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Ovarian Follicular Development during the Rat Estrous Cycle: Gonadotropin Receptors and Follicular Responsiveness[1]

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ABSTRACT

The LH and FSH receptor content in thecal and granulosa cells of large antral follicles was measured by *in vitro* binding assays and *in vivo* autoradiography during the rat estrous cycle. Specific binding of [^{125}I]-hCG to granulosa cells increased from <1000 cpm/μg DNA on estrus, metestrus and diestrus to 6000 CPM/μg DNA on proestrus. Specific binding of [^{125}I]-hFSH to granulosa cells was consistently between 2000 and 3000 cpm/μg DNA throughout the cycle. Specific hCG binding to thecal cells also increased between diestrus and proestrus, while specific hFSH binding to thecal cells was essentially nondetectable.

The increase in LH receptors in large antral follicles from diestrus to proestrus could be correlated with an increased ability of these follicles to produce cyclic AMP and estradiol during *in vitro* incubations with increasing concentrations (0.06–60 μg/ml) of oLH. Incubation of antral follicles collected on metestrus, diestrus and proestrus with and without testosterone revealed a progressive increase in both follicular aromatase activity and endogenous androgen production.

It is concluded that in the presence of low and unchanging LH and FSH concentrations from metestrus to proestrus large antral follicles become more responsive to LH. This responsiveness is associated with an increase in LH receptors both in thecal and granulosa cells and increased production of estradiol. Although the hormonal stimulus for the increase in granulosa cell LH receptor on proestrus is not known, it is suggested that increased follicular estradiol might play a pivotal role.

INTRODUCTION

The growth of preovulatory follicles during each estrous cycle in the rat appears to require the periovulatory surge of gonadotropins at the end of the previous cycle (Welschen, 1973; Schwartz, 1974). Antisera against follicle stimulating hormone (FSH) injected into rats on proestrus (Welschen and Dullaart, 1976) or administration of pentobarbital (Hirschfield and Midgley, 1978b) prevented the growth of a new group of antral follicles normally seen on estrus. These data have suggested that preovulatory follicles are selected from a pool of growing follicles most of which become atretic. However, once "selected" the continued growth and differentiation of preovulatory follicles occurring from estrus to proestrus proceeds in the presence of low unchanging concentrations of gonadotropins (Gay et al., 1970; Butcher et al., 1974; Smith et al., 1975). Thus, final differentiation might depend on a change in response of follicular cells to the gonadotropins.

Granulosa cells from small and large antral follicles bind specifically radioiodinated FSH to the same extent, as demonstrated by autoradiography (Midgley, 1973; Zeleznik et al., 1974) and by *in vitro* specific binding studies (Nimrod et al., 1976) and contain FSH-responsive adenylate cyclase which is not different in preantral and antral follicles (Zeleznik et al., 1977). Specific luteinizing hormone (LH) binding has been demonstrated in thecal cells from preantral and antral follicles (Midgley, 1973), in thecal and granulosa cells from large preovulatory follicles derived from pigs (Channing and Kammerman, 1973; Lee, 1976) and from hormonally primed rats (Zeleznik et al., 1974; Richards et al., 1976). Further, the increase in LH receptor in granulosa cells from small to large antral follicles has been shown to be associated with an increased ability of large

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follicles to produce cyclic AMP and progesterone in response to the gonadotropins (Lee, 1976; Zeleznik et al., 1977; Richards et al., 1978, 1979; Hillier et al., 1978). The increase in LH receptor has been shown to be dependent on the combined effects of estradiol and FSH (Richards et al., 1976).

It was the aim of the present study to determine the LH and FSH receptor content in granulosa and thecal cells of large antral follicles during the rat estrous cycle and to correlate possible changes in receptor content with the ability of follicles to produce cyclic AMP and estradiol in response to LH stimulation.

MATERIALS AND METHODS

Animals

Adult female rats, 3–5 months of age, were obtained from Holtzman Company, Madison, WI. Although these animals show 4- or 5-day estrous cycles as assessed by vaginal smearing, only those exhibiting consistent 5-day estrous cycles were used in this study.

In vitro hCG and hFSH Binding

Animals were killed on various days of the estrous cycle: at 1400 h on estrus, metestrus and diestrus and at 0900, 1400 and 1900 h on proestrus. The ovaries were dissected out and the 10–15 largest follicles were isolated with fine forceps under a dissecting microscope.

By puncturing the follicles and applying gentle pressure, we released granulosa cells leaving a thecal shell. Granulosa cells were transferred by a Pasteur pipette to a separate tube and collected by centrifugation at 800 × g at 4°C for 10 min. The thecal shell was homogenized in 0.01 M phosphate buffered 0.14 M NaCl, pH 7.0, containing 0.1% gelatin (PBS-gel) and a membrane fraction was obtained by centrifugation at 30,000 × g at 4°C for 10 min. Granulosa cells and thecal membranes were resuspended in 1 ml PBS-gel. LH and FSH receptors were determined in these preparations by measuring specific [^{125}I]-hCG and [^{125}I]-hFSH binding as described elsewhere (Richards et al., 1976). Data have been expressed as cpm radioiodinated hormone bound/μg DNA. DNA was measured by the method described by Burton (1956).

In vivo Autoradiography

At 1400 h on various days of the estrous cycle, animals were injected i.v. (tail vein) with 0.2 ml PBS containing 0.2 μg [^{125}I]-hCG or 0.04 μg [^{125}I]-hFSH (specific activity 13 μCi/μg and 23 μCi/μg, respectively). Two h later, the animals were killed. The ovaries were dissected out, fixed in Bouin's fluid and embedded in paraffin. Tissue sections (8 μm) were heavily stained with hematoxylin and eosin, dipped in Kodak NTB-2 emulsion (Eastman Kodak, Rochester, NY), stored at -20°C for 2–4 weeks and developed.

In vitro Incubation Studies

Individual whole follicles isolated on metestrus, diestrus I and proestrus were incubated in 500 μl Medium-199 (GIBCO) containing 10 mM HEPES (N-2 hydroxyethylpiperazine-N'-2-ethanesulphonic acid Sigma Chemicals) and 0.5 mM MIX (1-methyl-3-isobutylxanthine) with and without various amounts of ovine LH (LER 1733-3, LH activity 1.64 × NIH-LH-S1; FSH activity <0.04 × NIH-FSH-S1). Incubations were carried out in glass culture tubes in 95% O_2:5% CO_2 at 37°C in a shaking water bath.

After 15 min of incubation 500 μl of distilled water were added and the incubates immediately placed in a boiling water bath for 20 min. Each boiled follicle and its medium were homogenized together and a supernatant was prepared by centrifugation at 30,000 × g at 4°C for 20 min. The supernatant was stored at -20°C for measurement of cyclic AMP.

To measure the estradiol production of whole follicles, diestrous I and proestrous follicles were incubated as described above. After 4 h of incubation the follicle was removed and the medium was frozen and stored at -20°C for measurement of estradiol.

To assess the ability of whole follicles to convert testosterone to estrogen, individual follicles collected on metestrus, diestrus I and proestrus were incubated in Medium-199 + HEPES with and without testosterone in a final concentration of 50 ng/ml or 200 ng/ml. After 4 h of incubation at 37°C the follicle was removed and the medium was frozen and stored at -20°C for measurement of estradiol.

Radioimmunoassays

Cyclic AMP concentrations were measured by double antibody radioimmunoassay using 2'-0'-mono-succinyl-adenosine-3',5'-monophosphate-tyrosine-methyl-ester (Sigma Chemicals) for iodination and an antiserum generated against a succinyl-cyclic AMP-hemocyanin conjugate, provided by Dr. J. L. Vaitukatis. Procedures and validation of this assay are described elsewhere (Vaitukaitis et al., 1975; Richards et al., 1979). Briefly, samples and standards were acetylated by the procedures of Harper and Brooker (1975). At a final tube dilution of this antiserum at 1:100,000, the 50% inhibition point was 50 femtomoles and the sensitivity 2 femtomoles.

Estradiol concentrations were measured by radioimmunoassay as described by England et al. (1974).

Statistical analysis of data was performed by analysis of variance and Student's t test. A difference was considered as significant if the double tail probability was ≤0.05.

RESULTS

In vitro hCG and hFSH Binding

Specific binding of hCG to expressed granulosa cells shows a steep increase between diestrus II and proestrus (Fig. 1). Specific hFSH binding remained constant on all days of the estrous cycle. Thecal tissue also showed an increase in hCG binding between diestrus II and proestrus. Although this binding might reflect

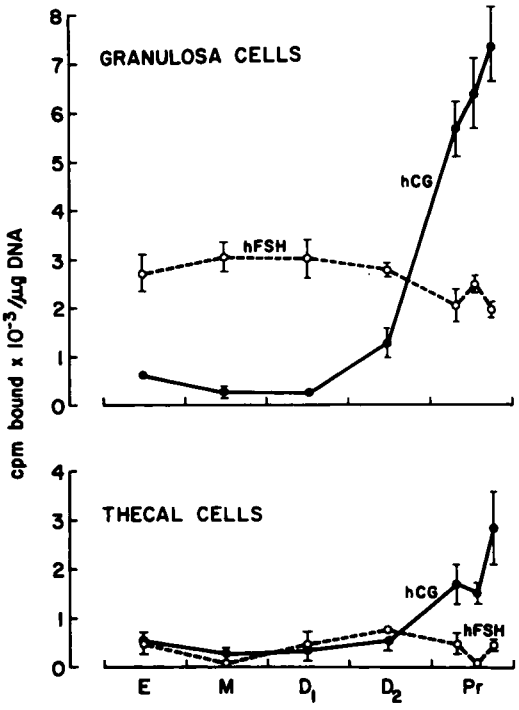

FIG. 1. Specific hCG and hFSH binding to granulosa and thecal cells of the 10–15 largest follicles collected on each day of the estrous cycle. Each point represents the mean ± SEM of 4–6 rats.

contamination of the thecal preparation with granulosa cells, this seems unlikely, because specific hFSH binding to this preparation was nearly nondetectable.

In vivo Autoradiography

Binding of the injected [^{125}I]-labeled hCG was observed in thecal cells of all healthy antral follicles both at diestrus (400–500 μm in diameter) and proestrus (>500 μm in diameter), but the amount (relative number of silver grains) was greater in large antral follicles present on proestrus (Fig. 2a, b). In these large antral follicles the binding of hCG to granulosa cells was observed only in the mural layer adjacent to the basement membrane. The number of silver grains over granulosa cells of small follicles could not be distinguished from background level. Neither theca nor granulosa of atretic follicles bound hCG. Binding of hFSH to granulosa cells was present in small and large antral follicles, but hFSH binding to the thecal cells was absent in all types of follicles (Fig. 2c, d).

Follicular Responsiveness to LH

This experiment was designed to correlate the amount of LH receptor in antral follicles with the ability of these follicles to produce cyclic AMP and estradiol in response to LH stimulation. Single metestrous, diestrous and proestrous follicles were incubated with oLH at concentrations ranging from 0.06–60 μg/ml. Cyclic AMP content in the follicle plus medium was measured after 15 min incubation. The results are shown in Fig. 3. Cyclic AMP content after incubation in the absence of LH was higher in diestrous follicles compared with metestrous follicles (2.53 ± 0.15 vs 1.23 ± 0.24, respectively, mean ± SEM expressed as pmole/15min/follicle; $P<0.01$) and higher in proestrous follicles compared with diestrous follicles (3.61 ± 0.21 vs 2.53 ± 0.15; $P<0.01$). Addition of oLH increased cyclic AMP accumulation in the 3 types of follicles, but with a dose of oLH higher than 0.6 μg/ml the response was much greater in proestrous follicles than in diestrous and metestrous follicles. With a dose of 60 μg/ml the difference between the cyclic AMP content between diestrous and metestrous follicles was no longer significant.

Estradiol accumulation in the medium after 4 h of incubation showed a marked difference between diestrous and proestrous follicles (Fig. 4). In the absence of LH, estradiol production was 4-fold higher in the proestrous follicles than in the diestrous follicles. After addition of increasing amounts of oLH, estradiol production remained consistently low in the diestrous follicles. In contrast, estradiol production by proestrous follicles was already maximal with the lowest dose (0.06 μg) of oLH.

Follicular Aromatase Activity

To assess the ability of small and large antral follicles to convert testosterone to estrogen, individual follicles collected at metestrus, diestrus I and proestrus were incubated in the absence or presence of 50 ng testosterone/ml. Metestrous follicles incubated with or without substrate produced little estradiol (Fig. 5). After addition of testosterone to diestrous follicles, estradiol accumulation in the incubation medium was increased. These results suggest that aromatase activity is low in metestrous follicles, but present in diestrous follicles. Proestrous follicles produced a substantial amount of estradiol without addition of sub-

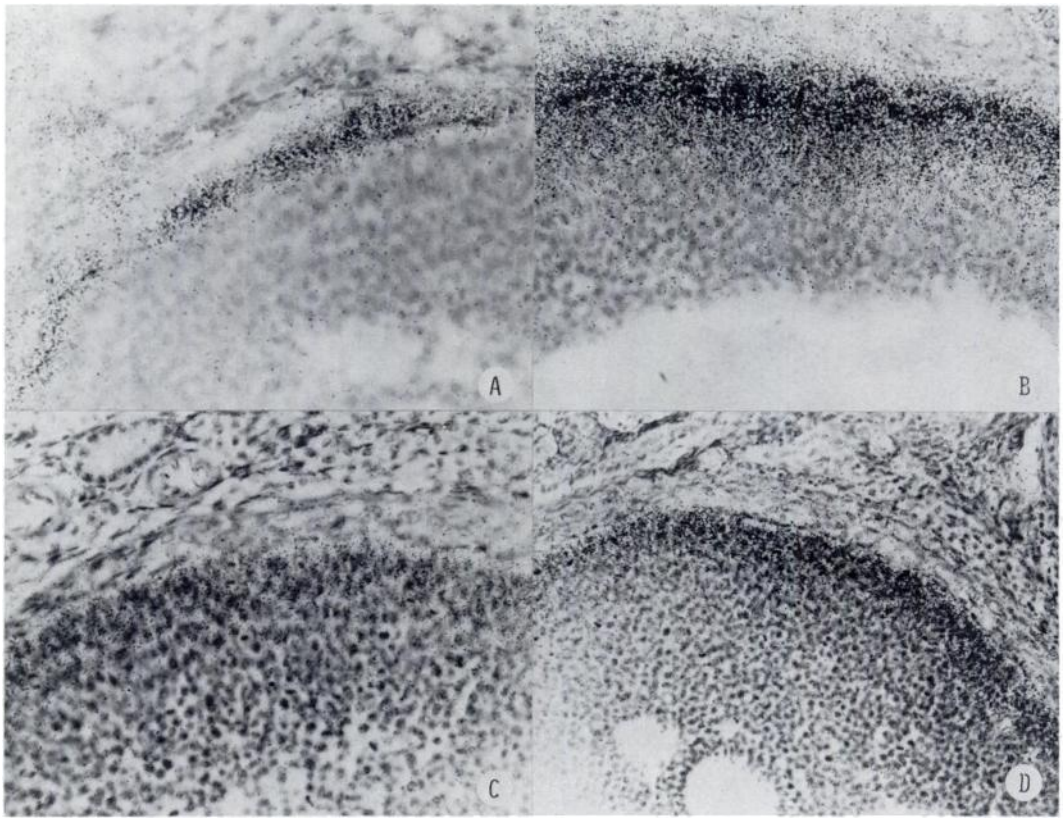

FIG. 2. Autoradiographic localization of [^{125}I]-hCG (upper panels) and [^{125}I]-hFSH (lower panels) over thecal cells and granulosa cells of an antral follicle at diestrus (400–500 μm in diameter; Fig. 2a, c) and a preovulatory follicle at proestrus (>500 μm in diameter; Fig. 2b, d). [^{125}I]-hCG and [^{125}I]-hFSH were administered i.v. 2 h earlier. X 534.

strate; supplementation of 50 ng testosterone/ml did not cause a greater accumulation. However, in an additional experiment using a much higher dose of testosterone (200 ng/ml), estradiol production by proestrous follicles was also increased (4.1 ± 0.4 ng/follicle to 6.6 ± 0.2 ng/follicle, $0.01<P<0.02$). These data suggest that in proestrous follicles aromatase activity and endogenous substrate production are increased.

DISCUSSION

The data presented demonstrate that the increase in LH receptors both in granulosa and thecal cells during the 5 day rat estrous cycle is coincident with the growth of preovulatory follicles. The presence of LH receptors in granulosa cells of large antral follicles has been reported earlier in the pig (Channing and Kammerman, 1973; Lee, 1976) and in hormonally-primed immature rats (Zeleznik et al., 1974; Richards et al., 1976). In a recent report Nimrod et al. (1977) demonstrated in 4 day cycling rats an increase in the number of LH receptors in granulosa cells prior to the LH surge. The increase in hCG binding to thecal cells, although questionable in the *in vitro* binding studies is supported by the autoradiographic studies, demonstrating a greater binding to the thecal cells of large antral follicles. The changes in LH receptors in the granulosa cells occur without corresponding changes in FSH receptors. Accordingly, in hormonally-primed immature rats and proestrous adult rats only a slight increase was found in the number of FSH binding sites from medium to large antral follicles (Nimrod et al., 1976). The *in vivo* autoradiographic data support the relative distribution of LH and FSH specific binding sites as revealed by the *in vitro* binding assays. FSH binding was observed only to granulosa

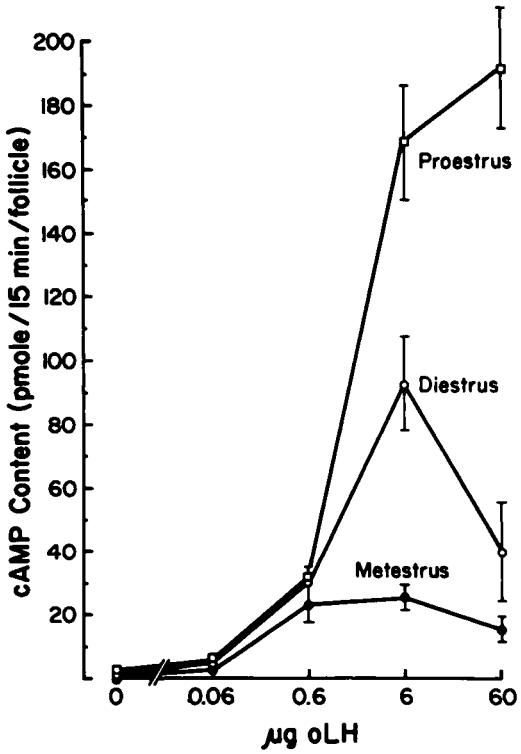

FIG. 3. Cyclic AMP content in follicles + media after 15 min of incubation of single metestrous, diestrous and proestrous follicles with oLH. Each point represents the mean ± SEM of 4 follicles.

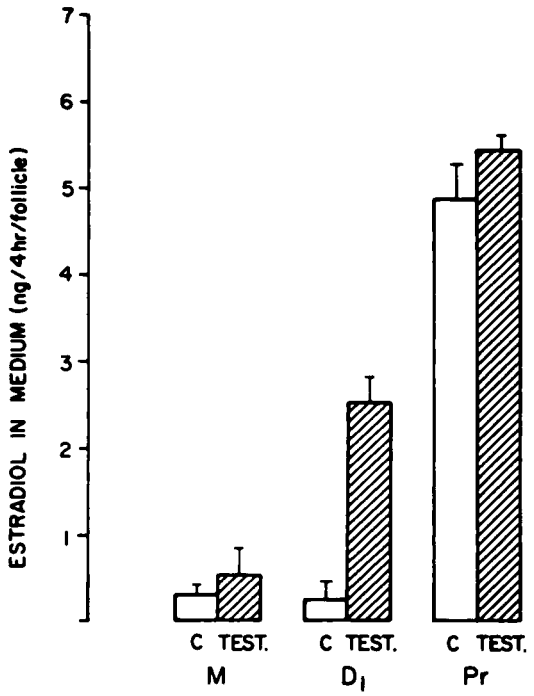

FIG. 5. Estradiol accumulation in the medium after 4 h of incubation of single metestrous, diestrous and proestrous follicles with and without testosterone (50 ng/ml). Each point represents the mean ± SEM of 4–8 follicles.

cells and not to the thecal cells; only granulosa cells of large antral follicles showed hCG binding. However, the hCG binding to granulosa cells was restricted to the layer adjacent to the basement membrane. Similar observations were made by Midgley (1973) with topically applied radioiodinated hCG and by Amsterdam et al. (1975) after hCG injection. Whether this is due to uneven distribution of LH receptors in the granulosa cells is not known.

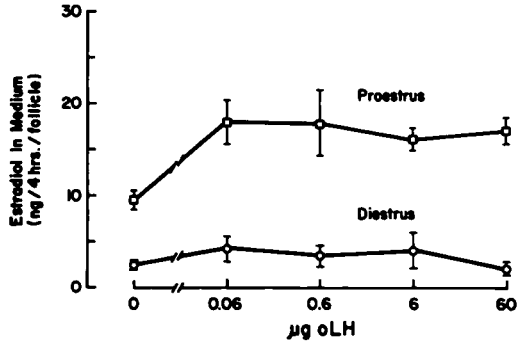

FIG. 4. Estradiol accumulation in the medium after 4 h of incubation of diestrous and proestrous follicles with oLH. Each point represents the mean ± SEM of 4 follicles.

The increase in LH receptors in the Graafian follicles was associated with an increased responsiveness of these follicles to LH as demonstrated by an increased ability to produce cyclic AMP and estradiol after LH stimulation. An increase in the responsiveness of follicular adenyl cyclase activity to LH prior to ovulation has also been reported by Hunzicker-Dunn and Birnbaumer (1976) using broken membrane preparations. Thus it appears that the LH binding sites are effectively coupled to an adenyl cyclase system to provide cyclic AMP for intracellular processes.

Even more marked than the ability of follicles to produce cyclic AMP is the ability of developing follicles to produce estradiol. It has been well documented that the theca under the influence of LH produces androgen (Fortune and Armstrong, 1977). Androgen can then be

converted to estradiol by the granulosa cells (Fortune and Armstrong, 1978) provided that their aromatizating system has been induced by FSH (Moon et al., 1975; Erickson and Hsueh, 1978). Our experiments suggest that developing follicles gain an increased ability to produce androgen as well as an increased ability to aromatize androgen to estradiol. According to the above mentioned theory, the increased estradiol production after LH stimulation is the result of an increased androgen production by the thecal cells. Although supplementation of 50 ng testosterone/ml to proestrous follicles did not increase estradiol production, a dose of 200 ng testosterone/ml resulted in a 60% increase.

The interpretation of the data is based on the assumption that the 10–15 largest follicles which are isolated on each day of the estrous cycle are the follicles destined to ovulate during the next estrus. This assumption is justified by the observation that on estrus, metestrus and diestrus I all antral follicles are smaller than 500 μm in diameter (Welschen, 1973; Hirschfield and Midgley, 1978a). On proestrus, 8–12 large antral follicles (> 500 μm) are present, which correspond with the number of ova in the oviduct on estrus, while follicles of the 400–500 μm size range are absent. On the basis of these observations it has been suggested that in the rat the small antral follicles present on estrus and metestrus either become atretic or continue to grow to preovulatory size and ovulate. Because early atretic follicles cannot be distinguished macroscopically from healthy follicles, it is possible that atretic follicles are included in the present study, which may account for some of the variation in cyclic AMP and estradiol production seen after follicle incubation.

It is likely that the following sequence of events occurs in the developing preovulatory follicles. The high FSH concentrations during proestrus-estrus stimulate the growth of a number of small antral follicles. During the following days from estrus to proestrus, these follicles acquire an increased ability to produce estrogen in response to basal concentrations of LH and FSH. The progressive changes involve an enhanced ability of LH to act on thecal cells to stimulate androgen production and an enhanced ability of FSH to act on granulosa cells to stimulate aromatase activity. The enhanced responsiveness of the theca cells to LH appears to be related, in part, to an increased number of LH receptors. As thecal androgen production increases, follicular estrogen production and aromatase activity also increase. Because estradiol has been shown to play a key role in the induction of LH receptors (Richards et al., 1976), it is likely that the increased follicular production of estradiol together with basal concentrations of FSH stimulate the appearance of LH receptors in granulosa cells of preovulatory follicles. Thus, once selected, developing preovulatory follicles can become more responsive to LH by an increase in LH receptors in the theca and granulosa cells without major changes in the gonadotropin concentrations.

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REFERENCES

Amsterdam, A., Koch, Y., Lieberman, M. E. and Lindner, H. R. (1975). Distribution of binding sites for human chorionic gonadotropin in the preovulatory follicle of the rat. J. Cell. Biol. 67, 894–900.

Burton, K. (1956). A study of the conditions and mechanism of the diphenylamine reactions for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62, 315–323.

Butcher, R. L., Collins, W. E. and Fugo, N. W. (1974). Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinology 94, 1704–1708.

Channing, C. P. and Kammerman, S. (1973). Characteristics of gonadotropin receptors of porcine granulosa cells during follicle maturation. Endocrinology 92, 531–540.

England, B. G., Niswender, G. D. and Midgley, A. R. (1974). Radioimmunoassay of estradiol-17β without chromatography. J. Clin. Endocrinol. Metab. 38, 42–50.

Erickson, G. F. and Hsueh, A.J.W. (1978). Stimulation of aromatase activity by follicle stimulating hormone in rat granulosa cells *in vivo* and *in vitro*. Endocrinology 102, 1275–1282.

Fortune, J. E. and Armstrong, D. T. (1977). Androgen production by theca and granulosa isolated from proestrous rat follicles. Endocrinology 100, 1341–1347.

Fortune, J. E. and Armstrong, D. T. (1978). Hormonal control of 17β-estradiol biosynthesis in proestrous rat follicles: Estradiol production by isolated theca versus granulosa. Endocrinology 102, 227–235.

Gay, V. L., Midgley, A. R. and Niswender, G. D. (1970). Patterns of gonadotrophin secretion

associated with ovulation. Fed. Proc. 29, 1880–1887.

Harper, J. F. and Brooker, G. (1975). Femtomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2′-o-acetylation by acetic anhydride in aqueous solution. J. Cyclic. Nucl. Res. 1, 207–218.

Hillier, S. G., Zeleznik, A. J. and Ross, G. T. (1978). Independence of steroidogenic capacity and luteinizing hormone receptor induction in developing granulosa cells. Endocrinology 102, 937–946.

Hirschfield, A. N. and Midgley, A. R. (1978a). Morphometric analysis of follicular development in the rat. Biol. Reprod. pp. 19, 597–605.

Hirschfield, A. N. and Midgley, A. R. (1978b). The role of FSH in the selection of large ovarian follicles in the rat. Biol. Reprod. pp. 19, 606–611.

Hunzicker-Dunn, M. and Birnbaumer, L. (1976). Adenylyl cyclase activities in ovarian tissues. III. Regulation of responsiveness to LH, FSH and PGE_1, in the prepubertal, cycling, pregnant and pseudopregnant rat. Endocrinology 99, 198–210.

Lee, C. Y. (1976). The porcine ovarian follicle: III Development of chorionic gonadotropin receptors associated with increase in adenyl cyclase activity during follicle maturation. Endocrinology 99, 42–48.

Midgley, A. R. (1973). Autoradiographic analysis of gonadotropin binding to rat ovarian tissue sections. Adv. Exp. Med. Biol. 36, 365–378.

Moon, Y. S., Dorrington, J. H. and Armstrong, D. T. (1975). Stimulatory action of follicle-stimulating hormone on estradiol-17β secretion by hypophysectomized rat ovaries in organ culture. Endocrinology 97, 244–247.

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.

Nimrod, A., Erickson, G. F. and Ryan, K. J. (1976). A specific FSH receptor in rat granulosa cells: Properties of binding *in vitro*. Endocrinology 98, 56–64.

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

Richards, J. S., Jonassen, J. J., Rolfes, A. I., Kersey, K. and Reichert, L. E. (1979). Cyclic AMP, luteinizing hormone receptor, and progesterone during granulosa cell differentiation: effects of estradiol and follicle stimulating hormone. Endocrinology. In Press.

Richards, J. S., Rao, M. C. and Ireland, J. J. (1978). Actions of pituitary gonadotrophins on the ovary. In: Control of Ovulation. (D. B. Crighton, ed.). Butterworths, Sevenoaks, U.K. pp. 197–216.

Schwartz, N. B. (1974). The role of FSH and LH and of their antibodies on follicle growth and on ovulation. Biol. Reprod. 10, 236–272.

Smith, M. S., Freeman, M. E. and Neill, J. D. (1975). The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: Prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 96, 219–226.

Vaitukaitis, J. L., Lee, C. Y., Ebersole, E. R. and Lerario, A. C. (1975). New evidence for an acute role of protein kinase in hCG action. Endocrinology 97, 215–222.

Welschen, R. (1973). Amounts of gonadotropins required for normal follicular growth in hypophysectomized adult rats. Acta Endocrinol. 72, 137–155.

Welschen, R. and Dullaart, J. (1976). Administration of antiserum against ovine follicle-stimulating hormone or ovine luteinizing hormone at proestrus in the rat: Effects of follicular development during the oncoming cycle. J. Endocrinol. 70, 301–306.

Zeleznik, A. J., Keyes, P. L., Menon, K.M.J., Midgley, A. R. and Reichert, L. E. (1977). Development-dependent responses of ovarian follicles to FSH and hCG. Am. J. Physiol. 233, E299–E234.

Zeleznik, A. J., Midgley, A. R. and Reichert, L. E. (1974). Granulosa cell maturation in the rat: Increased binding of human chorionic gonadotropin following treatment with follicle-stimulating hormone *in vivo*. Endocrinology 95, 818–825.

RECOMMENDED REVIEWS

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

Lindner, H. R., Amsterdam, A., Salomon, Y., Tsafriri, A., Nimrod, A., Lamprecht, S. A., Zor, U. and Koch, Y. (1977). Intraovarian factors in ovulation: Determinants of follicular response to gonadotrophins. J. Reprod. Fert. 51, 215–235.