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Effect of Neutralization of Endogenous Follicle Stimulating Hormone (FSH) or Luteinizing Hormone (LH) on Ovarian Lipids in the Hamster: A Histochemical and Biochemical Evaluation

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ABSTRACT

The effect of neutralizing FSH or LH on ovarian lipids in the cycling hamster was studied. In the normal cycling hamster on the day of proestrus, histochemical examination revealed the presence of sudanophilic lipids in the granulosa cells of the follicles and in the interstitium. A clear reduction in the intensity of lipid staining was observed on proestrus in the ovary of hamsters treated with FSH antiserum on the previous proestrus. Similar treatment with antiserum to LH, on the other hand, caused an accumulation of lipids in these structures.

Estimation of the free and esterified fractions of cholesterol and triglycerides in the nonluteal tissue of the ovary of hamsters on proestrus following treatment with FSH antiserum on the previous proestrus revealed a significant reduction in all 3 lipid components. Even a short term deprivation of FSH caused a similar reduction in these lipids in the ovary. In contrast, treatment with LH antiserum either on the previous proestrus or on the previous day (diestrus-2) resulted in an enhancement in esterified cholesterol and triglycerides, while it caused a reduction in the free cholesterol fraction of the ovary on proestrus.

It is suggested that though treatment with antisera to either FSH or LH causes a disruption in follicular maturation, their effect on lipid metabolism is different. A positive role for FSH and LH in maintaining normal sterol and triglyceride levels in the nonluteal ovarian tissue of cycling hamster is indicated.

INTRODUCTION

Earlier studies from our laboratory have shown that neutralization of endogenous luteinizing hormone (LH) with LH antiserum affects not only steroid biosynthesis, but also sterol metabolism and biosynthesis in the luteal tissue of pregnant and pseudopregnant rats and hamsters (Moudgal et al., 1972; Behrman et al., 1972; Mukku and Moudgal, 1975, 1976). However, virtually nothing is known regarding the involvement of LH and follicle stimulating hormone (FSH) in sterol metabolism in nonluteal ovarian tissue. Hence, it was of considerable interest to examine in the present study the effect of neutralizing endogenous LH or FSH for short or long periods on sterol and triglyceride levels in the nonluteal tissue of the ovary of the cycling hamster.

A preliminary histochemical study was undertaken to examine if sudanophilic lipids in specific compartments of the ovary are influenced by the lack of endogenous FSH or LH. This was later followed by biochemical estimations of ovarian cholesterol (free and esterified) and triglycerides.

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MATERIALS AND METHODS

Histochemical Methods

Regularly cycling female hamsters (*Mesocricetus auratus*), maintained under a lighting schedule of 14L:10D (lights on at 0600 h) in a well ventilated room, were either untreated or were given an intracardiac injection of 0.1 ml FSH antiserum or 0.2 ml LH antiserum (Sheela Rani and Moudgal, 1977a) at 1300 h on the day of proestrus. On the next proestrus (4 days later), they were killed by decapitation at

1000 h and 1 ovary from each animal (n = 3/group) was immediately frozen in isopentane at -70°C. Frozen sections were cut in a cryostat (Ames Labtech) at 14 μm and mounted on clean glass slides. After slight air drying, they were fixed for 24 h in Baker's calcium formal (40% formalin:10% $CaCl_2$:water [1:1:8] and a piece of chalk for neutralization). The sections were then washed with several changes of distilled water over a period of 24 h and were then stained for lipids with Sudan Black B for 15 min (Baker, 1946). The stained sections were then differentiated in 50% alcohol, washed in water and mounted in glycerine jelly (7.5% gelatin in 50% glycerol). The sections were examined under a microscope.

Biochemical Estimations

Groups of regularly cycling female hamsters were given by intracardiac route 50 μl FSH antiserum or 0.2 ml LH antiserum at 1300 h of proestrus or 0900 h of diestrus-2. The animals treated with antisera on 1 proestrus were killed 4 days later on the next proestrus and those given antisera on diestrus-2 were killed on the next day (proestrus) at 1700 h. A control group of hamsters was untreated and killed at 1700 h of proestrus. The ovaries were removed and carefully cleaned of the surrounding fat and other extraneous tissue and weighed to the nearest 0.1 mg. It should be mentioned here that in the cycling hamster by the evening of proestrus the luteal tissue is almost completely regressed and is not discernible even structurally (Lukaszewska and Greenwald, 1970).

The method used for the extraction of lipids with chloroform:methanol (1:2) and separation by TLC on silica gel plates with the use of 2 solvent systems (hexane:ether:acetic acid, 75:25:2 and 90:10:1, respectively, in the same direction) was essentially based on that described by Major et al. (1967) and Pokel et al. (1972). The extent of recovery of sterols was traced using [^{14}C]-labeled cholesterol oleate added during extraction and appropriate corrections were made.

Both free and esterified cholesterol were estimated by the method of Glick et al. (1964). By this method, cholesteryl ester was estimated directly without saponification and hence the values are reported as cholesterol equivalents (Mukku and Moudgal, 1975). For the quantitation of triglycerides, glyceride glycerol was estimated after saponifying the samples with 2% alcoholic KOH at 60–70°C for 30 min. The liberated glycerol was estimated by the method of Jover (1963). The results are expressed as μg glycerol equivalents/mg tissue.

The significance of differences between the means of control and experimental groups was determined using Student's t test.

RESULTS

Histochemical Observations

The sections of the ovary of hamster on proestrus stained with Sudan Black B showed a higher concentration of lipids in the interstitial cells than in the granulosa cells. The lipid content in the thecal cells was negligible (Fig. 1). In the ovary of hamsters treated with FSH antiserum, the lipid content of the granulosa cells as well as the interstitial cells was considerably reduced as compared to the normal proestrous ovaries (Fig. 2). In contrast, in the ovary of hamsters treated with LH antiserum, the sudanophilic material in the interstitial and the granulosa cells was markedly enhanced (Fig. 3).

Effect of Antisera to FSH or LH on Free and Esterified Cholesterol and Triglycerides in the Ovaries of Hamster on Proestrus

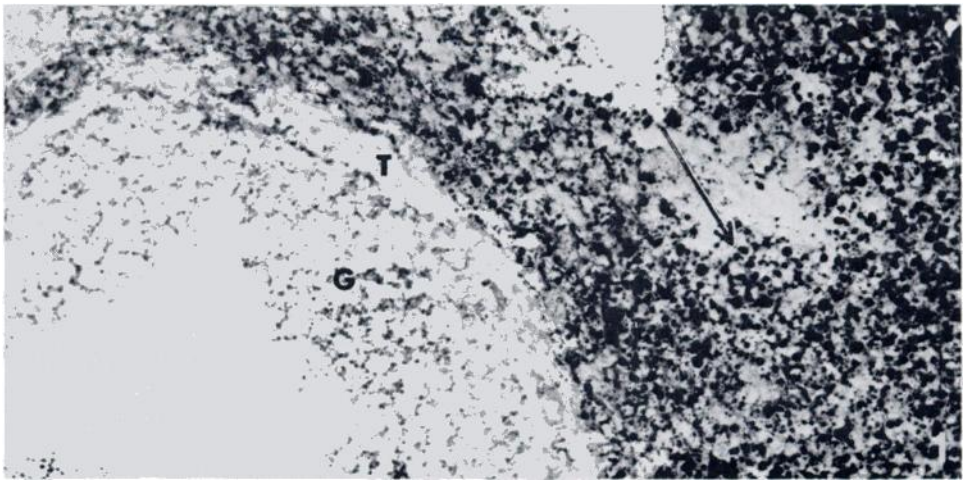
Administration of FSH antiserum either on proestrus (long term lack of FSH) or on diestrus-2 (acute lack of FSH) resulted in a significant reduction in both free and esterified cholesterol as well as in triglycerides in the ovary on proestrus (Table 1; Fig. 4). The reduction was marked in the free cholesterol fraction, whereas esterified cholesterol showed a marginal, though significant decrease. Interestingly, the extent of reduction in these lipids was more or less similar in the ovaries of animals treated with FSH antiserum either 4 days earlier, on the previous proestrus or on the previous day. The ovarian triglycerides on the other hand showed a greater reduction in the animals treated with FSH antiserum on the previous proestrus than those treated on the day prior to killing.

The effect of LH antiserum on these 3 fractions of lipids was different. While LH antiserum caused a significant decrease in the free cholesterol fraction, there was a tremendous accumulation of esterified cholesterol in

FIG. 1. Ovary of untreated hamster on proestrus revealing the pattern of lipid localization. Note the dense staining in the interstitial cells (arrow). The theca (T) has very little sudanophilic material. The granulosa cells have a fair amount of lipid. Sudan Black B staining. X 1500.

FIG. 2. Pattern of lipid localization in the ovary of hamster treated with FSH antiserum on the previous proestrus. The sudanophilic material is seen in the interstitium as well as in the granulosa cells. However, the intensity of staining of lipids is considerably reduced. Sudan Black B staining. X925.

FIG. 3. Pattern of lipid localization in the ovary of hamster treated with LH antiserum on the previous proestrus. Note the presence of large droplets of sudanophilic material in the interstitial cells (arrow). The granulosa cells also show a much higher intensity of staining of the lipid contents. Sudan Black B staining. X925.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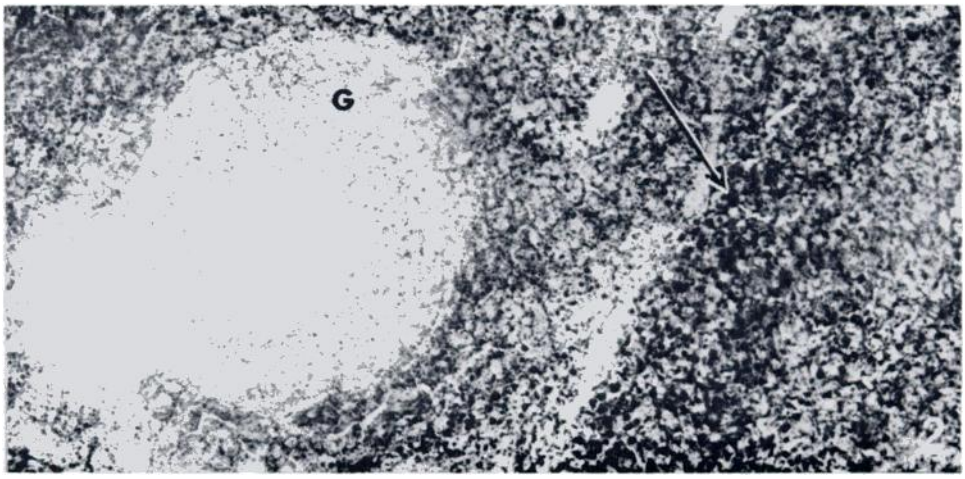
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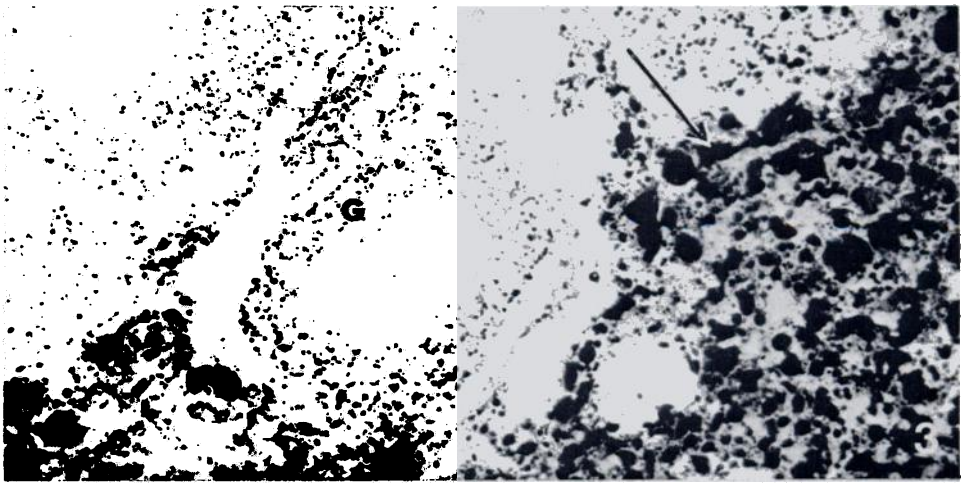
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TABLE 1. Effect of treatment with antisera to FSH or LH at proestrus or diestrus-2 on ovarian cholesterol and triglyceride concentration at 1700 h of proestrus. (Mean ± SEM; numbers in parentheses = N/group.)[a]

Treatment	Cholesterol (μg/mg tissue)		Triglycerides (μg glycerol/ mg tissue)
	Free	Esterified	
Untreated control	8.37 ± 1.70 (5)	17.34 ± 1.04 (11)	1.38 ± 0.12 (11)
FSH antiserum			
on proestrus[b]	3.85 ± 0.56 (8)*	14.30 ± 0.32 (8)*	0.68 ± 0.02 (7)**
on diestrus[c]	4.22 ± 0.32 (7)*	15.68 ± 1.19 (7)***	1.16 ± 0.01 (7)
LH antiserum			
on proestrus[b]	4.71 ± 0.56 (7)*	26.14 ± 4.29 (8)*	1.82 ± 0.17 (7)*
on diestrus[c]	3.83 ± 0.47 (3)*	20.14 ± 0.79 (3)*	1.45 ± 0.08 (3)***

[a]The values are significantly different from those of corresponding controls at *P<0.01, **P<0.001, ***not significant.

[b]The animals were given antisera at 1300 h of proestrus and killed at 1700 h of next proestrus, 4 days later.

[c]The animals were given antisera at 0900 h of diestrus-2 and killed on the next day (proestrus) at 1700 h.

these ovaries. Esterified cholesterol was more marked in the group exposed to LH antiserum on the previous proestrus than in the group treated with LH antiserum on the previous day (diestrus-2). Also in the former group there was a significant increase in ovarian triglycerides, while this component was found to be unchanged in the latter group (Table 1; Fig. 4).

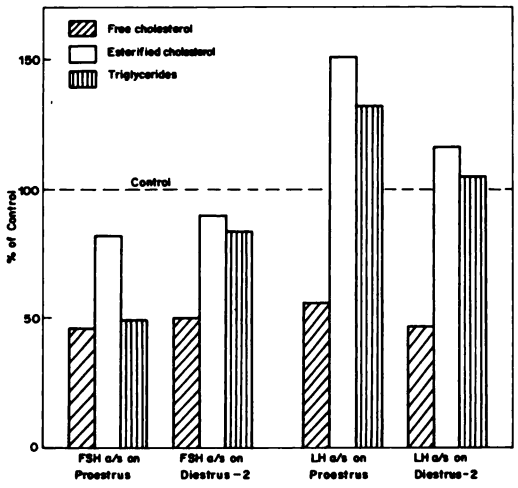

FIG. 4. Effect of antiserum (a/s) to FSH or LH on ovarian lipid constituents on proestrus. Treatment was given either at 1300 h of proestrus or at 0900 h of diestrus-2 and the animals were killed after 4 days or 1 day, respectively, at 1700 h of proestrus. Redrawn from mean values of free and esterified cholesterol and triglycerides in the ovaries (Table 1) as % of control, considering the values from untreated hamsters as 100%.

DISCUSSION

The present study, using both histochemical and biochemical methods, has demonstrated that deprivation of FSH or LH either for short or long duration produces specific effects on lipid metabolism in the ovary of the cycling hamster.

In studies involving the use of antisera to delineate the role of gonadotropins in physiological processes, it is essential to determine the specificity, cross reactivity and the neutralizing efficacy of the antisera used. In the present study, the above crieteria were established for both antisera used. Thus, it was found that following administration of the minimal effective dose (50 μl) of FSH antiserum on the afternoon of proestrus, the presence of FSH antibody could be detected in the serum of the hamsters for the next 4 days of the cycle; however, since just the presence of antibody itself does not necessarily mean that the hormone is neutralized, an attempt was made to measure free FSH in the sera of animals injected with FSH antiserum (Sheela Rani, 1977). This was possible only after removal of the interfering excess FSH antibody and/or the antigen-antibody complex by treatment of these sera with a polymer of the second antibody, goat antiserum to monkey gamma globulin (Sheela Rani and Moudgal, 1977b). It was found that in animals given 50 μl FSH antiserum at 1400 h of proestrus, no free FSH could be detected in the serum at other periods of the same day as well as on the next day, showing the neutralizing efficacy of

the antiserum. On the next 2 days of diestrus, some amount of FSH lower than in the controls (controls, 242.5 ± 32.5 ng/ml vs antiserum treated, 41.6 ± 13.1 ng/ml; mean ± SD) could be measured, indicating only a partial neutralization of FSH at these periods (Sheela Rani, 1977). To determine the specificity of the antiserum effect in the sera of animals treated with FSH antiserum LH was measured by radioimmunoassay. It was found that administration of FSH antiserum at 1400 h of proestrus had no effect on the occurrence of the LH surge on that day, since peak serum levels were seen at 1700 h as in control hamsters (Sheela Rani, 1977). Serum LH levels on subsequent days of the cycle also were found to be normal in these hamsters treated with FSH antiserum, thereby showing the specificity of antiserum effect. Similarly for LH antiserum, the criteria of specificity and efficacy were established as described above (Sheela Rani, 1977).

Our earlier studies had shown that treatment with minimal effective doses of antiserum to either FSH or LH on proestrus caused a blockade of follicular maturation, as indicated by the inability of these animals to ovulate at next estrus even in response to an exogenous dose of LH (Sheela Rani and Moudgal, 1977a). However, the effect of injecting the minimal effective doses of antisera was found to be confined to only 1 cycle, since these animals were found to ovulate normally at the third estrus (Sheela Rani, 1977). Additional evidence for an interference with follicular growth in these animals treated with FSH or LH antiserum was obtained by histological examination of the ovaries on the next proestrus. The characteristic feature of the ovary of a normal hamster on the evening of proestrus is the presence of at least 4–5 large preovulatory antral follicles. Such antral follicles were predominantly absent from the ovaries of animals treated with either FSH or LH antiserum on the previous proestrus (Sheela Rani, 1977). No follicles having more than 7–8 layers of granulosa cells were present in the ovaries of these hamsters on the next proestrus. The significant difference between the effect of treatment with FSH and LH antiserum was that while the former treatment did not result in any reduction in the size of the ovary, LH antiserum administration caused a reduction in the size of the ovaries by the next proestrus; further, these ovaries showed more extensive follicular atresia (Sheela Rani, 1977).

The present histochemical studies showed that the sudanophilic lipids in the follicles of the ovary were present exclusively in the granulosa and were absent from the theca interna cells. The interstitium, however, was the most densely stained portion of the ovary. These lipid droplets found in the hamster ovary have been reported to consist of triglycerides, cholesterol and/or its esters and little or no phospholipids (Guraya and Greenwald, 1965; Guraya, 1972). The significant observation in the histochemical study was FSH anstierum given on proestrus caused a decrease in the intensity of lipid staining with Sudan Black B at the next proestrus, particularly in the granulosa cells of the follicle. To get a quantitative picture of the changes in lipid constituents, biochemical estimations were carried out. The latter studies confirmed that the neutralization of FSH starting on proestrus had caused a drastic reduction in all 3 lipid components when examined on the next proestrus. Since it was known from our earlier studies that such a treatment also causes a blockade of follicular maturation (Sheela Rani and Moudgal, 1977b), it was not clear whether the reduction in levels of ovarian lipids is due to a general cessation of follicular growth or whether the lack of FSH specifically brings about such a decrease. To clarify this point, when the acute effect of withdrawal of FSH on this parameter was tested, it was found to cause a drastic reduction in unesterified cholesterol and triglycerides. These studies suggested that the lack of FSH even for short periods affected the ovarian cholesterol and triglyceride lipids add that these effects were accentuated by the long term withdrawal of FSH. The specificity of the effect of lack of FSH was further exemplified when the effect of LH antiserum treatment on these parameters was examined. The latter treatment brought about a reduction in free cholesterol, but produced an opposite effect on esterified cholesterol and triglycerides. A similar effect was seen even on a short term deprivation of LH.

It appears from these results that although both FSH and LH antisera bring about a cessation of follicular growth (Sheela Rani and Moudgal, 1977b), they could be operating by different mechanisms in affecting the lipid metabolism of the ovary.

In a steroid secreting tissue, cholesterol serves as a precursor for steroidogenesis and its availability appears to govern the rate of ste-

roidogenesis. Gonadotropin, particularly LH, is known to stimulate the esterase activity as well as to inhibit the activity of ester synthetase in luteal tissue (Armstrong, 1968; Behrman and Armstrong, 1969; Behrman et al., 1972). A converse situation, in which there is an accumulation of lipid droplets indicating a failure to utilize cholesterol, has been found to occur during luteolysis (Guraya and Greenwald, 1965; Guraya, 1973; Deane et al., 1966; Hoffman and Fajer, 1973) and this has also been found to be associated with reduced steroidogenesis. In fact, in studies on luteolysis induced by LH antiserum in the pregnant rat (Moudgal et al., 1972) and hamster (Mukku and Moudgal, 1975), it was clearly demonstrated that there was an accumulation of ovarian or luteal cholesterol esters, the free cholesterol remaining unchanged. All these studies clearly demonstrate that LH specifically influences cholesterol turnover at many points and brings about an overall regulation of steroidogenesis in the luteal tissue.

In the present study, however, LH antiserum has been shown to cause a decrease in free cholesterol and an accumulation of esterified cholesterol. The question of luteolysis does not arise in the present case, because in these animals the LH antiserum treatment given on proestrus not only blocked ovulation and hence luteal formation but also blocked follicular development during the cycle. This treatment was also found to result in reduced steroidogenesis (Sheela Rani and Moudgal, 1978). It is then possible that in the hamster ovary, a long term deprivation of LH totally arrests steroidogenesis by intefering with cholesterol turnover. It is also interesting that in the nonluteal tissue of the ovary of the cycling animal, similar results were also obtained by a short term deprivation of LH, as in the case of luteal tissue in the pregnant rat and hamster.

In a study by Armstrong (1968), it was found that LH not only inhibited esterification of free fatty acids ([^{3}H]-palmitic acid) with cholesterol, but also inhibited the incorporation into triglycerides. In conformity with this, our present results show that lack of LH caused not only an accumulation of cholesterol esters, but also of triglycerides.

Until recently, there was a lack of clear understanding regarding the role of FSH in steroidogenesis. Only of late, several studies have established a specific role for FSH in the aromatization step of estrogen biosynthesis and more recently FSH has been shown to stimulate progesterone synthesis in combination with androgen (Moon et al., 1975; Armstrong and Papkoff, 1976; Dorrington et al., 1975; Sheela Rani and Moudgal, 1978; Nimrod and Lindner, 1976; Armstrong and Dorrington, 1976; Schomberg et al., 1976). Our results with FSH antiserum, however, have indicated that neutralization of endogenous FSH either in the cycling or pregnant hamster had no effect on progesterone levels (Sheela Rani and Moudgal, 1977a) or on ovarian androgen levels, the effect of FSH antiserum being specific to estrogen biosynthesis (Sheela Rani and Moudgal, 1978). As already mentioned, virtually nothing is known about the involvement of FSH, if any, in sterol metabolism and maintenance of steroid precursor pools. The present study is perhaps the first report on the influence of FSH on cholesterol and triglycerides in the ovary. In view of the fact that FSH does not influence steroidogenesis in terms of progesterone and androgen synthesis, its effect on sterol and triglycerides is enigmatic. Whether this effect of FSH antiserum is a consequence of a general disruption in cellular metabolism due to the lack of trophic hormonal support for follicles is open to question. A detailed examination of other metabolic parameters may shed light on this issue.

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REFERENCES

Armstrong, D. T. (1968). Gonadotropins, ovarian metabolism and steroid biosynthesis. Recent Prog. Horm. Res. 24, 255–308.

Armstrong, D. T. and Dorrington, J. H. (1976). Androgens augment FSH induced progesterone secretion by cultured rat granulosa cells. Endocrinology 99, 1411–1418.

Armstrong, D. T. and Papkoff, H. (1976). Stimulation of aromatization of exogenous and endogenous androgens in ovaries of hypophysectomized rats *in vivo* by FSH. Endocrinology 99, 1144–1151.

Baker, J. R. (1946). Histochemical recognition of lipines. Quart. J. Micr. Sci. 7, 447–456.

Behrman, H. R. and Armstrong, D. T. (1969). Cholesterol esterase stimulation by luteinizing hormone in luteinized rat ovaries. Endocrinology 85, 474–480.

Behrman, H. R. Moudgal, N. R. and Greep, R. O. (1972). Studies with antisera to luteinizing hormone *in vivo* and *in vitro* on luteal steroidogenesis and enzyme regulation of cholesteryl ester turnover in rats. J. Endocrinol. 52, 419–426.

Deane, H. W., Hay, M. F., Moor, R. M., Rowson, L.E.A. and Short, R. V. (1966). The corpus luteum of the sheep; relationships between the morphology and function during the estrous cycle. Acta Endocrinol. 51, 245–263.

Dorrington, J. H., Moon, Y. S. and Armstrong, D. T. (1975). Estradiol-17β biosynthesis in cultured granulosa cells from hypophysectomized immature rats: stimulation by FSH. Endocrinology 97, 1328–1331.

Glick, D., Fell, B. F. and Sjolin, K. E. (1964). Spectrophotometric determination of nanogram amounts of total cholesterol in microgram quantities of tissue or microlitre volumes of serum. Anal. Chem. 36, 1119–1121.

Guraya, S. S. (1972). Comparative studies on the histochemical features of ovarian compartments in the rat and golden hamster with special reference to steroid hormone synthesis. Acta Anat. 82, 284–304.

Guraya, S. S. and Greenwald, G. S. (1965). Histochemical study of the hamster ovary. Am. J. Anat. 116, 257–266.

Guraya, S. S. (1973). Morphology, histochemistry and biochemistry of follicular growth and atresia. Ann. Biol. Anim. Bioch. Biophys. 13, 229–240.

Hoffman, D. C. and Fajer, A. B. (1973). Free and esterified cholesterol concentrations in the hamster ovary during the estrous cycle, pregnancy and lactation. J. Reprod. Fert. 32, 267–275.

Jover, A. (1963). A technique for the determination of serum glycerides. J. Lipid Res. 4, 228–230.

Lukaszewska, J. H. and Greenwald, G. S. (1970). Progesterone levels in the cycling and pregnant hamster. Endocrinology 86, 1–9.

Major, P. W., Armstrong, D. T. and Greep, R. O. (1967). Effect of luteinizing hormone *in vivo* and *in vitro* on cholesterol conversion to progestins in rat corpus luteum tissue. Endocrinology 81, 19–28.

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

Moudgal, N. R., Behrman, H. R. and Greep, R. O. (1972). Effect of luteinizing hormone antiserum on progesterone and 20α-dihydroprogesterone secretion in the pregnant rat. J. Endocrinol. 52, 413–418.

Mukku, V. R. and Moudgal, N. R. (1975). Studies on luteolysis: Effect of antiserum to luteinizing hormone on sterol and steroid levels in pregnant hamsters. Endocrinology 97, 1455–1459.

Mukku, V. R. and Moudgal, N. R. (1976). Relative sensitivity of the corpus luteum of different days of pregnancy to LH deprivation in the rat and hamster. Mol. Cell. Endocrinol. 6, 71–80.

Nimrod, A. and Lindner, H. R. (1976). A synergistic effect of androgen on the stimulation of progesterone secretion by FSH in cultured rat granulosa cells. Mol. Cell. Endocrinol. 5, 315–320.

Pokel, J. D., Moyle, W. R. and Greep, R. O. (1972). Depletion of esterified cholesterol in mouse testes and Leydig cell tumors by luteinizing hormone. Endocrinology 91, 323–325.

Schomberg, D. W., Stouffer, R. L. and Tyrey, L. (1976). Modulation of progestin secretion in ovarian cells by dihydrotestosterone: A direct demonstration in monolayer culture. Biochem. Biophys. Res. Comm. 68, 77–81.

Sheela Rani, C. S. (1977). Studies on Gonadotropins–Gonadotropic regulation of follicular maturation in cyclic hamsters using antisera to FSH and LH. Ph.D. Thesis. Indian Institute of Science, Bangalore, India.

Sheela Rani, C. S. and Moudgal, N. R. (1977a). Examination of the role of FSH in periovulatory events in the hamster. J. Reprod. Fert. 50, 37–45.

Sheela Rani, C. S. and Moudgal, N. R. (1977b). Role of the proestrous surge of gonadotropins in the initiation of follicular maturation in the cyclic hamster: A study using antisera to FSH and LH. Endocrinology 101, 1484–1494.

Sheela Rani, C. S. and Moudgal, N. R. (1978). Examination of the role of follicle stimulating hormone in estrogen biosynthesis *in vivo* and *in vitro* in the ovary of cycling hamster. Steroids. 00, 000–000.