J. Reprod. Fert., Suppl. **30** (1981), 183–189 *Printed in Great Britain*

Factors regulating receptors for LH on ovine luteal cells

G. D. Niswender, D. E. Suter and H. R. Sawyer

Department of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado 80523, U.S.A.

Introduction

Biological regulation of the function and lifespan of the corpus luteum in ruminant animals appears to be due to the complex interaction of a number of factors including pituitary hormones, uterine hormones, and factors which originate from the embryo. There appears to be unanimous agreement that luteinizing hormone (LH) is an absolute requirement for normal luteal function (Short, 1964; Niswender, Menon & Jaffe, 1972; Hansel, Concannon & Lukaszewska, 1973; Nalbandov, 1973; Niswender, Sawyer, Chen & Endres, 1980). Numerous studies have also suggested that the luteolytic effect of the non-pregnant uterus in ruminants is mediated via secretion of prostaglandin (PG) F-2α (Goding, 1974; Horton & Poyser, 1977). However, the exact mechanisms whereby the luteolytic effects of the non-pregnant uterus and the luteotrophic effects of the pregnant uterus are exerted remain controversial (Ellinwood, Nett & Niswender, 1979).

The purpose of the present paper is to review existing data regarding the role of receptors for LH in the function of the corpus luteum in ruminants and to present new data which provide information regarding the cellular mechanisms involved in loss and renewal of receptors for LH.

Role of receptors for LH

In sheep, receptors for LH appear first in thecal cells of small follicles and as the follicle enlarges there is a slight decrease in the capacity of thecal cells to bind LH concomitant to a dramatic increase in binding capacity of LH to granulosa cells (Carson, Findlay, Burger & Trounson, 1979). Contrary to data from rat studies, there is no evidence that prolactin plays a role in the regulation of LH receptors in sheep in either the follicle or corpus luteum. As the ovine corpus luteum develops, the number of receptors for LH and the peripheral concentration of progesterone appear to be highly correlated (Diekman, O'Callaghan, Nett & Niswender, 1978a). A partial summary of the data of Diekman *et al.* (1978) for non-pregnant ewes is presented in Table 1. Both the total luteal content of receptors for LH and the proportion of total receptors occupied by endogenous LH were highly correlated ($P < 0{\cdot}01$) with serum values of progesterone. The number of luteal granulosa cells does not increase after Day 2 of the oestrous cycle (McClellan, Diekman, Abel & Niswender, 1975) so there is a 40-fold increase in receptor numbers per luteal cell between Days 4 and 14 of the oestrous cycle. During this same period there was a 6-fold increase in the proportion of receptors occupied by endogenous LH which represented an estimated increase of 3×10^{10} molecules of LH. During the late luteal phase of the cycle the number of LH receptors and serum levels of progesterone declined dramatically (Table 1). In a second experiment (Diekman *et al.*, 1978b), designed to determine whether a loss in the number of LH receptors preceded the decreased levels of progesterone in serum, luteolysis was induced with PGF-2α on Day 9 of the cycle. Serum concentrations of progesterone had

0449-3087/81/020183-07$02.00/0

declined significantly within 7·5 h of the PGF-2α injection while the number of receptors for LH did not decrease until 22·5 h (Table 2). These data suggest that PGF-2α does not exert its luteolytic action via a direct alteration in the number of receptors for LH. However, the number of receptors does decrease as a result of luteolysis.

Table 1. Serum levels of progesterone, weight of corpora lutea (CL) and number of occupied and unoccupied receptors for LH throughout the oestrous cycle in ewes (from Diekman *et al.*, 1978a)

Day of cycle	Serum progesterone (ng/ml)	CL weight (mg)	Unoccupied LH receptors (pmol/CL)	Occupied LH receptors (fmol/CL)
2	$0·35 \pm 0·11^{a}$	100 ± 17^{a}	$0·3 \pm 0·1^{a}$	11 ± 2
4	$0·90 \pm 0·13^{a}$	246 ± 28^{b}	$0·5 \pm 0·1^{a}$	14 ± 2
6	$2·27 \pm 0·28^{b}$	$344 \pm 21^{b,c}$	$2·3 \pm 0·2^{b}$	16 ± 2
10	$3·04 \pm 0·20^{c}$	546 ± 41^{e}	$11·2 \pm 1·5^{c}$	68 ± 5
12	$3·28 \pm 0·28^{c}$	$512 \pm 40^{d,e}$	$6·7 \pm 1·7^{c}$	63 ± 1
14	$3·38 \pm 0·35^{c}$	609 ± 36^{e}	$12·2 \pm 3·0^{c}$	66 ± 5
16	$0·49 \pm 0·17^{a}$	$420 \pm 20^{c,d}$	$3·6 \pm 1·2^{b}$	16 ± 4

Values are mean ± s.e.m. for 6 observations/day.
Within columns, values with different superscripts are different ($P < 0·05$).

Table 2. Serum levels of progesterone, weight of corpora lutea (CL) and number of occupied and unoccupied receptors for LH after PGF-2α administration on Day 9 of the oestrous cycle (from Diekman *et al.*, 1978b)

Hours after PGF-2α	% Pretreatment serum progesterone	CL weight (mg)	Unoccupied LH receptors (pmol/CL)	Occupied LH receptors (fmol/CL)
Control	100 ± 5^{a}	546 ± 41^{a}	$11·2 \pm 1·5^{a}$	65 ± 5^{a}
2·5	90 ± 8^{a}	460 ± 30^{a}	$9·9 \pm 1·2^{a}$	68 ± 4^{a}
7·5	37 ± 8^{b}	453 ± 35^{a}	$6·3 \pm 1·4^{a}$	46 ± 4^{a}
22·5	13 ± 3^{b}	288 ± 22^{b}	$1·0 \pm 0·2^{b}$	9 ± 1^{b}

Values are mean ± s.e.m. for 6 observations/day.
Within columns, values with different superscripts are different ($P < 0·05$).

A final observation of Diekman *et al.* (1978a) was that neither the total number of receptors for LH nor the proportion occupied by LH changed during early pregnancy. Thus, it does not appear that alterations in the number of LH receptors or the extent to which they are occupied by endogenous LH is the signal for maintenance of the corpus luteum of pregnancy. However, it seems likely that a concentration of receptors of LH similar to that observed during the mid-luteal phase of the cycle is required in conjunction with the luteotrophic effects of the embryo for normal luteal function during pregnancy.

Turnover of receptors for LH

The results of the studies described above suggest that the number of luteal receptors for LH varies with different reproductive states and that the concentration of receptors is highly correlated with the functional state of the corpus luteum. However, the cellular mechanisms whereby the numbers of receptors are regulated remain unclear. A variety of factors appear to influence the concentrations of LH receptors in the gonads of rats (Richards & Midgley, 1976; Zipf, Payne & Kelch, 1978) but the primary factor appears to be LH itself. Exposure to high

concentrations of LH or human chorionic gonadotrophin (hCG) results in a dramatic loss of receptors for LH in follicular (Richards *et al.*, 1976; Rao, Richards, Midgley & Reichert, 1977), luteal (Conti, Harwood, Hsueh, Dufau & Catt, 1976; Conti, Harwood, Dufau & Catt, 1977a, b; Harwood, Conti, Conn, Dufau & Catt, 1978; Dufau & Catt, 1978) or testicular (Hsueh, Dufau & Catt, 1976, 1977; Tsuruhara, Dufau, Cigorra & Catt, 1977; Purvis, Torjesin, Haug & Hansson, 1977) tissue. However, similar studies for ruminants have not yet been published.

We have conducted two experiments to study the effects of alterations in serum concentrations of LH on the numbers of receptors for this hormone in ovine luteal tissue. In the first experiment, 25 ml equivalents of an ammonium sulphate precipitated gamma-globulin fraction of anti-ovine LH serum or normal rabbit serum characterized by Reimers & Niswender (1975) were injected into ewes on Day 9 of the oestrous cycle. Within 12 h there was a significant decrease in both the total number of luteal receptors for LH and the number occupied by hormone (Table 3). Serum concentrations of progesterone had declined ($P < 0.05$) within 6 h. These data suggest that normal circulating levels of LH are required for maintenance of the normal complement of LH receptors in ovine luteal tissue. However, they provide little insight into the mechanisms involved in the regulation of receptors for LH at the cellular level.

Table 3. Serum levels of progesterone, weight of corpora lutea (CL) and number of occupied and unoccupied receptors for LH after administration of antiserum to LH (from Diekman, 1978)

Hours after anti-LH serum	% Pretreatment serum progesterone	CL weight (mg)	Unoccupied LH receptors (pmol/CL)	Occupied LH receptors (fmol/CL)
NRS*	100 ± 2[a]	487 ± 57	6·4 ± 0·7	64 ± 4
6	62 ± 10[b]	452 ± 32	6·8 ± 0·8	65 ± 5
12	54 ± 3[b]	384 ± 8	2·9 ± 0·4	42 ± 4
18	35 ± 2	400 ± 40	1·4 ± 0·3	15 ± 1

Values are mean ± s.e.m. for 6 observations/day.

* Control ewes were treated with the ammonium sulphate-precipitate fraction equal to 25 ml normal rabbit serum 18 h before collection of luteal tissue.

Within columns, values with different superscripts are different ($P < 0.05$).

In a second experiment, serum levels of LH were elevated to study the influence of supraphysiological levels of LH on luteal receptors for this hormone (Suter, Fletcher, Sluss, Reichert & Niswender, 1980). Ewes on Day 10 of the oestrous cycle were injected i.v. with 1 mg ovine LH (LER-1929, approximately 1·0 NIH-LH-S1 units/mg) and samples of jugular-venous blood and corpora lutea were collected at frequent intervals up to 72 h after injection. Serum concentrations of LH were increased 1000-fold within 10 min of the LH injection but had declined by 90% within 2 h and reached preinjection levels within 24 h. The number of receptors occupied by LH increased dramatically ($P < 0.05$) within 10 min but had returned to basal levels within 6 h. A partial summary of these data is presented in Table 4. The total number of receptors (occupied plus unoccupied) had increased ($P < 0.05$) within 10 min, returned to preinjection levels within 2 h and had decreased ($P < 0.05$) by 66% within 12 h of the LH injection. Total LH receptors had returned to preinjection levels within 48 h. Serum concentrations of progesterone were maximal at 10 min, remained elevated for 2 h but had returned to basal levels within 6 h. It was concluded from these results that high concentrations of LH in the blood of sheep are followed by decreased numbers (down-regulation) of luteal receptors for LH, as observed for rats. However, a major difference between the data from ewes and those in rats was the close agreement between the number of receptors occupied by LH at 10 min (5·4 pmol/corpus luteum) and the number lost at 24 h (6·8 pmol/corpus luteum) after injection of LH. Conti *et al.* (1977b) reported that occupancy of as few as 1% of luteal receptors

for LH resulted in 50% loss of receptor sites by 24 h. However, these investigators did not study occupied receptors until 6 h after treatment of the rats with hCG, a time when occupied receptors had already returned to basal levels in the present study.

Table 4. Serum levels of progesterone, weight of corpora lutea (CL) and number of occupied and unoccupied receptors for LH after intravenous injection of 1 mg ovine LH (from Suter *et al.*, 1980)

Time after LH (h)	Serum progesterone (ng/ml)	Unoccupied LH receptors (pmol/CL)	Occupied LH receptors (pmol/CL)	Total LH receptors (pmol/CL)
0	2·61	2·6 ± 0·5	0·4 ± 0·7	3·0 ± 0·9
0·17 (10 min)	4·12*	2·5 ± 0·5	5·4 ± 0·7†	7·9 ± 1·0†
2	3·57*	1·5 ± 0·5	1·5 ± 0·7	3·0 ± 1·0
6	3·51	1·5 ± 0·5	0·5 ± 0·7	2·1 ± 0·9
12	3·20	0·9 ± 0·5†	1·0 ± 0·7	1·9 ± 0·9
24	3·73	1·0 ± 0·5†	0·2 ± 0·7	1·2 ± 1·0†
48	3·81	3·3 ± 0·5	0·4 ± 0·7	3·7 ± 1·0
72	5·27	3·3 ± 0·5	0·5 ± 0·7	3·8 ± 1·0

Values are mean ± s.e.m. for 6 observations at each time.
* Significantly different from values in 4 saline-injected control ewes, $P < 0·05$.
† Significantly different from value at time 0, $P < 0·05$.

Several mechanisms could account for the loss of luteal receptors for LH following exposure to a desensitizing dose of LH. The most likely appears to be that the LH–receptor complexes are internalized (Chen, Abel, McClellan, Sawyer & Niswender, 1977; Conn, Conti, Harwood, Dufau & Catt, 1978; Anderson, Kong, Perotti, Bramley & Ryan, 1979; Amsterdam, Kohen, Nimrod & Lindner, 1979) and degraded in lysosomes (Abel *et al.*, 1978; Ascoli, 1978; Ascoli & Puett, 1978; Conn *et al.*, 1978; Niswender *et al.*, 1980). Internalization of the hormone–receptor complex appears to be a degradatory process rather than a mechanism for action of the hormone because lysosomal inhibitors block degradation of the hormone–receptor complex by target cells but do not reduce steroid secretion (Ascoli & Puett, 1978).

Release of protein-containing secretory granules from ovine luteal cells is stimulated by LH and closely parallels release of progesterone (Gemmell, Stacy & Thorburn, 1974; Gemmell & Stacy, 1977; Sawyer, Abel, McClellan, Schmitz & Niswender, 1979). The limiting membrane of these granules is incorporated into the plasma membrane of the luteal cell during exocytosis. Therefore, if the membrane of the secretory granule contained receptors for LH, this mechanism could result in the increased number of receptors seen at 10 min when serum levels of progesterone were highest. Another possible explanation for the increased number of receptors observed 10 min after LH injection is that it was due to technical error. The total number of receptors was obtained by addition of the number of occupied plus unoccupied receptors, each of which was measured by different methods. Although the methods used for these studies were validated very carefully (Diekman *et al.*, 1978a) there were a number of assumptions made which could not be tested directly. Therefore, a final experiment was designed using isoproterenol to enhance progesterone secretion (Jordan, Caffrey & Niswender, 1978) so that LH receptors could be measured using a single method (Scatchard analysis). On Day 10 of the cycle 4 ewes were selected and each had three corpora lutea in one ovary. One corpus luteum was removed 2 min before injection of 100 μg isoproternol-D-bitartrate (Sigma Chemical Co., St Louis, Missouri) into the ovarian artery over a 2-min interval. The two remaining corpora lutea were removed at 15 and 30 min after the end of the injection and LH receptor numbers were determined by Scatchard analyses (Diekman *et al.*, 1978a). Since serum levels of progesterone would not be meaningful in samples collected from ewes which had corpora lutea removed at different times, 4 additional ewes with a single corpus luteum were injected with 100 μg

isoproterenol under similar conditions and jugular blood samples were collected at 15-min intervals for 90 min before and 1 h after the isoproterenol injection. Serum concentrations of progesterone were increased by 149 and 143% ($P < 0.01$) over preinjection levels at 15 and 30 min respectively after the isoproterenol injection, but had returned to preinjection levels at 45 and 60 min (Table 5). Numbers of LH receptors increased from 7·4 to 8·2 pmol/corpus luteum at 10 min and were 11·1 pmol/corpus luteum ($P < 0.01$) at 30 min. This increase in the number of LH receptors is similar to that occurring after injection of LH. These data indicate that the increased number of receptors for LH seen at 10 and 30 min is real and that the increase is closely associated with increased progesterone secretion. Careful morphological evaluation of the tissues for granule release will be necessary before it can be concluded that the increased receptor numbers are associated with granule release.

Table 5. Effect of isoproterenol (100 μg) on serum levels of progesterone and luteal receptors for LH

Time (min)	Serum progesterone (% pretreatment)	LH receptors† (pmol/CL)
Preinjection	100	7·45
10		8·15
15	149*	
30	143*	11·1**
45	117	
60	94	

† Determined by Scatchard analysis.
Significantly different from preinjection value: *$P < 0.05$; **$P < 0.01$.

Conclusions

Based on the preceding and other data we propose the following model for the effects of LH on its receptor and progesterone secretion. First, LH binds to its plasma membrane receptor and initiates a biological response. This response appears to involve the activation of adenylate cyclase, production of cyclic AMP (Marsh, 1975), activation of protein kinase (Ling & Marsh, 1977; Dufau & Catt, 1978), phosphorylation of steroidogenic enzymes (Caron, Goldstein, Savard & Marsh, 1975; Caffrey, Fletcher, Diekman, O'Callaghan & Niswender, 1979), phosphorylation of ribosomes (Azhar & Menon, 1975) and enhanced protein synthesis. All of these actions appear to be involved in modulation of the steroidogenic response of the luteal cell to LH. After initial binding to a receptor a portion of all of the LH bound to the receptor is internalized via endocytosis and degraded in lysosomes. As a result of the stimulation by LH, progesterone secretion and release of protein containing secretory granules is enhanced. We suggest that the limiting membrane of these secretory granules contains new or recycled LH receptors and, therefore, incorporation of the limiting membrane with their LH receptors into the plasma membrane of the luteal cell via exocytosis completes the cycle and results in maintenance of a full complement of luteal receptors for LH.

This research was supported by a grant from the National Institutes of Health, No. HD11590.

References

Abel, J.H., Jr, Chen, T.T., Endres, D.B., McClellan, M.C., Diekman, M.A., Sawyer, H.R., Niswender, G.D., Schmitz, M.H.J. & Pineda, M.P. (1978) Sites of binding and metabolism of gonadotropic hormones in the mammalian ovary. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach*, pp. 183–202. Eds R. W. Straub & L. Bolis. Raven Press, New York.

Amsterdam, S., Kohen, F., Nimrod, A. & Lindner, H.R. (1979) Lateral mobility and internalization of hormone receptor to human chorionic gonadotropin in cultured rat granulosa cells. In *Ovarian Follicular and Corpus Luteum Function*, pp. 69–76. Eds C. P. Channing, J. Marsh & W. A. Sadler. Plenum Press, New York.

Anderson, W., Kong, Y.H., Perotti, M.E., Bramley, T.A. & Ryan, R.J. (1979) Interactions of gonadotropins with corpus luteum membranes. III. Electron microscopic localization of [^{125}I]hCG binding to sensitive and desensitized ovaries seven days after PMSG-hCG. *Biol. Reprod.* **20**, 362–376.

Ascoli, M. (1978) Demonstration of a direct effect of inhibitors of the degradation of receptor-bound human choriogonadotropin on the steroidogenic pathway. *J. biol. Chem.* **253**, 7839–7843.

Ascoli, M. & Puett, D. (1978) Inhibition of the degradation of receptor-bound human choriogonadotropin by lysosomotropic agents, protease inhibitors, and metabolic inhibitors. *J. biol. Chem.* **253**, 7832–7838.

Azhar, S. & Menon, K.M.J. (1975) Adenosine 3′, 5′-monophosphate dependent phosphorylation of ribosomes and ribosomal subunits from bovine corpus luteum. *Biochim. Biophys. Acta* **392**, 64–74.

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

Caron, M.G., Goldstein, S., Savard, K. & 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., Burger, H.G. & Trounson, A.O. (1979) Gonadotrophin receptors of the ovine follicle during follicular growth and atresia. *Biol. Reprod.* **21**, 75–87.

Chen, T.T., Abel, J.H., Jr, McClellan, M.C., Sawyer, H.R. & Niswender, G.D. (1977) Localization of gonadotropic hormones in lysosomes of ovine luteal cells. *Cytobiologie* **14**, 412–420.

Conn, P.M., Conti, M., Harwood, J.P., Dufau, M.L. & Catt, K.J. (1978) Internalization of gonadotrophin-receptor complex in ovarian luteal cells. *Nature, Lond.* **274**, 598–600.

Conti, M., Harwood, J.P., Hsueh, A.J.W., Dufau, M.L. & Catt, K.J. (1976) Gonadotrophin-induced loss of hormone receptors and desensitization of adenylate cyclase in the ovary. *J. biol. Chem.* **251**, 7729–7731.

Conti, M., Harwood, J.P., Dufau, M.L. & Catt, K.J. (1977a) Effect of gonadotrophin induced receptor regulation on biological responses of isolated rat luteal cells. *J. biol. Chem.* **252**, 8869–8874.

Conti, M., Harwood, J.P., Dufau, M.L. & Catt, K.J. (1977b) Regulation of luteinizing hormone receptors and adenylate cyclase activity by gonadotrophin in the rat ovary. *Mol. Pharmacol.* **13**, 1024–1032.

Diekman, M.A. (1978) *Quantification of receptors for luteinizing hormone in the ovine corpus luteum.* Ph.D. thesis, Colorado State University, Fort Collins.

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

Diekman, M.A., O'Callaghan, P.L., Nett, T.M. & Niswender, G.D. (1978b) Effect of prostaglandin F2α on the number of LH receptors in ovine corpora lutea. *Biol. Reprod.* **19**, 1010–1013.

Dufau, M.L. & Catt, K.J. (1978) Gonadotropin receptors and regulation of steroidogenesis in the testis and ovary. *Vitams Horm.* **36**, 462–592.

Ellinwood, W.E., Nett, T.M. & Niswender, G.D. (1979) Maintenance of the corpus luteum of early pregnancy in the ewe. II. Prostaglandin secretion by the endometrium *in vitro* and *in vivo*. *Biol. Reprod.* **21**, 845–856.

Gemmell, R.T. & Stacy, B.D. (1977) Effects of colchicine on the ovine corpus luteum: role of microtubules in the secretion of progesterone. *J. Reprod. Fert.* **49**, 115–117.

Gemmell, R.T., Stacy, B.D. & Thorburn, G.D. (1974) Ultrastructural study of secretory granules in the corpus luteum of the sheep during the estrous cycle. *Biol. Reprod.* **11**, 447–462.

Goding, J.R. (1974) The demonstration that $PGF_{2\alpha}$ is the uterine luteolysin in the ewe. *J. Reprod. Fert.* **38**, 261–271.

Hansel, W., Concannon, P.W. & Lukaszewska, J.H. (1973) Corpora lutea of the large domestic animal. *Biol. Reprod.* **8**, 222–245.

Harwood, J.P., Conti, M., Conn, P.M., Dufau, M.L. & Catt, K.J. (1978) Receptor regulation and target cell response: studies in the ovarian luteal cell. *Mol. cell. Endocr.* **11**, 121–135.

Horton, E.W. & Poyser, N.L. (1977) Uterine luteolytic hormone: a physiological role for prostaglandin $F_2\alpha$. *Physiol. Rev.* **56**, 595–651.

Hsueh, A.J.W., Dufau, M.L. & Catt, K.J. (1976) Regulation of luteinizing hormone receptors in testicular interstitial cells by gonadotrophin. *Biochem. Biophys. Res. Commun.* **72**, 1145–1152.

Hsueh, A.J.W., Dufau, M.L. & Catt, K.J. (1977) Gonadotrophin-induced regulation of luteinizing hormone receptors and desensitization of testicular 3′:5′-cyclic AMP and testosterone response. *Proc. natn. Acad. Sci. U.S.A.* **74**, 592–595.

Jordan, A.W.J., III, Caffrey, J.L. & Niswender, G.D. (1978) Catecholamine-induced stimulation of progesterone and adenosine 3′,5′-monophosphate production by dispersed ovine luteal cells. *Endocrinology* **103**, 385–392.

Ling, W.Y. & Marsh, J.M. (1977) Re-evaluation of the role of cyclic adenosine 3′-5′-monophosphate and protein kinase in stimulation of steroidogenesis by luteinizing hormone in bovine corpus luteum slices. *Endocrinology* **100**, 1571–1578.

Marsh, J.M. (1975) The role of cyclic AMP in gonadal function. In *Advances in Cyclic Nucleotide Research*, pp. 137–199. Eds P. Greengard & G. A. Robison. Raven Press, New York.

McClellan, M.C., Diekman, M.A., Abel, J.H., Jr & Niswender, G.D. (1975) Luteinizing hormone, progesterone and the morphological development of normal and superovulated corpora lutea in sheep. *Cell Tiss. Res.* **164**, 291–307.

Nalbandov, A.V. (1973) Control of luteal function in mammals. In *Handbook of Physiology. Endo-*

crinology, Vol. II, pp. 153–167. Ed. R. O. Greep. Am. Physiol. Soc., Washington, D.C.

Niswender, G.D., Menon, K.M.J. & Jaffe, R.B. (1972) Regulation of the corpus luteum during the menstrual cycle and early pregnancy. *Fert. Steril.* **23**, 432–442.

Niswender, G.D., Sawyer, H.R., Chen, T.T. & Endres, D.B. (1980) Action of luteinizing hormone at the luteal cell level. In *Advances in Sex Hormone Research*, Vol. 4, pp. 153–185. Eds J. A. Thomas & R. L. Singhal. Urban & Schwarzenberg, Baltimore.

Purvis, K., Torjesin, P.A., Haug, E. & Hansson, V. (1977) hCG suppression of LH receptors and responsiveness of testicular tissue to hCG. *Mol. cell. Endocr.* **8**, 73–80.

Rao, M.C., Richards, J.S., Midgley, A.R., Jr & Reichert, L.E. (1977) Regulation of gonadotrophin receptors by LH in granulosa cells. *Endocrinology* **101**, 512–523.

Reimers, T.J. & Niswender, G.D. (1975) Effects of specific neutralization and luteinizing hormone and prolactin on ovarian blood flow and progesterone secretion. In *Immunization with Hormones in Reproductive Research*, pp. 94–105. Ed. E. Nieschlag. North Holland Publishing Co., Amsterdam.

Richards, J.S. & Midgley, A.R., Jr (1976) Protein hormone action: a key to understanding ovarian follicular and luteal development. *Biol. Reprod.* **14**, 82–94.

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

Sawyer, H.R., Abel, J.H., Jr, McClellan, M.C., Schmitz, M. & Niswender, G.D. (1979) Secretory granules and progesterone secretion by ovine corpora lutea *in vitro*. *Endocrinology* **104**, 476–486.

Short, R.V. (1964) Ovarian steroid synthesis and secretion *in vivo*. *Recent Prog. Horm. Res.* **20**, 303–339.

Suter, D.E., Fletcher, P.W., Sluss, P.M., Reichert, L.E., Jr & 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.

Tsuruhara, T., Dufau, M.L., Cigorra, S. & Catt, K.J. (1977) Hormone regulation of testicular luteinizing hormone receptors. *J. biol. Chem.* **252**, 9002–9009.

Zipf, W.B., Payne, A.H. & Kelch, R.P. (1978) Prolactin, growth hormone, and luteinizing hormone in the maintenance of testicular luteinizing hormone receptors. *Endocrinology* **103**, 595–600.