamur et al. (1973) even removal of the uterus did not preclude impaired luteal formation and function in the hypophysectomized ewe. Thus, the anterior pituitary is necessary for normal luteal development in the ewe.

Results of numerous studies strongly suggest that LH is the primary luteotropin in ruminants. The functional life span of the CL of the ewe during the estrous cycle was extended by infusion of LH (Karsch et al., 1971), serum progesterone levels were increased by injections of LH (Carlson et al., 1971) and LH increased progesterone synthesis by bovine (Simmons et al., 1976) and ovine luteal tissue (Kaltenbach et al., 1967) *in vitro* . Futhermore, administration of antiserum to LH in cows resulted in luteal regression (Snook et al., 1969).

Rabbits differ from laboratory rodents and domestic ruminants which rely on prolactin and LH for luteal maintenance, respectively . Does are reflex ovulators, with the ovulatory LH surge induced by cervical stimulation. Maintenance of CL structure and function in the doe requires estradiol, although there is no direct effect of this steroid on progesterone production (Hilliard, 1973). The CL of the doe also contains LH receptors coupled to an adenylate cyclase system that can be activated by LH receptor occupancy (Hunzicker-Dunn and Birnbaumer, 1976). Thus, the mechanism by which estradiol maintains luteal function in the rabbit is not clear.

BIOCHEMISTRY AND MOLECULAR BIOLOGY

Luteinizing hormone is a glycoprotein, with a molecular weight of approximately 30,000 daltons (depending on the species) that is synthesized, stored and released from the anterior pituitary under the regulation of GnRH (Ryan et al., 1987). Secretion of LH from anterior pituitary gonadotropes is regulated by hypothalamic GnRH, as confirmed by pituitary-stalk section studies (Mallory et al., 1986; Hamernik and Nett, 1988). Luteinizing hormone along with human chorionic gonadotropin (hCG), FSH and thyroid-stimulating hormone (TSH) are heterodimers consisting of two polypeptide chains designated as alpha and beta that associate by noncovalent interactions. The alpha-subunit is common to all four hormones, however, the beta-subunit differs in amino acid sequence among the various hormones, thus conferring biological activity. Each subunit is glycosylated and internally cross-linked by disulfide bridges (Strickland et al., 1985).

The first report of the complete sequence of bovine LH was reported in 1971 (Liao and Pierce, 1971) and since then the amino acid sequence of LH in a variety of species has been determined (Pierce and Parsons, 1981). The oligosaccharide structure has been determined for bovine, ovine, porcine and human LH and hCG. The alpha-subunits contain two Asn N-linked oligosaccharide units located at Asn^{56} and Asn^{82} and beta-subunits contain a single N-linked oligosaccharide moiety at Asn^{13}. There are two additional N-linked and four O-linked carbohydrate units in the beta-subunit of

hCG. The higher order structure of LH, especially placement of the disulfide bridges remains equivocal (Strickland et al., 1985).

Because x-ray diffraction models of LH are not yet available, selective chemical reactions have been used to probe structure-function relationships. It has been well documented that dissociation of LH or hCG into subunits results in complete loss of receptor binding and loss of biological activity. It appears that physical separation of the subunits results in a conformational change that prevents binding to the LH receptor (Catt et al., 1973). Limited proteolysis has also been used to determine the portions of the molecule that are important for biological activity. Specifically, removal of the five C-terminal amino acids from the alpha-subunit of bovine LH results in complete loss of activity (Cheng et al., 1973). Effect of degylcosylation has also been investigated by use of anhydrous fluoride or trifluoromethane sulfonic acid treatment, which removes 70-85% of the carbohydrate of LH. Deglycosylation results in decreased activation of adenylate cyclase and attenuation of steroidogenesis. However, there is an increase in subunit interaction and receptor binding indicating that degylcosylated LH can act as an LH-antagonist (Goverman et al., 1982).

There are separate mRNAs encoding the individual subunits. Complementary deoxyribonucleic acid clones (cDNAs) have been made from mRNAs for the alpha-subunit of the cow (Erwin et al., 1983), mouse (Chin et al., 1981), rat (Godine et al., 1982) and human (Boothby et al., 1981) and from mRNA for the beta-subunit of bovine (Keller et al., 1980), rat (Chin et al., 1983), human LH and

hCG (Fiddes and Goodman, 1980). These cDNAs have been used to probe the structure of the subunit genes and in both the bovine and human, a separate gene encodes the alpha-subunit for LH, FSH and TSH (Erwin et al., 1983; Boothby et al., 1981). There is also a single gene that codes for beta-subunit of bovine, human, rat and mouse LH (Talmadge et al., 1984).

The correlation of LH subunit mRNA quantities with serum LH concentrations has been examined in ewes (Landefeld et al., 1984), rats (Counis et al., 1982) and cows (Keller et al., 1980). Effects of gonadectomy, steroid replacement and estradiol treatment were also reported. These studies and others have demonstrated that ovarian steroids can exert an effect at the level of gene transcription. Ovariectomy removes the suppressive effects of estradiol, leading to increased serum levels of LH and LH subunit gene expression, which can be reversed by estradiol replacement in rats (Shupnik et al., 1988). These effects on gene transcription are consistent with observed effects of chronic or short term treatment of ewes with estrogens as previously discussed (pp. 20-21).

Several studies suggest a role for GnRH in regulating LH biosynthesis. Landefeld et al. (1984) observed that in ewes alpha-subunit mRNA levels were elevated immediately prior to and during the preovulatory LH surge and remained elevated after serum and pituitary LH decreased. Quantity of LH beta-subunit mRNA also increased during the preovulatory LH surge and remained elevated after pituitary and serum LH concentrations decreased (Landefeld et al., 1985a). In another experiment administration of GnRH (1 or

2 pulses/hr) to ovariectomized, progesterone-treated, anestrous ewes resulted in increased beta-subunit mRNA (Landefeld et al., 1985b). Lalloz et al. (1988a) found that alpha-subunit mRNA levels in intact or gonadectomized animals were not affected by treatment with GnRH, however, GnRH preferentially modulated beta-subunit mRNA, greatly increasing serum LH and beta-subunit mRNA postcastration. Lalloz et al. (1988b) also observed that in GnRH-desensitized rat gonadotropes LH beta-subunit gene expression was inhibited and LH biosynthesis was reduced. Collectively, these results suggest that LH subunits are regulated by steroids and that GnRH plays a role in LH biosynthesis, presumably by regulating beta-subunit gene expression.

MECHANISM OF ACTION AT THE OVARY

Cholesterol is the major substrate for progesterone biosynthesis and arises from three sources; (1) *de novo* synthesis from acetyl CoA, (2) hydrolysis of cholesterol esters stored in lipid droplets and (3) lipoprotein-cholesterol complexes. The latter source of cholesterol is an integral constituent of low density lipoproteins (LDL) or high density lipoproteins (HDL). Transport of the HDL or LDL-cholesterol complexes into the cell involves binding of these complexes to LDL or HDL plasma membrane receptors, which are internalized and degraded by lysosomal enzymes to liberate free cholesterol (Payne et al., 1985). Specific LDL and HDL receptors have been detected in the rat ovary and treatment with LH *in vivo* or *in vitro* has been

shown to increase the number of LDL and HDL receptors, thus increasing the availability of cholesterol (Hwang and Menon, 1983). Increasing the amount of substrate available for steroid bioynthesis may be one mechanism by which LH increases progesterone production.

The first step in progesterone synthesis, when cholesterol is derived from sources other than the *de novo* pathway, occurs at the mitochondria, where cholesterol is converted to pregnenolone by the cytochrome P-450 cholesterol side chain cleavage enzyme complex (cytochromeP-450$_{ssc}$; Savard, 1973). Cytochrome P-450$_{ssc}$ is located on the inner surface of the inner mitochondrial membrane where it binds cholesterol, catalyzing hydroxylations at carbons 20 and 22, followed by cleavage at the C_{20}-C_{22} bond to yield pregnenolone and isocaproaldehyde. In the smooth ER, pregnenolone is coverted by the actions of 3ß-hydroxysteroid dehydrogenase and 3-ketosteroid-isomerase to progesterone (Payne et al., 1985). There is great interspecies variation in the diversity of steroids produced by the CL. In species studied, the smallest array of steroids are found in the bovine CL which synthesizes progesterone, pregnenolone and 20ß-hydroxy-4-pregnen-3-one (Mason et al., 1962) and at the other extreme the human CL produces progesterone, 17α-hydroxy-progesterone, pregnenolone, 20α-hydroxy-4-pregnen-3-one, 4-androstenedione, estrone and 17ß-estradiol (Huang and Pearlman, 1963). The major pathway by which progesterone is inactivated is via the action of 20α-hydroxysteroid dehydrogenase; however, in the cow the ß-

form of this enzyme is responsible for the formation of 20ß-hydroxy-4-pregnen-3-one (Mason et al., 1962).

The hormone responsible for the regulation of progesterone synthesis and secretion is LH, which has been shown to stimulate progesterone secretion both *in vivo* (Schomberg et al., 1967) and *in vitro* (Armstrong and Black, 1966; Kaltenbach et al., 1967). The first step in the mechanism of LH action at the luteal cell is the binding of this protein hormone to its plasma membrane receptor. The CL contains receptors that specifically bind LH as shown by radioligand studies (Catt et al., 1971; Gospodarowicz, 1973). Binding of LH to its receptor results in the activation of adenylate cyclase, the multi-subunit enzyme to which the LH receptor is coupled (Marsh, 1975).

The message carried by LH is transmitted into the cell by the stimulation of the adenylate cyclase enzyme complex, which increases the production of cAMP from ATP. Cyclic AMP then interacts with specific intracellular proteins, the cAMP-dependent protein kinases, which catalyze the phosphorylation of other proteins causing a biological response (Ling and Marsh, 1977). To terminate the actions of these second messengers, cAMP-phosphodiesterases degrade cAMP to 5'-AMP (Marsh, 1975) and cAMP dissociates from the cAMP-dependent kinases regulatory subunit (Krebs and Beavo, 1979).

The adenylate cyclase enzyme complex is composed of a catalytic subunit (C), a stimulatory guanine nucleotide binding protein (G_s) and many complexes contain an inhibitory guanine

nucleotide binding protein (G_i) (Hunzicker-Dunn and Birnbaumer, 1985). The G-proteins (which are also referred to as N-proteins) are signal-transducing proteins that regulate the activity of C. The two types of G-proteins are G_s, which mediate the effects of a stimulatory hormone-receptor complex and G_i which mediate the effects of an inhibitory hormone-receptor interaction (Hildebrandt et al., 1983). The C-subunit is responsible for catalyzing the formation of cAMP from ATP-Mg^{2+} (Rodbell et al., 1980).

There is little biochemical information known about C because it has not been purified from a mammalian source. However, both G_s and G_i have been purified and characterized (Northup et al., 1980). Both G-proteins are distinct in amino acid sequence, but do share many similarities in overall structure and function. Both have molecular weights of approximately 100,000 daltons, both bind GTP and its analogs, both require Mg^{2+} for activation and both have intrinsic GTPase activity. The G-protein consists of three subunits referred to as alpha, beta and gamma. The alpha subunit is responsible for binding guanine nucleotides and is affected by bacterial toxins. The alpha subunit of G_s is ADP-ribosylated by cholera toxin, while the alpha subunit of G_i is ADP-ribosylated by *Bordetella pertussis* toxin (Hunzicker-Dunn and Birnbaumer, 1985). The ADP-ribosylation of $alpha_s$ by cholera toxin results in the inhibition of the GTPase activity of G_s, whereas the ADP-ribosylation of $alpha_i$ by *B. pertussis* toxin blocks the action of G_i. The result of both toxins, although by different mechanisms of action, is the increased production of cAMP.

Stimulation of C by G_s, which is GTP and Mg^{2+}-dependent, results in increased catalytic activity. Inhibition of C by G_i is also GTP and Mg^{2+}-dependent and appears to prevent the alpha subunit of Gs from complexing with C, but may also involve a direct action of G_i (Gilman, 1984). Addition of a stimulatory hormone to the G_sG_iC complex in a membrane results in the stimulation of a guanine nucleotide-exchange system. In this system the conversion of G_s-GDP to G_s-GTP occurs and the G_s bound to GTP can then activate C. As a result of intrinsic cycling, the rate of which is increased by the presence of a stimulatory hormone, GTPase activity inherent to G_s causes the hydrolysis of GTP to GDP. This results in the inactivation of G_s and the cycle continues (Jakobs et al., 1982).

Actions of cAMP appear to be mediated by cAMP-dependent protein kinases that use ATP-Mg^{2+} to phosphorylate regulatory proteins that alter cellular function. The cAMP-dependent protein kinase is a tetramer composed of two catalytic subunits and two regulatory subunits. Cyclic AMP activates this enzyme by binding to the regulatory subunits that normally inhibit the activity of the catalytic subunits. Catalytic subunits, released from regulatory subunit inhibition by the binding of cAMP, phosphorylate other proteins (Hunzicker-Dunn and Birnbaumer, 1985).

The bovine CL contains two types of cAMP-dependent protein kinase, classified on the basis of their elution properties during DEAE-cellulose chromatography. Type II enzyme is present in follicular tissue, whereas Type I enzyme appears at the time of

luteinization and disappears at the time of luteal regression (Hunzicker-Dunn and Birnbaumer, 1985). In the rabbit, an injection of hCG causes the activation of only Type I enzyme in the CL (Hunzicker-Dunn, 1981). These observations suggest that Type I cAMP-dependent protein kinase may be involved in luteal steroidogenesis.

The precise mechanism by which increased cAMP-dependent protein kinase activity influences progesterone production by small luteal cells is not known, but several mechanisms have been proposed. It appears that continuous protein synthesis is required for steroidogenesis, due in part to the short biological half-life of sterol-carrier proteins (Schulster et al., 1974). Transport of cholesterol to the cytochrome P-450_{ssc} complex, which must be phosphorylated to be activated, is dependent on the presence of a sterol-carrier protein (Caron et al., 1975). Influence of cAMP-dependent protein kinases on sterol-carrier protein synthesis and phosphorylation of the cytochrome P-450_{ssc} complex may be steps in LH-induced stimulation of progesterone synthesis. Cholesterol esterase, another enzyme involved in steroid biosynthesis is also phosphorylated, and thus activated by cAMP-dependent protein kinase (Caffrey et al., 1979). Additionally, the availability of substrate for steroid biosynthesis may be enhanced by cAMP-dependent protein kinase activation of microfilaments involved in LDL/HDL uptake (Niswender and Nett, 1988).

The previous discussion of LH-stimulated progesterone production focused on small luteal cells, which contain the

majority of the LH receptors in the CL. Secretion of progesterone by large luteal cells appears to be mediated independently of cAMP, because direct activation of adenylate cyclase by the cardiotonic diterpene forskolin or cholera toxin results in increased cAMP levels without altering progesterone secretion (Hoyer et al., 1984). Niswender and Nett (1988) suggest that large luteal cells may have lost their ability to inhibit cAMP-dependent protein kinase activity, resulting in continuous stimulation of steroidogenesis.

In addition to its well established ability to activate adenylate cyclase and thus increase intracellular concentrations of the second messenger cAMP, LH may stimulate progesterone synthesis through activation of phospholipid turnover. The actions of many hormones depend on the hydrolysis of membrane phosphotidylinositol 4,5-bisphosphate to yield diacylglycerol and inositol trisphosphate, which serve as second messengers as reviewed by Berridge (1984). Davis et al. (1981) demonstrated that in bovine luteal cells LH stimulated changes in phospholipid metabolism, increasing the incorporation of $^{32}PO_4$ into phosphotidylinositol. It was suggested that these changes in phospholipid metabolism may play a role in the steroidogenic actions of LH. Strauss (1982) proposed that LH-stimulated alteration of phospholipids may change the composition of mitochrondrial membranes, thus enhancing the conversion of pregnenolone to progesterone. The role of LH-induced phospholipid turnover in luteal steroidogenesis, as well as its relative importance as a second messenger in this system remains to be

determined.

RECEPTOR REGULATION

Formation of the CL after ovulation involves the luteinization of follicular cells caused by the synergistic actions of estrogen and the pituitary gonadotropins. Induction of LH receptors occurs as ovarian follicles mature and increase in size. The initial appearance and subsequent increase in the number of LH receptors appears to occur as a result of the actions of estrogens and FSH on granulosa cells. The following model of luteal development has been proposed by Richards et al. (1976): Estradiol acts on granulosa cells inducing FSH receptors and increasing the number of estrogen receptors, after which FSH acts on the granulosa cell increasing its own receptor and inducing LH receptors. Subsequently, LH acting on the granulosa cell decreases the number of estrogen receptors. Once granulosa cells have acquired LH receptors, they can respond to this gonadotropin with increased cAMP production and morphological luteinization (Adashi and Hsueh, 1984). This model accounts for luteal development in sows (Kammerman and Ross, 1975) and ewes, however, LH receptors appear first in thecal cells of ewes (Carson et al., 1979). This model also describes luteal development in rats, however, prolactin is also required for the induction and maintenance of LH receptors in this species (Hilliard, 1973; Nimrod et al., 1977). .

Presence of specific LH receptors, located in plasma membranes of luteal cells, has been reported for women (Lee et al.,

1973), rats (Lee and Ryan, 1972), cows (Rao, 1974) and ewes (Diekman et al., 1978). In an early report the molecular weight of the partially purified receptor isolated from the bovine CL was presumed to be a glycoprotein of about 30,000 to 70,000 daltons (Haour and Saxena, 1974). There have been later reports (Wimalasena et al., 1985) describing the purified porcine LH receptor with an estimated molecular weight of 68,000 daltons. More recent studies of the purified bovine LH receptor suggest that the receptor may exist as subunits with an overall molecular weight from 240,000 to 280,000 daltons (Dattatreyamurty et al., 1983). In contrast to the oligometric structure proposed by others (Dattatreyamurty et al., 1983; Kusuda and Dufau, 1986), Dufau and Kusuda (1987) report that the rat LH receptor is a single polypeptide with an apparent molecular weight of 71,000 to 75,000 daltons. Although the existence of specific plasma membrane receptors for LH have been clearly demonstrated for luteal tissue of a number of species, the precise chemical characteristics of this molecule require further investigation.

The concentration of luteal LH receptors changes during the estrous cycle. Diekman et al. (1978) quantified occupied and unoccupied receptors in the ewe throughout the estrous cycle. These researchers observed that the number of occupied receptors increased from days 2 to 10 of the cycle, remaining at this high level until day 14, then decreased rapidly from days 14 until 16. Changes in unoccupied receptors followed this same pattern. There was a high correlation between occupied receptors and serum progesterone levels, however, on day 10 when serum progesterone

and occupied LH receptors were at a maximum, less than 1% of the total luteal LH receptors were occupied. This observation suggests that only a very small percentage of the luteal LH receptors need be occupied to elicit maximal progesterone secretion.

An important aspect in the biological effect of LH appears to be its modulation of the number of its own receptors. Homologous receptor regulation was first observed in the rat ovary (Conti et al., 1976) and testis (Hsueh et al., 1977) using radiolabeled hCG. Because hCG can be radioiodinated to higher specific activity, is more stable when stored labeled, binds with the same affinity to the LH receptor and exhibits lower nonspecific binding then LH (Niswender et al., 1985), it has been used frequently to study the effects of LH on luteal function. In addition, hCG has a longer biological half-life in blood than LH (hCG $t_{1/2}$=48 hr vs. LH $t_{1/2}$=25 min; Niswender et al., 1985). Effects of ovine LH (oLH) on ovine luteal LH receptors, serum LH and progesterone were investigated by Suter et al. (1980). These investigators injected ewes with 1mg oLH on day 10 of the cycle and collected corpora lutea and blood from 10 min until 72 hr post-injection. Total number of LH receptors increased to 260% of control levels by 10 min post-injection and returned to control levels by 2 hr, then subsequently decreased to 63% of control levels by 24 hr and returned to control levels by 48 hr post-injection. Total number of occupied receptors did not change throughout the study, thus the increases and decrease observed in the total LH receptor population represented changes in the number of unoccupied receptors. After injection of

LH, serum concentrations of this gonadotropin increased 1000-fold by 10 min post-injection and returned to control values by 2 hr. Also, serum progesterone concentrations increased by 10 min post-injection, remained elevated for 2 hr and returned to control values by 4 hr. Although there were dramatic changes in the number of LH receptors, progesterone levels never decreased compared with controls, which may be anticipated because the number of occupied receptors did not change. These data are in agreement with Hsueh et al. (1976) and Sharpe (1977) who reported that serum testosterone concentrations were unaffected in rats after hCG-induced decrease in testicular LH receptors.

It has been observed that there is a marked increase in LH receptors shortly after administration of hCG or LH (Hsueh et al., 1976; Suter et al., 1980) which prompted investigations into the mechanisms by which receptors are lost and replaced. Several hypotheses concerning the increase (up-regulation) of LH receptors have been proposed. Hsueh et al (1976) suggested that the increase in testicular LH receptors in rats after administration of hCG resulted from the "unmasking" of surface receptors induced by this gonadotropin. On the other hand, Willingham et al. (1984) suggested that receptors were recycled and inserted into the plasma membrane.

The pathway by which LH receptors are lost (down-regulated) appears to occur by endocytosis of the hormone-receptor complex by luteal cells (Conn et al., 1978). The internalized complex appears to be degraded because when radioiodinated LH or hCG is administered iodotyrosine is the major radioactive product

(Ascoli and Puet, 1978). Ahmed et al. (1980) further investigated the kinetics of the internalization process by pulse labeling ovine luteal cells with ^{125}I-hCG and found that by 24 hr over 60% of the radioactive hormone had been internalized. These observations suggest that internalization and degradation are major pathways by which the activity of this hormone is terminated and appears to be the way in which down-regulation occurs. Furthermore, Suter and Niswender (1983) suggested that once internalized the hormone and receptor separate, followed by subsequent recylcing of the receptor.

An alternate hypothesis regarding LH receptor down-regulation has been proposed by Schwall and Erickson (1984). These investigators suggested that LH causes down-regulation of the receptor by inhibiting protein synthesis and that receptor degradation is only a minor component of receptor loss. They proposed that the key step in protein synthesis at which LH exerts its inhibitory action is during post-translational modification, namely glycosylation of the LH receptor, because tunicamycin was most effective at inhibiting ^{125}I-hCG binding. It seems likely that both internalization followed by degradation and inhibition of protein synthesis play roles in LH or hCG-induced LH receptor down-regulation.

Several differences in the biological effect of LH and hCG have been observed. Mock and Niswender (1983) conducted an experiment to determine if hCG and LH are internalized at the same rate. Ovine luteal cells were pulse labeled with radioiodinated LH

and hCG and it was found that LH is internalized 60 times faster than hCG. Bourdage et al. (1984) conducted an experiment to determine whether there is a difference in the ability of hCG and LH to stimulate progesterone secretion. Dispersed ovine luteal cells were incubated with maximally stimulating doses of LH or hCG and progesterone secretion was measured. These researchers observed that after reaching a maximum level, LH-stimulated progesterone secretion declined, in contrast to hCG-stimulated progesterone secretion, which remained elevated. The LH receptor responds differently to occupancy by hCG and LH. It appears that the slower rate of hCG internalization results in prolonged stimulation of the steroidogenic response of ovine luteal cells.

In summary, LH and hCG are capable of promoting LH receptor down-regulation, although the receptor responds to these two hormones differently in terms of kinetics. Down-regulation in ewes and rats does not appear to inhibit progesterone secretion because the decrease in the LH receptor population is due to loss of unoccupied receptors.

EXPERIMENTS 1 AND 2: RESPONSE OF THE BOVINE CORPUS LUTEUM TO EXOGENOUS GONADOTROPIN-RELEASING HORMONE DURING THE ESTROUS CYCLE

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) has been shown to promote or inhibit gonadal function in various species depending on the dosage and duration of administration. Luteal progesterone synthesis is altered by treatment with GnRH in women (Casper and Yen, 1979) and rats (Jones and Hsueh, 1980); however, the effects of GnRH on bovine luteal function have been variable. In cows, repeated injections of GnRH during the midluteal phase of the estrous cycle have been shown to increase serum progesterone concentrations (Kittok et al, 1973; Milvae et al, 1984). However, it has been demonstrated that a single injection of GnRH during the early luteal phase of the cycle causes subsequent attenuation of progesterone secretion, but does not result in premature luteal regression (Ford and Stormshak, 1978; Lucy and Stevenson, 1986; Rodger and Stormshak, 1986). Administration of GnRH to ewes during the early luteal phase of the estrous cycle has also been shown to attenuate serum progesterone concentrations without promoting premature luteal regression (Slayden et al., 1987). Suprisingly, there is a lag period of several days between the injection of GnRH and the observed decrease in progesterone secretion.

Exogenous GnRH acts directly on the ovary to impair luteal

function in the rat (Hsueh and Erickson, 1979). Harwood et al. (1980) demonstrated the presence of high affinity binding sites for this decapeptide in rat ovarian tissue. However, in the cow GnRH is believed to act indirectly via the release of gonadotropins to attenuate progesterone secretion. This mode of action is indirectly supported by the absence of ovarian GnRH receptors (Brown and Reeves, 1983) and by the fact that administration of GnRH is followed by down-regulation of the LH receptor (Rodger and Stormshak, 1986). Brief exposure of the rat and rabbit CL to high concentrations of LH results in desensitization of the tissue to further stimulation by the homologous hormone (Hunzicker-Dunn and Birnbaumer, 1976) and LH receptor down-regulation (Conti et al., 1976). Further, basal production of steroids was not altered in LH-desensitized Leydig cells; only LH-stimulated steroid production was inhibited (Hsueh et al., 1977).

Because a single injection of GnRH during the early luteal phase of the cycle subsequently attenuates progesterone secretion, it was anticipated that a subsequent injection of GnRH during the same cycle would either increase or further decrease progesterone secretion. The present experiments were therefore conducted to further investigate the effects of exogenous GnRH on bovine luteal function when administered during the early and midluteal phases of the same estrous cycle.

MATERIALS AND METHODS

Experiment 1

Mature Hereford and Hereford x Angus cows were utilized in experiments 1 and 2. Eight cows were observed twice daily for estrus using vasectomized bulls. After exhibiting two consecutive estrous cycles of normal length, cows were injected by jugular venipuncture with saline on days 2 and 8 of the cycle (day of estrus=day 0 of the cycle), then with 100ug GnRH (CEVA Laboratories, Overland Park, KS) on days 2 and 8 of the subsequent cycle. The design of experiment 1 is presented in Figure 1. Jugular blood samples were collected immediately prior to an injection (t=0), at 15, 30, 45, 60, 120, 240 min post-injection and thereafter on alternate days through day 16 of the cycle. Blood samples (10ml) were stored at 4 C for 24 hr and centrifuged to obtain sera, which were stored at -20C, until assayed for LH and progesterone.

Experiment 2

Effects of *in vivo* treatment with GnRH on *in vitro* luteal function were examined in five cows exhibiting normal estrous cycles. The design of experiment 2 is shown in Figure 2. Cows were injected i.v. with 100ug GnRH on day 2 of the cycle, with saline on days 2 and 8 of the succeeding cycle and then with 100ug GnRH on days 2 and 8 of a subsequent cycle. Corpora lutea were collected per vaginam on day 10 of each cycle after rectal palpation. The CL were weighed, sliced (.3mm thickness) and

washed three times with 40ml of culture medium, blotted on filter paper and divided into 200-300mg aliquots. Aliquots of luteal tissue were placed into three sets of duplicate flasks containing 2.7ml Dulbecco's Modified Eagles Minimum Medium (Sigma, St. Louis, MO) supplemented with antibiotic/antimycotic (Sigma). Tissue samples in sets of flasks were subjected to the following treatments: 1) none (unincubated control); 2) none (incubated control); 3) incubated with LH (10ng/ml). The LH (USDA-bLH-I-1, Beltsville, MD) was dissolved in saline and equivalent volumes (300ul) of this vehicle were added to control flasks. All flasks were flushed with 95% O2:5% CO2 and appropriate flasks were incubated in a Dubnoff metabolic incubator at 37 C for 2 hr. Six milliliters of absolute ethanol (-20 C) was added to the unincubated flasks at the initiation of the incubation and to the incubated flasks to terminate the incubation. Subsequently samples were stored at -20 C pending extraction and quantification of progesterone.

Experiment 1

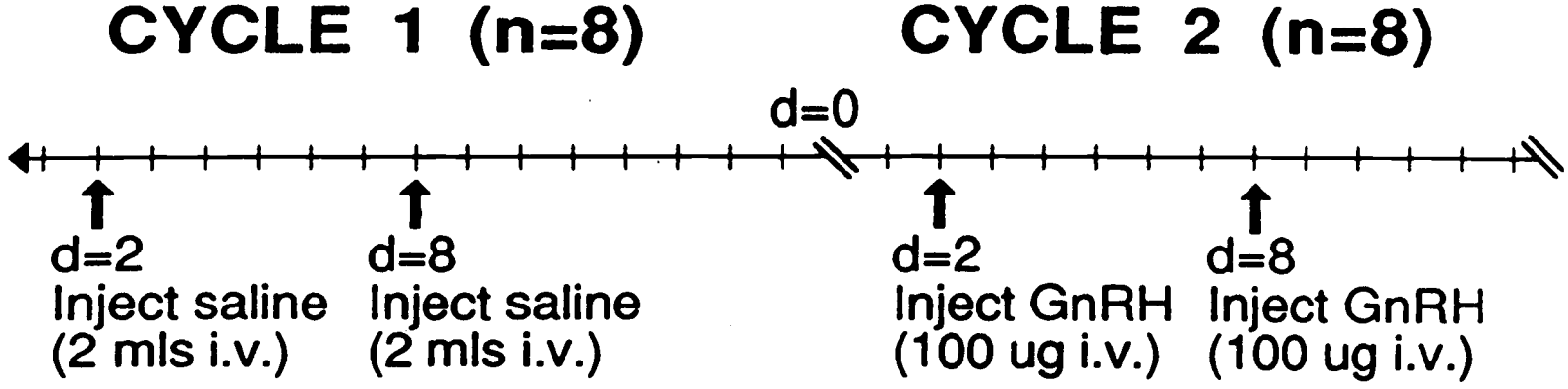

Figure 1. Design of Experiment 1. Cows were injected with saline on days 2 and 8 of the cycle (cycle 1) then with 100ug GnRH (cycle 2). Jugular blood samples were collected immediately prior to an injection and at 15, 30, 45, 60, 120 and 240 min post-injection to characterize serum LH and on alternate days thereafter to determine serum progesterone.

Experiment 2

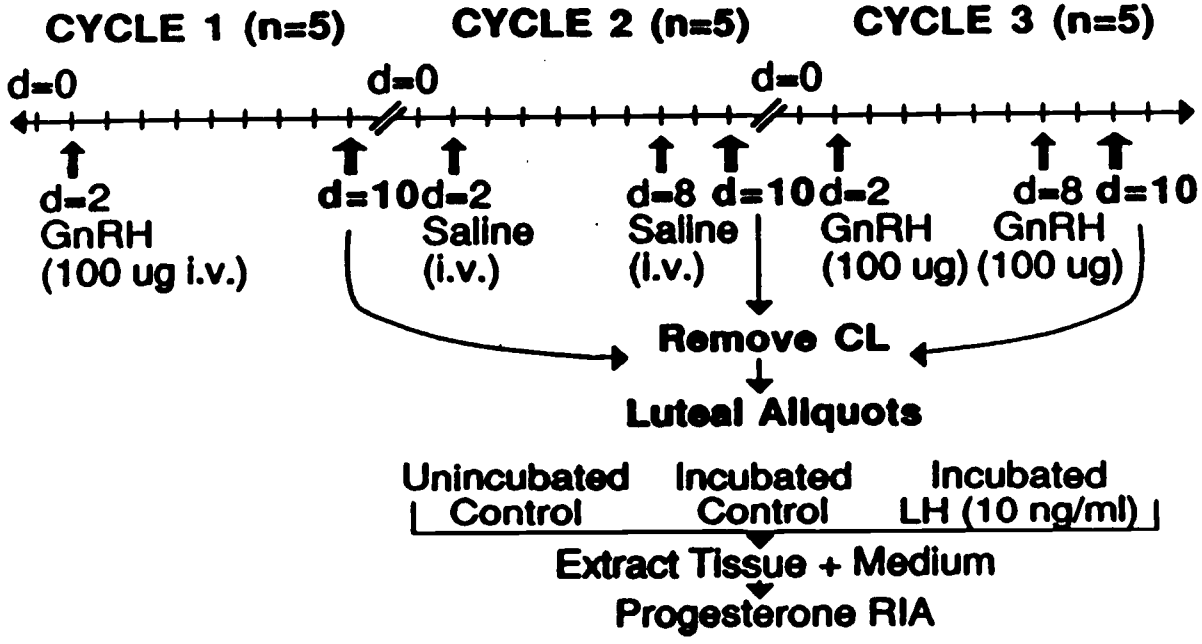

Figure 2. Design of Experiment 2. Cows were injected with 100ug GnRH on day 2 of the cycle (cycle 1), with saline on days 2 and 8 (cycle 2) and with 100ug GnRH on days 2 and 8 (cycle 3). Corpora lutea were collected per vaginam on day 10 of each cycle, weighed, sliced and divided into aliquots. Luteal aliquots served as an unincubated control or were incubated for 2 hr with LH (10ng/ml) or saline (300ul). Tissue + medium were extracted and analyzed for progesterone.

Radioimmunoassays

Hormone assays of sera were performed as previously described for LH (McCarthy and Swanson, 1976) and progesterone (Koligian and Stormshak, 1976). Extraction of tissue + medium for progesterone was as follows: To each sample 33,333 dpm ^{3}H-progesterone (115 Ci/mmol; New England Nuclear, Boston, MA) was added to assess the extraction efficiency and the sample (tissue + medium + ethanol) was homogenized and filtered. The ethanol extract was dried under vacuum in a water bath at 45 C, the residue was dissolved in gel-PBS and allowed to equilibrate for 1 hr. Twenty milliliters of hexane-benzene (2:1 v/v) was added and the sample was vortexed for 2 min then stored at -20 C for 24 hr. The organic solvent was decanted and dried under air in a water bath at 45 C, the residue was redissolved in absolute ethanol and stored at -20 C until a 50ul aliquot (1:100 v/v) was assayed for progesterone as described by Koligian and Stormshak (1976). The average extraction efficiency was 90.6 $\pm$ .6%. Intra- and interassay coefficients of variation for serum progesterone were 8.0% and 8.7%, respectively. Intra- and interassay coefficients of variation for tissue + medium progesterone were 8.8% and 10.6%, respectively. The intrassay coefficient of variation for serum LH was 8.1%.

Statistics

Data of experiment 1 on serum progesterone and LH levels were analyzed by split-split-plot analysis of variance and data on cycle

length were analyzed by analysis of variance. Data for experiment 2 on *in vitro* progesterone concentrations were analyzed by a randomized block-split-plot analysis of variance, with differences between means tested for significance by orthogonal contrasts. Data on corpora lutea weights were analyzed by analysis of variance.

RESULTS

Changes in progesterone secretion after injection of GnRH on days 2 and 8 of the estrous cycle are presented in Figure 3. Although there was a trend for serum concentrations of progesterone to be lower in treated compared with control animals during days 8 through 12 of the cycle, these differences were not significant statistically. Administration of GnRH caused a release of LH on days 2 and 8, with the quantity of LH released on day 8 being greater ($P<0.025$) than on day 2 (Figure 4). As might be anticipated from the pattern of progesterone secretion, treatment did not affect the duration of the estrous cycle (control 20.4 ± .5 vs. treated 20.4 ± .4 days).

Response of luteal tissue to *in vitro* incubation after treatment with GnRH on day 2 or days 2 and 8 of the estrous cycle are depicted in Figure 5. Luteal tissue from cows treated with GnRH on day 2 or days 2 and 8 responded to *in vitro* incubation with a marked increase in basal progesterone concentrations compared with that of tissue removed during the control cycle (control, 37.45 ± 2.93 vs. GnRH day 2, 43.97 ± 5.03 vs. GnRH day 2 and 8, 53.18 ± 3.30 ug/g; $P<0.001$). Relative to progesterone concentrations attained during incubation alone, addition of LH to the incubation medium caused a further increase in steroidogenesis by luteal tissue removed during the control cycle ($P<0.07$), but was without effect on luteal tissue removed after treatment with GnRH on day 2 or days 2 and 8. Treatment did not affect the initial luteal progesterone concentration as determined

by measurement of the steroid in unincubated samples (control, 23.99 ± 1.60 vs. GnRH day 2, 24.08 ± 2.15 vs. GnRH days 2 and 8, 29.80 ± 3.65 ug/g). Treatment with GnRH did not affect weights of corpora lutea (control, 5.06 ± .37 vs. GnRH day 2, 4.48 ± .97 vs. GnRH days 2 and8, 5.97 ± .48 g). Neither treatment with GnRH on day 2 or days 2 and 8 resulted in the formation of accessory corpora lutea as determined by rectal palpation.

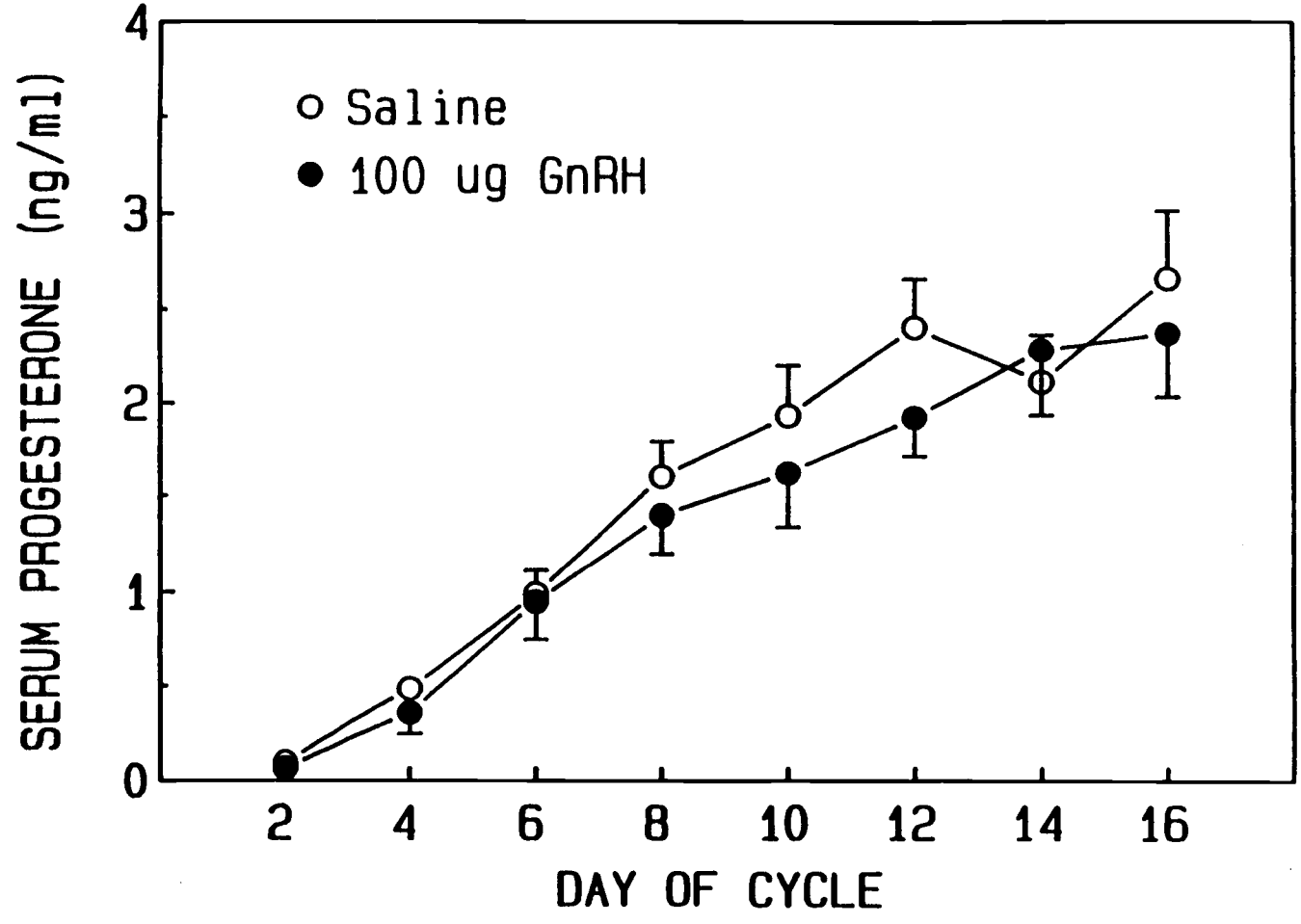

Figure 3. Serum progesterone concentrations (mean ± SE) after administration of GnRH on days 2 and 8 of the cycle.

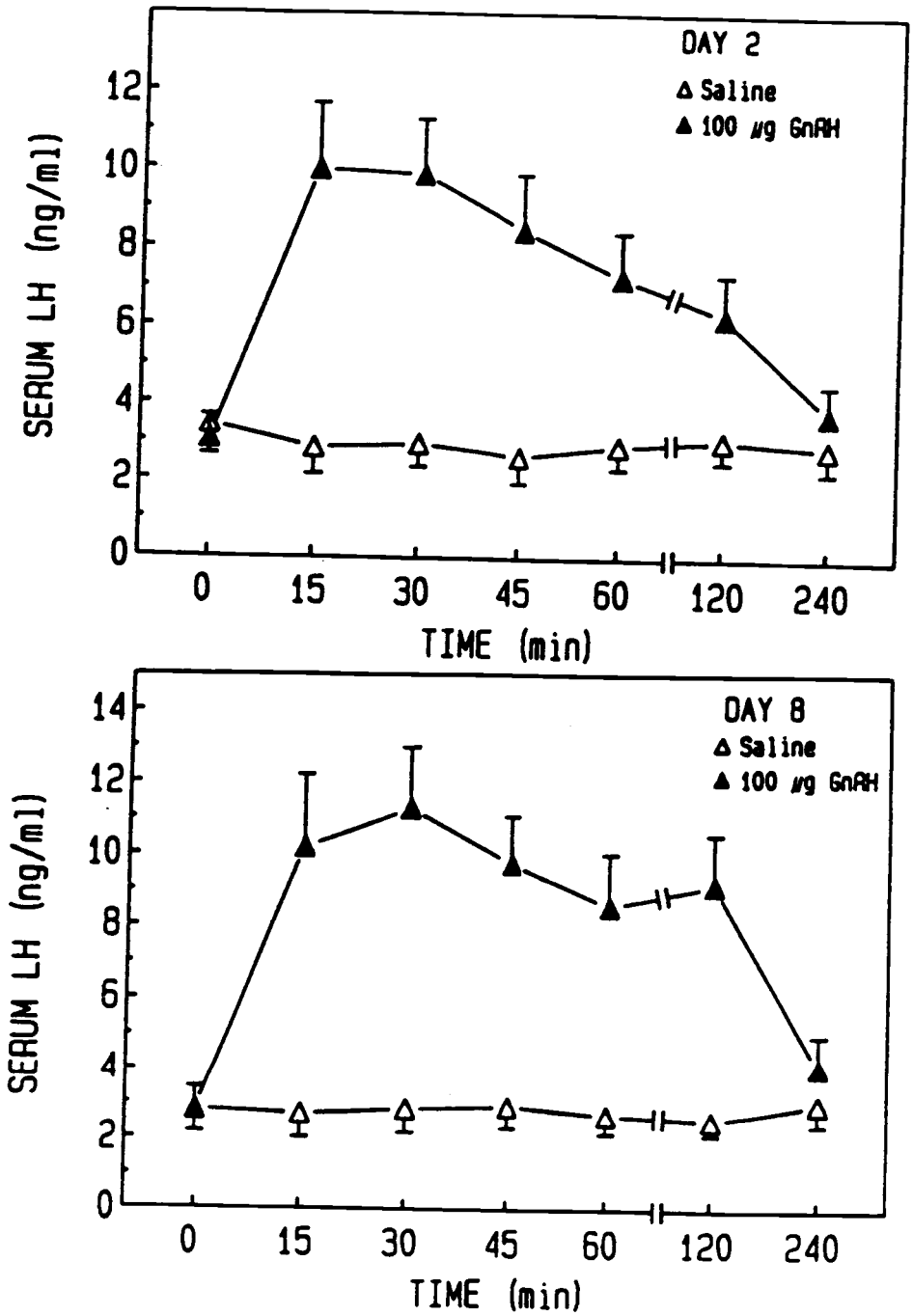

Figure 4. Serum LH concentrations (mean ± SE) after injection of GnRH on days 2 and 8 of the cycle.

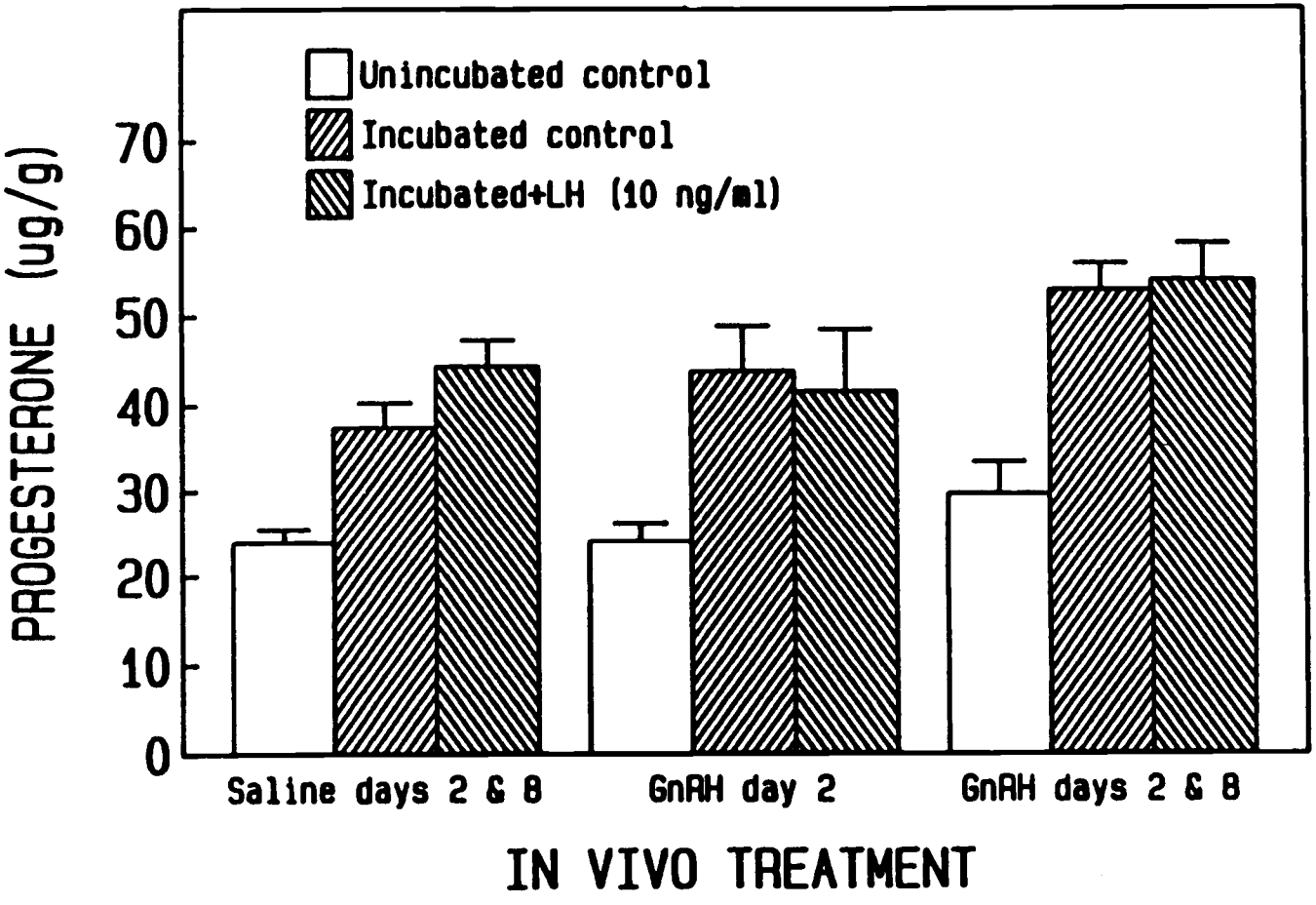

Figure 5. *In vitro* progesterone production (mean ± SE) of luteal tissue after previous *in vivo* administration of GnRH on day 2 or days 2 and 8 of the cycle.

DISCUSSION

Results of Experiment 1 indicate that administration of GnRH on days 2 and 8 of the estrous cycle stimulates LH release, but does not alter serum progesterone concentrations or cycle duration. These data are in agreement with reports, that in general, a single injection or repeated injections of GnRH during the midluteal phase does not alter serum progesterone levels or cycle length (Britt, 1975; Seguin et al., 1977; Milvae et al., 1984). However, these results are in marked contrast to those obtained when GnRH was administered early during the cycle. Injection of GnRH on day 2 of the cycle has been shown to significantly attenuate progesterone secretion during the midluteal phase of the same cycle (Ford and Stormshak, 1978; Rodger and Stormshak, 1986). In the present study, it is possible that LH, released in response to the second injection of GnRH on day 8, masked the detrimental effects of the first injection given on day 2, causing serum progesterone concentrations to return to normal levels. This possibility is supported by the data of Experiment 2 demonstrating that exogenous GnRH resulted in increased basal progesterone production. The bovine CL contains two cells types classified on the basis of size (O'Shea et al, 1979). Both large and small cell types are capable of synthesizing progesterone, however, large cells show higher basal progesterone production than small cells and are less responsive to LH stimulation than small cells (Koos and Hansel, 1981; Rodgers and O'Shea, 1982). Furthermore, the majority of LH receptors in the CL are found on the small luteal

cells (Fitz et al., 1982). The CL undergoes pronounced changes in cellular composition throughout the cycle. Composition of the ovine CL as determined by morphometric analysis revealed that luteal weight and number of steroidogenic cells increased throughout the luteal phase (Farin et al., 1986). Donaldson and Hansel (1965) proposed that under the influence of LH, small luteal cells differentiate into large cells as the CL matures. Cran (1983) reported that thecal (small cells) obtained from ovine cystic follicles exhibited characteristics of large luteal cells after treatment with PMSG and Gamboni et al. (1984) showed that there was a decrease in the number of small cells after treatment of ewes with human chorionic gonadotropin (hCG) on day 5 of the cycle. More recently, Farin et al. (1988) found that hCG injected on days 5 and 7.5 or pharamcological dosages of LH injected every 6 hr from day 5 to 10 caused a decrease in the number of small cells with a concomitant increase in the number of large cells in the ovine CL. There was no change in serum progesterone due to treatment with LH, however, there was an increase in serum progesterone beginning on day 10 after treatment with hCG. The foregoing reports support the hypothesis that LH promotes the conversion of small luteal cells into large luteal cells. In the current study it is possible that LH, secreted in response to GnRH, acted on the CL promoting the conversion of small cells to large cells. Occurrence of such a phenomenom could account for the observed increase in basal progesterone production, due to the increased number of large cells and failure of LH to stimulate progesterone secretion due to fewer small cells.

CONCLUSORY REMARKS

The data from experiments 1 and 2 demonstrate that administration of GnRH to cows during the early and midluteal phases of the same estrous cycle did not affect serum progesterone concentrations, in contrast to the results obtained when this decapeptide was administered either in the early or midluteal phase of the estrous cycle (Rodger and Stormshak, 1986). It appears that LH, released in response to the second injection of GnRH, was able to ameliorate the detrimental effects of the first injection of GnRH. Because it was hypothesized that LH promoted the conversion of small to large cells an experiment should be conducted to quantify each cell type in the bovine CL after *in vivo* treatment with GnRH on day 2 or days 2 and 8 of the cycle. It has been shown in ewes that administration of pharmacological doses of LH (120ug/6 hr from days 5 to 10 of the cycle) promotes the conversion of small to large luteal cells (Farin et al., 1988), however, it is not known if exposure of the CL to physiological levels of LH, as occurred in the current investigation, also induces these changes. The proposed study would answer this question.

BIBLIOGRAPHY

Abdelgadir, S. E., Swanson, L. V., Oldfield, J. E. and Stormshak, F. 1987. Prostaglandin $F_{2\alpha}$-induced release of oxytocin from bovine corpora lutea in vitro. Biol. Reprod. 37:550-555.

Adams, T. E., Kinder, J. E., Chakraborty, P. K., Estergreen, V. L. and Reeves J. J. 1975. Ewe luteal function influenced by pulsatile administration of synthetic LHRH/FSHRH. Endocrinology 97: 1460-1467.

Adashi, E. Y. and Hsueh, A. J. W. 1984. Hormonal induction of receptors during ovarian granulosa cell differentiation. The Receptors 1:587-634.

Ahmad, N., Lyons, W. R. and Papkoff, H. 1969. Maintenance of gestation in hypophysectomized rats with highly purified pituitary hormones. Anat. Rec. 164:291-303.

Ahmed, C. E., Sawyer, H. R. and Niswender, G. D. 1981. Internalization and degradation of human chorionic gonadotropin in ovine luteal cells: Kinetic studies. Endocrinology 109:1380-1387.

Alila, H. W. and Hansel, W. 1984. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol. Reprod. 31:1015-1025.

Andrews, W. V. and Conn, P. M. 1986. Gonadotropin-releasing hormone stimulates mass changes in phosphoinositides and diacylglycerol accumulation in purified gonadotrope cell cultures. Endocrinology 118:1148-1158.

Arimura, A., Matsuo, H., Baba, Y. and Schally, A. V. 1971. Ovulation induced by synthetic luteinizing hormone releasing hormone in the hamster. Science 174:511-512.

Armstrong, D. T. and Black, D. L. 1966. Influence of luteinizing hormone on corpus luteum metabolism and progesterone biosynthesis throughout the bovine estrous cycle. Endocrinology 78:937-944.

Armstrong, D. T. and Hansel, W. 1959. Alteration of the bovine estrous cycle with oxytocin. J. Dairy Sci. 42:533- 542.

Ascoli, M. and Puett, D. 1978. Degradation of receptor-bound human choriogonadotropin by murine Leydig tumor cells. J. Biol. Chem. 253:4892-4899.

Aten, R. F. and Behrman, H. R. 1988. The ovarian GnRH-like protein: Purification from bovine ovaries and characterization of biological activity. Abstracts of the 70th Annual Meeting of the Endocrine Society pg. 294.

Aten, R. F., Ireland, J. J., Weems, C. W. and Behrman, H. R. 1987. Presence of gonadotropin-releasing hormone-like proteins in bovine and ovine ovaries. Endocrinology 120:1727-1733.

Aten, R. F., Polan, M. L., Bayless, R. and Behrman, H. R. 1987. A gonadotropin-releasing hormone (GnRH)-like protein in human ovaries: Similarity to the GnRH-like ovarian protein of the rat. J. Clin. Endocrinol. Metab. 64:1288-1293.

Aten, R. F., Williams, A. T. and Behrman, H. R. 1986. Ovarian gonadotropin-releasing hormone-like protein(s): Demonstration and characterization. Endocrinology 118:961-967.

Auletta, F. J., Kamps, D. L., Pories, S., Bisset, J. and Gibson, M. 1984. An intra-corpus luteum site for the luteolytic action of $PGF_{2\alpha}$ in the rhesus monkey. Prostaglandins 27:285-298.

Babcock, J. C. 1966. Luteotrophic and luteolytic mechanisms in bovine corpora lutea. J. Reprod. Fertil. (Suppl. 1):47.

Badger, T. M., Loughlin, J. S. and Naddaff, P. G. 1983. The luteinizing hormone-releasing hormone (LHRH) desensitized rat pituitary: Luteinizing hormone responsiveness to LHRH *in vitro* . Endocrinology 112:793-799.

Baird, D. T. and Scaramuzzi, R. J. 1976. Changes in the secretion of ovarian steroids and pituitary luteinizing hormone in the peri-ovulatory period in the ewe: The effect of progesterone. J. Endocrinol. 70:237-245.

Beattie, C. W., Corbin, A., Cole, G., Corry, S., Jones, R. C., Koch, K. and Tracy, J. 1977. Mechanism of the postcoital contraceptive effect of LH-RH in the rat. I. Serum hormone levels during chronic LH-RH adminstration. Biol. Reprod. 16:322-332.

Beck, T. W. and Convey, E. M. 1977. Estradiol control of serum luteinizing hormone concentrations in the bovine. J. Anim. Sci. 45:1096-1101.

Bergquist, C., Nillius, S. J. and Wide, L. 1979. Inhibition of ovulation in women by intranasal treatment with a luteinizing hormone releasing hormone agonist. Contraception 19:497-506.

Berridge, M. J. 1984. Inositol trisphosphate and diacylglycerol as second messengers. Biochem. J. 220:345-360.

Bex, F. J. and Corbin, A. 1981. Luteinizing hormone-releasing hormone (LHRH) and LHRH agonist termination of pregnancy in hypophysectomized rats: Extrapituitary site of action. Endocrinology 108:273-280.

Birnbaumer, L., Shahabi, N., Rivier, J. and Vale, W. 1985. Evidence for a physiological role of gonadotropin-releasing hormone (GnRH) or GnRH-like material in the ovary. Endocrinology 116:1367-1370.

Boothby, M., Ruddon, R. W., Anderson, C., McWilliams, D. and Boime, I. 1981. A single gonadotropin α-subunit gene in normal and tumor-derived cell lines. J. Biol. Chem. 256:5121-5127.

Bourdage, R. J., Fitz, T. A. and Niswender, G. D. 1984. Differential steroidogenic response of ovine luteal cells to ovine luteinizing hormone and human chorionic gonadotropin. Proc. Soc. Exp. Biol. Med. 175:483-486.

Bradbury, J. T., Brown, W. E. and Gray, L. A. 1950. Maintenance of the corpus luteum and physiologic actions of progesterone. Rec. Prog. Horm. Rec. 5:151-194.

Britt, J. H. 1975. Ovulation and endocrine response after LH-RH in domestic animals. Ann. Biol. Anim. Biochem. Biophys. 15:221-231.

Brown, J. L. and Reeves, J. J. 1983. Absence of specific luteinizing hormone-releasing hormone receptors in ovine, bovine and porcine ovaries. Biol. Reprod. 29:1179-1182.

Burgus, R., Butcher, M., Amoss, M., Ling, N., Monahan, M. W., Rivier, J., Fellows, R., Blackwell, R., Vale, W. and Guillemin, R. 1972. Primary structure of the ovine hypothalamic luteinizing hormone releasing factor (LRF). Proc. Natl. Acad. Sci. USA 69:278-282.

Caffrey, J. L., Fletcher, P. W., Diekman, M. A., O'Callaghan, P. L. and Niswender, G. D. 1979. The activity of ovine luteal cholesterol esterase during several experimental conditions. Biol. Reprod. 21:601-608.

Caldwell, B. V., Mazer, R. S. and Wright, P. A. 1967. Luteolysis as affected by uterine transplantation in the Syrian hamster. Endocrinology 80:477-482.

Carlson, J. C., Norimoto, K. and Hansel., W. 1971. Effect of LH on peripheral progesterone concentrations in intact and hysterectomized heifers. Endocrinology 89:1530-1533.

Caron, M. G., Goldstein, S., Savard, K. and Marsh, J. M. 1975. Protein kinase stimulation of a reconstituted cholesterol side chain cleavage enzyme system in the bovine corpus luteum. J. Biol. Chem. 250:5137-5143.

Carson, R. S., Findlay, J. K. and Burger, H. G. 1979. Receptors for gonadotropins in the ovine follicle during growth and atresia. Adv. Exp. Med. Biol. 112:89-94.

Casida, L. E. and Warwick, E. J. 1945. The necessity of the corpus luteum for maintenance of pregnancy in the ewe. J. Anim. Sci. 4:34-36.

Casper, R. F. and Yen, S. S. C. 1979. Induction of luteolysis in the human with a long-acting analog of luteinizing hormone releasing factor. Science 205:408-410.

Catt, K. J., Dufau, M. L. and Tsuruhara, T. 1971. Studies on a radioligand-receptor assay system for luteinizing hormone and chorionic gonadotropin. J. Clin. Endocrinol. Metab. 32:860-863.

Catt, K. J., Dufau, M. L. and Tsuruhara, T. 1973. Absence of intrinsic biological activity in LH and hCG subunits. J. Clin. Endocrinol. Metab. 36:73-80.

Chakraborty, P. K., Adams, T. E., Tarnavsky, G. K. and Reeves, J. J. 1974. Serum and pituitary LH concentrations in ewes infused with LH-RH/FSH-RH. J. Anim. Sci. 39:1150-1157.

Chenault, J. R. 1983. Response of bovine corpora lutea to intrauterine prostaglandin E_2 infusion. J. Anim Sci. (Suppl. 1) 57:323-324.

Cheng, K. W., Glazer, A. N. and Pierce, J. G. 1973. The effects of modification of the COOH-terminal regions of bovine thyrotropin and its subunits. J. Biol. Chem. 248:7930-7937.

Chin, W. W., Godine, J. E., Klein, D. R., Chang, A. S., Tan, L. K. and Habener, J. F. 1983. Nucleotide sequence of the cDNA encoding the precusor of the ß-subunit of rat lutropin. Proc. Natl. Acad. Sci. USA 80:4649-4653.

Chin, W. W., Kronenberg, H. M., Dee, P. C., Maloof, F. and Habener, J. F. 1981. Nucleotide sequence of the mRNA encoding the pre-α-subunit of mouse thyrotropin. Proc. Natl. Acad. Sci. USA 78:5329-5333.

Clarke, I. J. and Cummings, J. T. 1985. Increased gonadotropin-releasing hormone pulse frequency associated with estrogen induced luteinizing hormone surges in ovariectomized ewes. Endocrinology 116:2376-2383.

Clayton, R. N., Harwood, J. P. and Catt, K. J. 1979. Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production. Nature 282:90-93.

Clayton, R. N. and Huhtaniemi, I. T. 1982. Absence of gonadotropin-releasing hormone receptors in human gonadal tissue. Nature 299:56-59.

Clayton, R. N., Shakespear, R. A., Duncan, J. A. and Marshall, J. C. 1979. Radioiodinated nondegradable gonadotropin-releasing hormone analogs: New probes for investigations of pituitary gonadotropin-releasing hormone receptors. Endocrinology 105:1369-1381.

Conn, P. M. 1983. Ligand dimerization: A technique for assessing receptor-receptor interactions. In Conn, P. M. (ed.), Methods in Enzymology, pp. 49-58. Academic Press, New York.

Conn, P. M., Conti, M., Harwood,. J. P., Dufau, M. L. and Catt, K. J. 1978. Internalisation of gonadotrophin receptor complex in ovarian luteal cells. Nature 274:598- 600.

Conn, P. M., Ganong, B. R., Ebeling, J., Staley, D., Neidel, J. E. and Bell, R. M. 1985. Diacylglycerols release LH: Structure activity relationship reveals a role for protein kinase C. Biochem. Biophys. Res. Commun. 126:532-539.

Conn, P. M. and Hazum, E. 1981. Luteinizing hormone release and gonadotropin-releasing hormone (GnRH) receptor internalization: Independent actions of GnRH. Endocrinology 109:2040-2045.

Conn, P. M., Rogers, D. C. and Seay, S. G. 1983. Structure-function relationships of calcium ion channel antagonists at the pituitary gonadotrope. Endocrinology 113:1592-1595

Conn, P. M., Rogers, D. C. and Sheffield, T. 1981. Inhibition of gonadotropin-releasing hormone-stimulated luteinizing hormone release by pimozide: Evidence for a site of action after calcium mobilization. Endocrinology 109:1122-1126.

Conn, P. M. and Venter, J. C. 1985. Radiation activation (target size analysis) of the gonadotropin-releasing hormone receptor: Evidence for a high molecular weight complex. Endocrinology 116:1324-1326.

Conti, M., Harwood, J. P., Dufau, M. L. and Catt, K. J. 1977. Effect of gonadotropin-induced receptor regulation on biological responses of isolated rat luteal cells. J. Biol. Chem. 252: 8869-8874.

Conti, M., Harwood, J. P., Hsueh, A. J. W., Dufau, M. L. and Catt, K. J. 1976. Gonadotropin-induced loss of hormone receptors and desensitization of adenylate cyclase in the ovary. J. Biol. Chem. 251:7729-7731.

Corner, G. W. 1919. On the origin of the corpus luteum of the sow from both granulosa and theca interna. Am. J. Anat. 26:117-183.

Counis, R., Corbani, M., Poissonnier, M. and Jutisz, M. 1982. Characterization of the precusors of α and ß subunits of follitropin following cell-free translation of rat and ovine mRNAs. Biochem. Biophys. Res. Commun. 107:998-1005.

Cran, D. G. 1983. Follicular development in sheep after priming with PMSG. J. Reprod. Fertil. 67:415-423.

Critser, E. S., Rutledge, J. J. and French, R. L. 1980. Role of the uterus and the conceptus in regulating luteal lifespan in the mouse. Biol. Reprod. 23:558-563.

Crowder, M. E. and Nett, T. M. 1984. Pituitary content of gonadotropins and receptors for gonadotropin-releasing hormone (GnRH) and hypothalamic content of GnRH during the periovulatory period of the ewe. Endocrinology 114:234-239.

Csapo, A. I., Puri, C. P. and Tano, S. 1981. Relationship between timing of ovariectomy and maintenance of pregnancy in the guinea pig. Prostaglandins 22:131-140.

Csapo, A. I. and Wiest, W. G. 1966. An examination of the quantitative relationship between progesterone and maintenance of pregnancy. Endocrinology 85:735-746.

Cusan, L., Auclair, C., Belanger, A., Ferland, L., Kelly, P. A., Seguin, C. and Labrie, F. 1979. Inhibitory effects of long term treatment with a luteinizing hormone releasing hormone agonist on the pituitary-gonadal axis in male and female rats. Endocrinology 104:1369-1376.

Dattatreyamurty, B., Rathnam, P. and Saxena, B. B. 1983. Isolation of the luteinizing hormone-chorionic gonadotropin receptor in high yield from bovine corpora lutea. Molecular assembly and oligometric nature J. Biol. Chem. 258:3140-3158.

Davis, J. S., Farese, R. V. and Marsh, J. M. 1981. Stimulation of phospholipid labeling and steroidogenesis by luteinizing hormone in isloated bovine luteal cells. Endocrinology 109:469-475.

Denamur, R., Martinet, J. and Short, R. V. 1973. Pituitary control of the ovine corpus luteum. J. Reprod. Fertil. 32:207-220.

Dewar, A. D. 1973. Effects of hysterectomy on corpus luteum activity in the cyclic, pseudopregnant and pregnant mouse. J. Reprod. Fertil. 33:77-89.

Diekman, M. A., O'Callaghan, P., Nett, T. M. and Niswender, G. D. 1978. Validation of methods and quantification of luteal receptors for LH throughout the estrous cycle and early pregnancy in ewes. Biol. Reprod. 19:999-1009.

Donaldson, L. and Hansel, W. 1965. Histological study of bovine corpora lutea. J. Dairy Sci. 48:905-909.

Donaldson, L. E., Hansel, W. and VanVleck, L. D. 1965. Luteotropic properties of luteinizing hormone and nature of oxytocin induced luteal inhibition in cattle. J. Dairy Sci. 48:331-337.

Dufau, M. L. and Kusuda, S. 1987. Purification and characterization of ovarian LH/hCG and prolactin receptors. J. Recept. Res. 7:167-193.

Durrant, E. P. 1972. Studies on vigor. XI. Relationship of hysterectomy to voluntary activity in the white rat. Am. J. Physiol. 82:14-18.

Erwin, C. R., Croyle, M. L., Donelson, J. E. and Maurer, R. A. 1983. Nucleotide sequence of cloned complementary deoxyribonucleic acid for the α-subunit of bovine pituitary glycoprotein hormones. Biochemistry 22:4856-4860.

Estergreen, V. L., Frost, O. L., Gomes, W. R., Erb, R. E. and Bullard, J. F. 1967. Effect of ovariectomy on pregnancy maintenance and parturition in dairy cows. J. Dairy Sci. 50:1293-1295.

Fairclough, R. J., Smith, J. F. and McGowan, L. T. 1981. Prolongation of the oestrous cycle in cows and ewes after passive immunization with PGF antibodies. J. Reprod. Fertil. 62:213-219.

Farin, C. E., Moeller, C. L., Mayan, H., Gamboni, F., Sawyer, H. R. and Niswender, G. D. 1988. Effect of luteinizing hormone and human chorionic gonadotropin on cell populations in the ovine corpus luteum. Biol. Reprod. 38:413-421.

Farin, C. E., Moeller, C. L., Sawyer, H. R., Gamboni, F. and Niswender, G. D. 1986. Morphometric analysis of cell types in the ovine corpus luteum throughout the estrous cycle. Biol. Reprod. 35:1299-1308.

Fiddes, J. C. and Goodman, H. M. 1980. The cDNA for the ß-subunit of human chorionic gonadotropin suggests evolution of a gene by readthrough into the 3'-untranslated region. Nature 286:684-687.

Fields, P. A., Eldridge, R. K., Fuchs, A. R., Roberts, R. F. and Fields, M. J. 1983. Human placental and bovine corpora luteal oxytocin. Endocrinology 112:1544-1546.

Fitz, T. A., Mayan, M. H., Sawyer, H. R. and Niswender, G. D. 1982. Characterization of two steroidogenic cell types in the ovine corpus luteum. Biol. Reprod. 27:703-711.

Flint, A. P. F. and Sheldrick, E. L. 1982. Ovarian secretion of oxytocin is stimulated by prostaglandin. Nature 297:587-588.

Flint, A. P. F. and Sheldrick, E. L. 1983. Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. J. Reprod. Fertil. 67:215-225.

Ford, S. P., Christenson, R. K. and Chenault, J. R. 1979. Patterns of blood flow to the uterus and ovaries of ewes during the period of luteal regression. J. Anim. Sci. 49:1510-1516.

Ford, S. P. and Stormshak, F. 1978. Bovine ovarian and pituitary responses to PMS and GnRH administered during metestrus. J. Anim. Sci. 46:1701-1706.

Foxcroft, G. R., Hamilton, J. W. and Nalbandov, A. V. 1975. Ovulation and luteal function in the rabbit in response to injection or infusion of synthetic gonadotropin releasing hormone (Gn-RH). Biol. Reprod. 12:284-288.

Gamboni, F., Fitz, T. A., Hoyer, P. B., Wise, M. E., Mayan, H. and Niswender, G. D. 1984. Effect of human chorionic gonadotropin on induced ovine corpora lutea during the anestrous season. Domes. Anim. Endocrinol. 1:79-88.

Gillim, S. W., Christensen, A. K. and McLennan, C. E. 1969. Fine structure of the human menstrual corpus luteum at its stage of maximum secretory activity. Am. J. Anat. 126:409-415.

Gilman, A. G. 1984. G proteins and dual control of adenylate cyclase. Cell 36:577-580.

Ginther, O. J. 1974. Internal regulation of physiological processes through local venoarterial pathways: A review. J. Anim. Sci. 39:550-564.

Ginther, O. J., Woody, C. O., Janakiraman, K. and Casida, L. E. 1966. Effect of an intra-uterine plastic coil on the oestrous cycle of the heifer. J. Reprod. Fertil. 12:193-198.

Ginther, O. J., Woody, C. O., Mahajan, S., Janakiraman, K. and Casida, L. E. 1967. Effect of oxytocin administration on the oestrous cycle of unilaterally hysterectomized heifers. J. Reprod. Fertil. 14:225-229.

Godine, J. E., Chin, W. W. and Habener, J. F. 1982. α-subunit of rat pituitary glycoprotein hormones. Primary structure of the precusor determined from the nucleotide sequence of cloned cDNAs. J. Biol. Chem. 257:8368-8371.

Godkin, J. D., Bazer, F. W., Thatcher, W. W. and Roberts, R. M. 1984. Proteins released by cultured day 15-16 conceptuses prolong luteal maintenance when introduced into the uterine lumen of cyclic ewes. J. Reprod. Fertil. 71:57-64.

Goodman, R. L., Bittman, E. L., Foster, D. L. and Karsch, F. J. 1981. The endocrine basis of the synergistic suppression of luteinizing hormone by estradiol and progesterone. Endocrinology 109:1414-1417.

Goodman, R. L. and Karsch, F. J. 1980. Pulsatile secretion of luteinizing hormone: Differential suppression by ovarian steroids. Endocrinology 107:1286-1290.

Gospodarowicz, D. 1973. Properties of the luteinizing hormone receptor of isolated bovine corpus luteum plasma membranes. J. Biol. Chem. 248:5042-5049.

Goverman, J. M., Parsons, T. F. and Pierce, J. G. 1982. Enzymatic deglycosylation of the subunits of chorionic gonadotropin: Effects on formation of tertiary structure and biological activity. J. Biol. Chem. 257:15059-15064.

Greeley, G. H., Muldoon, T. G. and Mahesh, V. B. 1975. Correlative aspects of luteinizing hormone-releasing hormone sensitivity and cytoplasmic estrogen receptor concentration in the anterior pituitary and hypothalamus of the cycling rat. Biol. Reprod. 13:505-512.

Greenwald, G. S. and Rothchild, I. 1968. Formation and maintenance of corpora lutea in laboratory animals. J. Anim. Sci. (Suppl. I.) 27:138-162.

Grimes, E. M., Taymor, M. L. and Thompson, I. E. 1975. Induction of timed ovulation with synthetic luteinizing hormone releasing hormone in women undergoing insemination therapy. I. Effect of a single parentral adminstration at midcycle. Fertil. Steril. 26:277-282.

Gulyus, B. J., Stouffer, R. L. and Hodgen, G. D. 1979. Progesterone synthesis and fine structure of dissociated monkey *(Macaca mulatta)* luteal cells maintained in culture. Biol. Reprod. 20:779-792.

Hamernik, D. L. and Nett, T. M. 1988. Gonadotropin-releasing hormone increases the amount of messenger ribonucleic acid for gonadotropins in ovariectomized ewes after hypothalamic-pituitary disconnection. Endocrinology 122:959-966.

Hansel, W. 1975. Luteal regression in domestic animals. Ann. Biol. Anim. Biochem. Biophys. 15:147-160.

Hansel, H., Concannon, P. W. and Lukaszewska, J. H. 1973. Corpora lutea of the large domestic animals. Biol. Reprod. 8:222-245.

Hansel, W. and Convey, E. M. 1983. Physiology of the estrous cycle. J. Anim. Sci. (Suppl. 2) 57:404-424.

Hansel, W. and Snook, R. B. 1970. Pituitary ovarian relationships in the cow. J. Dairy Sci. 53:945-961.

Hansen, P. J., Anthony, R. V., Bazer, F. W., Baumbach, G. A. and Roberts, R. M. 1985. *In vitro* synthesis and secretion of ovine trophoblast protein-1 during the period of maternal recognition of pregnancy. Endocrinology 117:1424-1430.

Haour, F. and Saxena, B. B. 1974. Characterization and solubilization of gonadotropin receptor of bovine corpus luteum. J. Biol. Chem. 249:2195-2205.

Haresign, W., Foster, J. P., Haynes, N. B., Crighton, D. B. and Lamming, G. E. 1975. Progesterone levels following treatment of seasonally anoestrus ewes with synthetic LH-releasing hormone. J. Reprod. Fertil. 43:269-279.

Haresign, W. and Lamming, G. E. 1978. Comparison of LH release and luteal function in cyclic and LH-RH treated anoestrous ewes pretreated with PMSG or oestrogen. J. Reprod. Fertil. 52:349-353.

Harms, P. G., Niswender, G. D. and Malven, P. V. 1969. Progesterone and luteinizing hormone secretion during luteal inhibiton by exogenous oxytocin. Biol. Reprod. 1:228-233.

Harwood, J. P., Clayton, R. N. and Catt, K. J. 1980. Ovarian gonadotropin-releasing hormone receptors. I. Properties and inhibition of luteal cell function. Endocrinology 107:407-413.

Harwood, J. P., Clayton, R. N., Chen, T. T., Knox, G. and Catt, K. J. 1980. Ovarian gonadotropin-releasing hormone receptors. II. Regulation and effects on ovarian development. Endocrinology 107:414-421.

Helmer, S. D., Hansen, P. J., Anthony, R. V., Thatcher, W. W., Bazer, F. W. and Roberts, R. M. 1987. Identification of bovine trophoblast protein-1, a secretory protein immunologically related to ovine trophoblast protein-1. J. Reprod. Fertil. 79:83-91.

Hildebrandt, J. D., Sekura, R. D., Codina, J., Iyengar, R., Manclark, C. R. and Birnbaumer, L. 1983. Stimulation and inhibition of adenylyl cyclases mediated by distinct proteins. Nature 302: 706-709.

Hilliard, J. 1973. Corpus luteum function in guinea pigs, hamsters, rats, mice and rabbits. Biol. Reprod. 8:203-221.

Hirst, J. J., Rice, G. E., Jenkin, G. and Thornburn, G. D. 1988. Control of oxytocin secretion by ovine corpora lutea: Effects of arachidonic acid, phospholipases, and prostaglandins. Endocrinology 122:774-781.

Hixon, J. E., Gengenbach, D. R. and Hansel, W. 1975. Failure of prostaglandin $F_{2\alpha}$ to cause luteal regression in ewes after destruction of ovarian follicles by X-irradiation. Biol. Reprod. 13:126-135.

Hixon, J. E. and Hansel, W. 1974. Evidence for preferential transfer of prostaglandin $F_{2\alpha}$ to the ovarian artery following intrauterine administration in cattle. Biol. Reprod. 11: 543-552.

Hoffman, B., Schams, D., Bopp, R., Ender, M. L., Gimenez, T. and Karg, H. 1974. Luteotropic factors in the cow: Evidence for LH rather prolactin. J. Reprod. Fertil. 40:77-85.

Hopkins, C. R. and Walker, A. M. 1978. Calcium as a second messenger in the stimulation of luteinizing hormone secretion. Mol. Cell. Endocrinol. 12:189-208.

Hoyer, P. B., Fitz, T. A. and Niswender, G. D. 1984. Hormone independent activation of adenylate cyclase in large steroidogenic ovine luteal cells does not result in increased progesterone secretion. Endocrinology 114:604-608.

Hsueh, A. J. W., Dufau, M. L. and Catt, K. J. 1976. Regulation of luteinizing hormone receptors in testicular interstitial cells by gonadotropins. Biochem. Biophys. Res. Commun. 72:1145-1152.

Hsueh, A. J. W., Dufau, M. L. and Catt, K. J. 1977. Gonadotropin induced regulation of luteinizing hormone receptors and desensitization of testicular 3':5'-cyclic AMP and testosterone response. Proc. Natl. Acad. Sci. USA 74:592-595.

Hsueh, A. J. W. and Erickson, G. F. 1979. Extrapituitary action of gonadotropin-releasing hormone: Direct inhibition of ovarian steroidogenesis. Science 204:854-855.

Hsueh, A. J. W. and Jones, P. B. C. 1981. Extrapituitary actions of gonadotropin-releasing hormone. Endocrin. Rev. 2:437-460.

Huang, W. Y. and Pearlman, W. H. 1963. The corpus luteum and steroid hormone formation. J. Biol. Chem. 28:1308-1315.

Huckle, W. R. and Conn, P. M. 1987. The relationship between gonadotropin-releasing hormone-stimulated luteinizing hormone release and inositol phosphate production: Studies with calcium antagonists and protein kinase C activators. Endocrinology 120:160-169.

Huie, J. M., Magness, R. R., Reynolds, L. P., Hoyer, G., Hueckstead, T., Colcord, M., Stalcup,. B., Whysong, G. L. and Weems, C. W. 1981. Effect of chronic ipsilateral or contralateral infusion of prostaglandin E_1 (PGE_1) on luteal function of unilaterally ovariectomized ewes. Prostaglandins 21:945-955.

Humphrey, R. R., Dermody, W. C., Brink, H. O., Bousley, F. G., Schottin, N. H., Sakowski, R., Vaitkus, J. W., Veloso, H. T. and Reel, J. R. 1973. Induction of luteinizing hormone (LH) release and ovulation in rats, hamsters, and rabbits by synthetic luteinizing hormone releasing factor (LRF). Endocrinology 92:1515-1526.

Humphrey, R. R., Windsor, B. L., Bousley, F. G. and Edgren, R. A. 1976. Anti-fertility effects of an analog of luteinizing hormone releasing hormone (LHRH) in rats. Contraception 14:625-629.

Hunzicker-Dunn, M. 1981. Selective activation of rabbit ovarian protein kinase isozymes in rabbit ovarian follicles and corpora lutea. J. Biol. Chem. 256:12185-12193.

Hunzicker-Dunn, M. and Birnbaumer, L. 1976. Adenylyl cyclase activities in ovarian tissues. IV. Gonadotropin-induced desensitization of the luteal adenylyl cyclase throughout pregnancy and pseudopregnancy in the rabbit and rat Endocrinology 99:211-222.

Hunzicker-Dunn, M. and Birnbaumer, L. 1985. The involvement of adenylyl cyclase and cylic AMP-dependent protein kinases in luteinizing hormone actions. In Ascoli, M. (ed.), Luteinizing Hormone Action and Receptors, pp. 57-134, CRC Press, Boca Raton, Florida.

Hwang, J. and Menon, K. M. J. 1983. Characterization of low density and high density lipoprotein receptors in the rat corpus luteum and regulation by gonadotropin. J. Biol. Chem. 258: 8020-8027.

Imakawa, K., Anthony, R. V., Kazemi, M., Marotti, K. R., Polites, H. G. and Roberts, R. M. 1987. Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm. Nature 330:377-380.

Inskeep, E. K. and Butcher, R. L. 1966. Local component of utero-ovarian relationships in the ewe. J. Anim. Sci. 25:1164-1168.

Ireland, J. J., Aten, R. F. and Behrman, H. R. 1988a. GnRH-like proteins in cows: Concentrations during corpora lutea development and selective localization in granulosa cells. Biol. Reprod. 38:544-550.

Ireland, J. J., Milvae, R. A., Aten, R. F. and Behrman, H. R. 1988b. The bovine ovarian GnRH-like protein has potent, reversible antigonadotropic effects on bovine luteal cells. Abstracts of the 70th Annual Meeting of the Endocrine Society pg. 294.

Ivell, R., Brackett, K. H., Fields, M. J. and Richter, D. 1985. Ovulation triggers oxytocin gene expression in the bovine ovary. FEBS Lett. 190:263-267.

Jakobs, K. H., Lasch, P., Minuth, M., Aktories, K. and Schultz, G. 1982. Uncoupling of α-adrenorecepto-mediated inhibition of human platelet adenylate cyclase by N-ethylmaleimide. J. Biol. Chem. 257:2829-2833.

Jones, R. C. 1980. Local antifertility effect of luteinizing hormone releasing hormone (LRH) in the rat: Mechanism of action. Endocr. Res. Commun. 7:107-112.

Jones, D. S. C. and Flint, A. P. F. 1986. Oxytocin-neurophysin mRNA in the corpus luteum of the sheep during the oestrous cycle and in pregnancy. J. Endocrinol. (Suppl. 1):140.

Jones, P. B. C., Conn, P. M., Marian, J. and Hsueh, A. J. W. 1980. Binding of gonadotropin-releasing hormone agonist to rat ovarian granulosa cells. Life Sci. 27:2125-2132.

Jones, P. B. C. and Hsueh, A. J. W. 1980. Direct inhibitory effect of gonadotropin-releasing hormone upon luteal luteinizing hormone receptor and steroidogenesis in hypophysectomized rats. Endocrinology 107:1930-1936.

Jones, P. B. C. and Hsueh, A. J. W. 1981. Direct effects of gonadotropin releasing hormone and its antagonist upon ovarian functions stimulated by FSH, prolactin, and LH. Biol. Reprod. 24:747-759.

Kaltenbach, C. C., Cook, B., Niswender, G. D. and Nalbandov, A. V. 1967. Effect of pituitary hormones on progesterone synthesis by ovine luteal tissue *in vitro* . Endocrinology 81:1407-1409.

Kaltenbach, C. C., Graber, J. W., Niswender, G. D. and Nalbandov, A. V. 1968. Effect of hypophysectomy on the formation and maintenance of corpora lutea in the ewe. Endocrinology 82:753-759.

Kammerman, S. and Ross, J. 1975. Increase in numbers of gonadotropin receptors on granulosa cells during follicle maturation. J. Clin. Endocrinol. Metab. 41:546-550.

Karsch, F. J., Cook, B., Ellicott, A. R., Foster, D. L., Jackson, G. L. and Nalbandov, A. V. 1971. Failure of infused prolactin to prolong the lifespan of the corpus luteum of the ewe. Endocrinology 89:272-275.

Karsch, F. J., Cummings, J. T., Thomas, G. B. and Clarke, I. J. 1987. Steroid feedback inhibition of pulsatile secretion of gonadotropin-releasing hormone in the ewe. Biol. Reprod. 36:1207-1218.

Karsch, F. J., Foster, D. L., Legan, S. J., Ryan, K. D. and Peter, G. K. 1979. Control of the preovulatory endocrine events in the ewe: Interrelationship of estradiol progesterone, and luteinizing hormone. Endocrinology 105:421-426.

Karsch, F. J., Roche, J. F., Noveroske, J. W., Foster, D. L., Norton, H. W. and Nalbandov, A. V. 1971. Prolonged maintenance of the corpus luteum of the ewe by continuous infusion of luteinizing hormone. Biol. Reprod. 4:129-136.

Karten, M. J. and Rivier, J. E. 1986. Gonadotropin-releasing hormone analog design. Structure-function studies toward the development of agonists and antagonists: Rationale and perspective. Endocrin. Rev. 7:44-66.

Keller, D., Fetherston, J. and Boime, I. 1980. Isolation of mRNA from bovine pituitary: The cell free synthesis of the α and ß subunits of luteinizing hormone. Eur. J. Biochem. 108:367-372.

Kittok, R. J., Britt, J. H. and Convey, E. M. 1973. Endocrine response after GnRH in luteal phase cows and cows with follicular ovarian cysts. J. Anim. Sci. 37:985-989.

Kledzik, G. C., Cusan, L., Auclair, C., Kelly, P. A. and Labrie, F. 1978. Inhibition of ovarian luteinizing hormone (LH) and follicle stimulating hormone (FSH) receptor levels by treatment with a LH-releasing hormone analog during the estrous cycle of the rat. Fertil. Steril. 30:348-353.

Knobil, E. 1981. Patterns of hypophysiotropic signals and gonadotropin secretion in the rhesus monkey. Biol. Reprod. 24:44-49.

Koligian, K. B. and Stormshak, F. 1976. Progesterone synthesis by ovine fetal cotyledons in vitro. J. Anim. Sci. 42:439-443.

Koligian, K. B. and Stormshak, F. 1977. Progesterone inhibition of estrogen receptor replenishment in ovine endometrium. Biol. Reprod. 17:412-416.

Koos, R. D. and Hansel, W. 1981. The large and small cells of the bovine corpus luteum: Ultrastructural and functional differences. In Schwartz, N. B. and Hunzicker Dunn. M. (eds.), Dynamics of Ovarian Function, pp. 197-203, Raven Press, NewYork.

Korda, A. R., Shutt, D. A., Smith, I. D., Shearman, R. P. and Lyneham, R. C. 1975. Assessment of possible luteolytic effect of intra ovarian injection of prostaglandin $F_{2\alpha}$ in the human. Prostaglandins 9:443-449.

Krebs, E. G. and Beavo, J. A. 1979. Phosphorylation-dephosphorylation of enzymes. Ann. Rev. Biochem. 48:923-959.

Kusuda, S. and Dufau, M. L. 1986. Purification and characterization of the rat ovarian receptor for luteinizing hormone: Structural studies of subunit interaction. J. Biol. Chem. 261:16161-16166.

Lalloz, M. R. A., Detta, A. and Clayton, R. N. 1988a. Gonadotropin releasing hormone is required for enhanced luteinizing hormone subunit gene expression *in vivo*. Endocrinology 122:1681-1688.

Lalloz, M. R. A., Detta, A. and Clayton, R. N. 1988b. Gonadotropin-releasing hormone desensitization preferentially inhibits expression of the luteinizing hormone ß-subunit gene *in vivo*. Endocrinology 122:1689-1694.

Lamprecht, S. A., Zor, U., Salomon, Y., Koch, Y., Ahren, K. and Lindner, H. R. 1977. Mechanism of hormonally induced refractoriness of ovarian adenylate cyclase to luteinizing hormone and prostaglandin E_2. J. Cycl. Nucleo. Res. 3:69-83.

Landefeld, T., Kaynard, A. and Kepa, J. 1985a. Pituitary α-subunit messenger ribonucleic acid remains elevated during the latter stages of the preovulatory luteinizing hormone surge. Endocrinology 117:934-938.

Landefeld, T., Kepa, J. and Karsch, F. 1984. Estradiol feedback effects on the α–subunit mRNA in the sheep pituitary gland: Correlation with serum and pituitary luteinizing hormone concentrations. Proc. Natl. Acad. Sci. USA 81:1322-1326.

Landefeld, T., Maurer, R. and Kepa, J. 1985b. Luteinizing hormone ß-subunit mRNA amounts increase during the preovulatory surge of luteinizing hormone in the ewe: The highest levels are observed at the completion of the peak. DNA 4:249-254.

Lasley, B. L., Wang, C. F. and Yen, S. S. C. 1975. The effects of estrogen and progesterone on the functional capacity of the gonadotrophs. J. Clin. Endocrinol. Metab. 41:820-826.

Lee, C. Y., Coulam, C. B., Jiang, N. S. and Ryan, R. J. 1973. Receptors for human luteinizing hormone in human corpora luteal tissue. J. Clin. Endocrinol. Metab. 36:148-152.

Lee, C. Y. and Ryan, R. J. 1972. Luteinizing hormone receptors: Specific binding of human luteinizing hormone to homogenates of luteinized rat ovaries. Proc. Natl. Acad. Sci. USA 69:3520-3523.

Liao, T. H. and Pierce, J. G. 1971. The primary structure of bovine thyrotropin. II. The amino acid sequences of the reduced, S-carboxymethyl α and ß chains. J. Biol. Chem. 246:850-865.

Ling, W. Y. and Marsh, J. M. 1977. Reevaluation of the role of cyclic 3', 5'-monophosphate and protein kinase in the stimulation of steroidogenesis by luteinizing hormone in bovine corpus luteum slices. Endocrinology 100:1571-1578.

Lucy, M. C. and Stevenson, J. S. 1986. Gonadotropin-releasing hormone at estrus: Luteinizing hormone, estradiol, and progesterone during the periestrual and postinsemination periods in dairy cattle. Biol. Reprod. 35:300-311.

Mallory, D. S., Gust, C. M. and Daily, R. A. 1986. Effects of pituitary stalk transection and type of barrier on pituitary and luteal function during the estrous cycle of the ewe. Dom. Anim. Endocrinol. 3:253-259.

Marian, J. and Conn, P. M. 1979. Gonadotropin-releasing hormone stimulation of cultured pituitary cells requires calcium. Mol. Pharmacol. 16:196-201.

Marian, J., Cooper, R. L. and Conn, P. M. 1981. Regulation of the rat pituitary gonadotropin-releasing hormone receptor. Mol. Pharmacol. 19:399-405.

Marsh, J. M. 1975. The role of cAMP in gonadal steroidogenesis. Biol. Reprod. 14:30-53.

Martal, J., Camous, S., Febre, J., Charlier, M. and Heyman, Y. 1984. Specificity of embryonic signals maintaining corpus luteum in early pregnancy in ruminants. Proc. 10th Int. Congr. Anim. Reprod. Artif. Insem. 3:510.

Martin, J. E., Tyrey, L., Everett, J. W. and Fellows, R. E. 1974. Estrogen and progesterone modulation of the pituitary response to LRF in the cyclic rat. Endocrinology 95:1664-1673.

Mason, N. R., Marsh, J. M. and Savard, K. 1962. An action of gonadotropin *in vitro* . J. Biol. Chem. 237:1801-1806.

Massicotte, J., Borgus, J. P., LaChance, R. and Labrie, F. 1981. Inhibition of hCG-induced cyclic AMP accumulation and steroidogenesis in rat luteal cells by an LHRH agonist. J. Steroid Biochem. 14:239-242.

Matsuo, H., Baba, Y., Nair, R. M. G., Airmura, A. and Schally, A. V. 1971. Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Commun. 43:1334-1339.

McArdle, C. A., Huckle, W. R. and Conn, P. M. 1987. Phorbol esters reduce gonadotrope responsiveness to protein kinase C activators but not to Ca^{2+} mobilizing secretagogues: Does protein kinase C mediate gonadotropin-releasing hormone action? J. Biol. Chem. 262:5028-5035.

McCarthy, M. S. and Swanson, L. V. 1976. Serum LH concentration following castration, steroid hormone and gonadotropin releasing hormone treatment in the male bovine. J. Anim. Sci. 43:151-158.

McCracken, J. A. 1980. Hormone receptor control of prostaglandin $F_{2\alpha}$ secretion by the ovine uterus. In Samuelsson, B., Ramwell, P. W. and Paoletti, R. (eds.). Advances in Prostaglandin and Thromboxane Research, pp. 1329-1344, Raven Press, New York.

McCracken, J. A., Baird, D. T. and Goding, J. R. 1971. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Rec. Prog. Horm. Res. 27:537-582.

Melampy, R. M., Anderson, L. L. and Kragt, C. L. 1964. Uterus and life span of rat corpora lutea. Endocrinology 74:501-504.

Meyer, H. H. D., Mittermeier, T. and Schams, D. 1988. Dynamics of oxytocin, estrogen and progestin receptors in the bovine endometrium during the estrous cycle. Acta Endocrinol. 118: 96-104.

Milvae, R. A. and Hansel, W. 1985. Inhibition of bovine luteal function by indomethacin. J. Anim. Sci. 60:528-531.

Milvae, R. A., Murphy, B. D. and Hansel, W. 1984. Prolongation of the bovine estrous cycle with a gonadotropin releasing hormone analog. Biol. Reprod. 31:664-670.

Mitchell, M. D., Flint, A. P. F. and Turnbull, A. C. 1975. Stimulation by oxytocin of prostaglandin F levels in uterine venous effluent in pregnant and puerperal sheep. Prostaglandins 9:47-56.

Mock, E. J. and Niswender, G. D. 1983. Differences in the rates of internalization of ^{125}I-labeled human chorionic gonadotropin, luteinizing hormone, and epidermal growth factor by ovine luteal cells. Endocrinology 113:259-264.

Moor, R. M. and Rowson, L. E. A. 1966a. The corpus luteum of the sheep: Effect of the removal of embryos on luteal function. J. Endocrinol. 34:497-502.

Moor, R. M. and Rowson, L. E. A. 1966b. Local uterine mechanisms affecting luteal function in sheep. J. Reprod. Fertil. 11:307-310.

Moss, G. E., Crowder, M. E. and Nett, T. E. 1981. GnRH receptor interaction. VI. Effect of progesterone and estradiol on hypophyseal receptors for GnRH, and serum and hypophyseal concentrations of gonadotropins in ovariectomized ewes. Biol. Reprod. 25:938-944.

Nalbandov, A. V. 1970. Comparative aspects of corpus luteum function. Biol. Reprod. 2:7-13.

Nestor, Jr., J. J. 1984. Development of LHRH analogs. In Vickery, B. H., Nestor, Jr., J. J. and Hafez, E. (eds.), LHRH and Its Analogs, pp. 3-31, MTP Press, Lancaster, England.

Nett, T. M., Akbar, A. M. and Niswender, G. D. 1974. Serum levels of luteinizing hormone and gonadotropin-releasing hormone in cycling, castrated and anestrous ewes. Endocrinology 94:713-718.

Nett, T. M., Crowder, M. E., Moss, G. E. and Duello, T. M. 1981. GnRH receptor interaction. V. Down-regulation of pituitary receptors for GnRH in ovariectomized ewes by infusion of homologous hormone. Biol. Reprod. 24:1145-1155.

Nett, T. M., McClellan, M. C. and Niswender, G. D. 1976. Effects of prostaglandins on the ovine corpus luteum: Blood flow, secretion of progesterone and morphology. Biol. Reprod. 15:66-78.

Nimrod, A., Bedrak, E. and Lamprecht, S. A. 1977. Appearance of LH receptors and LH-stimulable cyclic AMP accumulation in granulosa cells during follicular maturation in the rat ovary. Biochem. Biophys. Res. Commun. 78:977-984.

Niswender, G. D. and Nett, T. M. 1988. The corpus luteum and its control. In Knobil, E. and Neill, J. D. (eds.), The Physiology of Reproduction, pp. 489-525, Raven Press, New York.

Niswender, G. D., Schwall, R. H., Fitz, T. A., Farin, C. E. and Sawyer, H. R. 1985. Regulation of luteal function in domestic ruminants: New Concepts. Rec. Prog. Horm. Res. 41:101-142.

Northey, D. L. and French, L. R. 1980. The effect of embryo removal and intrauterine infusion of embryonic homogenates on the lifespan of the bovine corpus luteum. J. Anim. Sci. 50:298-302.

Northup, J. K., Sternweis, P. C., Smigel, M. D., Schleifer, L. S., Ross, E. M. and Gilman, A. G. 1980. Purification of the regulatory component of adenylate cyclase. Proc. Natl. Acad. Sci. USA 77:6516-6520.

O'Shea, J. D., Cran, D. G. and Hay, M. F. 1979. The small luteal cell of the sheep. J. Anat. 128:239-251.

Payne, A. H., Quinn, P. G. and Stalvey, J. R. D. 1985. The stimulation of steroid biosynthesis by luteinizing hormone. In Ascoli, M. (ed.), Luteinizing Hormone Action and Receptors, pp. 135-172, CRC Press, Boca Raton, Florida.

Pelletier, J. and Thimonier, J. 1975. Interactions between ovarian steroids or progesterone and LH release. Ann. Biol. Anim. Biochem. Biophys. 15:131-146.

Pharriss, B. B., Cornette, J. C. and Gutknecht, G. D. 1970. Vascular control of luteal steroidogenesis. J. Reprod. Fertil. (Suppl. 10) pp.97-103.

Phariss, B. B. and Wyngarden. L. 1969. The effect of prostaglandin $F_{2\alpha}$ on the progestogen content of ovaries from pseudopregnant rats. Proc. Soc. Exp. Biol. Med. 130:92-94.

Pieper, D. R., Richards, J. S. and Marshall, J. C. 1981. Ovarian gonadotropin-releasing hormone (GnRH) receptors: Characterization, distribution, and induction by GnRH. Endocrinology 108:1148-1155.

Pierce, J. G. and Parsons, T. F. 1981. Glycoprotein hormones: Structure and function. Ann. Rev. Biochem. 50:465-495.

Plante, C., Hansen, P. J. and Thatcher, W. W. 1988. Prolongation of luteal lifespan in cows by intrauterine infusion of recombinant bovine alpha-interferon. Endocrinology 122:2342-2344.

Rahe, C. H., Owens, R. E., Fleeger, J. L., Newton, H. J. and Harms, P. G. 1980. Patterns of plasma luteinizing hormone in the cyclic cow: Dependence upon period of the cycle. Endocrinology 107:498-503.

Rao, C. V. 1974. Properties of gonadotropin receptors in the cell membranes of bovine corpus luteum. J. Biol. Chem. 249:2864-2872.

Redding, T. W., Kastin, A. J., Gonzalez-Barcena, D., Coy, D. H., Coy, E. J., Schalch, D. S. and Schally, A. V. 1973. The half-life, metabolism and excretion of tritiated luteinizing hormone releasing hormone (LHRH) in man. J. Clin. Endocrinol. Metab. 37:626-631.

Reeves, J. J., Arimura, A. and Schally, A. V. 1971a. Changes in pituitary responsiveness to luteinizing hormone releasing hormone (LH-RH) in anestrous ewes pretreated with estradiol benzoate. Biol. Reprod. 4:88-92.

Reeves, J. J., Arimura, A. and Schally, A. V. 1971b. Pituitary responsiveness to purified luteinizing hormone releasing hormone (LH-RH) at various stages of the estrous cycle in sheep. J. Anim. Sci. 32:123-126.

Reynolds, L. P., Stigler, J., Hoyer, G. L., Magness, R. R., Huie, J. M., Huecksteadt, T. P., Whysong, G. L., Behrman, H. R. and Weems, C. W. 1981. Effect of PGE_1 or PGE_2 on $PGF_{2\alpha}$ induced luteolysis in nonbred ewes. Prostaglandins 21:957-972.

Richards, J. S., Ireland, J. J., Rao, M. C., Bernath, G. A., Midgely, A. R. and Reichert Jr., L. E. 1976. Ovarian follicular development in the rat: Hormone receptor regulation by estradiol, follicle stimulating hormone and luteinizing hormone. Endocrinology 99:1562-1570.

Roberts, J. S., McCracken, J. A., Gavagan, J. E. and Soloff, M. S. 1976. Oxytocin-stimulated release of prostaglandin F_2 from ovine endometrium *in vitro* . Correlation with estrous cycle and oxytocin-receptor binding. Endocrinology 99:1107-1114.

Rodbell, M. 1980. The role of hormone receptors and GTP regulatory proteins in membrane transduction. Nature 284:17-22.

Rodger, L. D. and Stormshak, F. 1986. Gonadotropin-releasing hormone-induced alteration of bovine corpus luteum function. Biol. Reprod. 35:149-156.

Rodgers, R. J. and O'Shea, J. D. 1982. Purification, morphology, and progesterone production and content of three cell types isolated from the corpus luteum of the sheep. Aust. J. Biol. Sci. 35: 441-455.

Ryan, R. J., Keutman, H. T., Charlesworth, M. C., McCormick, D. J Milius, R. P., Calvo, F. O. and Vutyavanich, T. 1987. Structure function relationships of gonadotropins. Rec. Prog. Horm. Res. 43:383-429.

Savard, K. 1973. The biochemistry of the corpus luteum. Biol. Reprod. 8:183-202.

Schally, A. V., Arimura, A., Baba, Y., Nair, R. M. G., Matsuo, H., Redding, T. W., Debeljuk, L. and White, W. F. 1971. Isolation and properties of the FSH and LH-releasing hormone. Biochem. Biophys. Res. Commun. 43:393-399.

Schams, D., Lahlou-Kassi, A. and Glatzel, P. 1982. Oxytocin concentrations in peripheral blood during the oestrous cycle and after ovariectomy in two breeds of sheep with low and high fecundity. J. Endocrinol. 92:9-13.

Schomberg, D. W., Coudert, S. P. and Short, R. V. 1967. Effects of bovine luteinizing hormone and human chorionic gonadotrophin on the bovine corpus luteum *in vivo*. J. Reprod. Fertil. 14: 277-285.

Schrey, M. P. 1985. Gonadotropin releasing hormone stimulates the formation of inositol phosphates in the rat anterior pituitary tissue. Biochem. J. 226:563-569.

Schulster, D., Richardson, M. C. and Palfreyman, J. W. 1974. The role of protein synthesis in adrenocorticotropin action: Effects of cyclohexamide and puromycin on the steroidogenic response of isolated adrenocortical cells. Mol. Cell. Endocrinol. 2:17-29.

Schwall, R. H. and Erickson, G. F. 1984. Inhibition of synthesis of luteinizing hormone (LH) receptors by a down-regulating dose of LH. Endocrinology 114:1114-1123.

Seeburg. P. H. and Adelman, J. P. 1984. Characterization of cDNA for precusor of human luteinizing hormone releasing hormone. Nature 311:666-668.

Seeburg, P. H., Mason, A. J., Stewart, T. A. and Nikolics, K. 1987. The mammalian GnRH gene and its pivotal role in reproduction. Rec. Prog. Horm. Res. 43:69-98.

Seguin, B. E., Oxender, W. D. and Britt, J. H. 1977. Effect of human chorionic gonadotropin and gonadotropin-releasing hormone on corpus luteum function and estrous cycle duration in dairy heifers. Am. J. Vet. Res. 38:1153-1156.

Sharma, S. C. and Fitzpatrick, R. J. 1974. Effect of oestradiol-17ß and oxytocin treatment on prostaglandin F alpha release in the anestrous ewe. Prostaglandins 6:97-105.

Sharpe, R. M. 1977. Relationship between testosterone, fluid content and luteinizing hormone in the rat testis. Biochem. Biophys. Res. Commun. 75:711-717.

Sheldrick, E. L. and Flint, A. P. F. 1981. Circulating concentrations of oxytocin during the estrous cycle and early pregnancy in the sheep. Prostaglandins 22:631-636.

Shemesh, M. and Hansel, W. 1975a. Levels of prostaglandin F (PGF) in bovine endometrium, uterine venous, ovarian arterial and jugular plasma during the estrous cycle. Proc. Soc. Exp. Biol. Med. 148:123-126

Shemesh, M. and Hansel, W. 1975b. Arachidonic acid and bovine corpus luteum function. Proc. Soc. Exp. Biol. Med. 148:243-246.

Shemesh, M., F., Milaguir, F., Ayalon, N. and Hansel, W. 1979. Steroidogenesis and prostaglandin synthesis by cultured bovine blastocysts. J. Reprod. Fertil. 56:181-185.

Shupnik, M. A., Gharib, S. D. and Chin, W. W. 1988. Estrogen suppresses rat gonadotropin gene transcription *in vivo*. Endocrinology 122:1842-1845.

Silvia, W. J., Fitz, T. A., Mayan, M. H. and Niswender, G. D. 1984. Cellular and molecular mechanisms involved in luteolysis and maternal recognition of pregnancy in the ewe. Anim. Reprod. Sci. 7:57-74.

Silvia, W. J., Ottobre, J. S. and Inskeep, E. K. 1984. Concentrations of prostaglandin E_2, $F_{2\alpha}$ and 6-keto prostaglandin $F_{1\alpha}$ in the utero-ovarian venous plasma of nonpregnant and early pregnant ewes. Biol. Reprod. 30:936-944.

Simmons, K. R., Caffrey, J. L., Phillips, J. L., Abel Jr., J. H. and Niswender, G. D. 1976. A simple method for preparing suspensions of luteal cells. Proc. Soc. Exp. Biol. Med. 152: 366-371.

Simmons, K. R. and Hansel, W. 1964. Nature of the luteotropic hormone in the bovine. J. Anim. Sci. 23:136-141.

Slayden, O. D., Archibong, A. E. and Stormshak, F. 1987. Attenuation of luteal function in the ewe by exogenous GnRH. J. Anim. Sci. (Suppl. 1) 65:375.

Snook, R. B., Brunner, M. A., Saatman, R. R. and Hansel, W. 1969. The effect of antisera to bovine LH in hysterectomized and intact heifers. Biol. Reprod. 1:49-58.

Sotrel, G., Helvacioglu, A., Dowers, S., Scommegna, A. and Auletta, F. J. 1981. Mechanism of luteolysis: Effect of estradiol and $PGF_{2\alpha}$ on corpus luteum LH/hCG receptors and cyclic nucleotides in the rhesus monkey. Am. J. Obstet. Gynecol. 139:134-140.

Speroff, L. and Ramwell, P. W. 1970. Prostaglandin stimulation of *in vitro* progesterone synthesis. J. Clin. Endocrinol. Metab. 30:345-350.

Strauss, J. F., Tanaka, T., MacGregor, L. and Tureck, R. E. 1982. Regulation of cholesterol acquisition and utilization in the corpus luteum. Adv. Exp. Med. Biol. 147:303-308.

Streb, H., Irvine, R. F., Berridge, M. J. and Schulz, I. 1983. Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1, 4, 5-trisphosphate. Nature 306:67-69.

Strickland, T. W., Parsons, T. F. and Pierce, J. G. 1985. Structure of LH and hCG. In Ascoli, M. (ed.), Luteinizing Hormone Action and Receptors, pp. 1-16, CRC Press, Boca Raton, Florida.

Suter, D. E., Fletcher, P. W., Sluss, P. M., Reichert Jr., L. E. and Niswender, G. D. 1980. Alterations in the number of ovine luteal receptors for LH and progesterone secretion induced by homologous hormone. Biol. Reprod. 22:205-210.

Suter, D. E. and Niswender, G. D. 1983. Internalization and degradation of human chorionic gonadotropin in ovine luteal cells: Effects of inhibition of protein synthesis. Endocrinology 112:838-845.

Swanson, L. V. and McCarthy, S. K. 1986. Steroid hormone feedback on LH in prepubertal holstein heifers. Steroids 47:101-114.

Talmadge, K., Vamvakopoulos, N. C. and Fiddes, J. C. 1984. Evolution of the genes for the ß-subunits of human chorionic gonadotropin and luteinizing hormone. Nature 307:37-40.

Thorson, J. A., Marshall, J. C., Bill, C. L. and Keyes, P. L. 1985. D-Ala^{6}-des-Gly^{10}-gonadotropin-releasing hormone ethylamide: Absence of binding sites and lack of direct effect in rabbit corpora lutea. Biol. Reprod. 32:226-231.

Turgeon, J. L. and Waring, D. W. 1986. Modification of luteinizing hormone secretion by activators of Ca^{2+}/phospholipid-dependent protein kinase. Endocrinology 118:2053-2058.

Vane, J. R. and Williams, K. I. 1973. The contribution of prostaglandin production to contractions of isolated uterus of the rat. Brit. J. Pharmacol. 48:629-639.

Villa-Godoy, A., Ireland, J. J., Wortman, J. A., Ames, N. K. and Fogwell, R. L. 1981. Luteal function in heifers following destruction of ovarian follicles. J. Anim. Sci. (Suppl. 1) 53:372.

Wathes, D. C. and Swann, R. W. 1982. Is oxytocin an ovarian hormone? Nature 297:225-227.

Wilks, J. W. and Hansel, W. 1971. Oxytocin and the secretion of luteinizing hormone in cattle. J. Anim. Sci. 33:1048-1052.

Willingham, M. C. and Pastan, I. 1984. Endocytosis and membrane traffic in cultured cells. Rec. Prog. Horm. Res. 40:569-587.

Wiltbank, J. N. and Casida, L. E. 1956. Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15:134-140.

Wimalasena, J., Moore, P., Wiebe, J. P., Abel, J. J. and Chen, T. T. 1985. The porcine LH/hCG receptor. Characterization and purification. J. Biol. Chem. 260:10689-10697.