# INITIATION OF OVULATION AND REGULATION OF LUTEAL FUNCTION

*by* N. R. MOUDGAL *and* A. JAGANNADHA RAO, *Endocrinology Laboratory, Department of Biochemistry, Indian Institute of Science, Bangalore*-560012

An attempt has been made in the present paper to review the current status of our knowledge on the subject of relative role of Luteinzing Hormone (LH) and Follicle Stimulating Hormone (FSH) in the initiation of ovolution and regulation of luteal function. Emphasis is laid on studies in laboratory rodents and primates and work from our laboratory and others are reviewed. The use of specific, well-characterized anti-gonadotropins in delineating the the role of LH and FSH in these has been highlighted. Evidence has been furnished in support of LH being the true physiological trigger for ovulation to occur. Data in support of the concept that both prolactin and LH are needed to maintain luteal function is furnished; the former responsible for providing adequate substrate stores as well as maintain a basic cell matrix needed for active steroidogenesis, while the latter regulates the quantum of steroid syhthesis by amplification of the signal.

Ovulation, the primary physiological event of the estrus and menstrual cycles, is preceeded by a period during which the follicles undergo maturation to an 'ovulable' stage, ovulation itself being achieved by additional gonadotropin stimulation. The process of follicular maturation, which is also under the control of FSH and LH, has been discussed earlier and as such it may just suffice here to stress the physiological significance of the raising titers of estradiol and 17α–hydroxy progesterone seen during the follicular phase. While the increase in the levels of these steroids (brought about by the tonic stimulation of FSH and LH) are suggested to indicate progress in follicular maturation and preparedness of the follicles to ovulate, the triggering action of estrogen to release from the pituitary a surge of gonadotropin, has been made clear by the use of specific estrogen antibodies (Ferin *et al.* 1969) (Fig. 1). The involvement of progesterone, if any, in this triggering action is disputable. There is a large body of evidence based on the use of hypothalamic depressing drugs, electrolytic lesions, etc. (Sawyer 1964), indicating that the mode of action of estrogen is via the hypothalamus and that the release of surge of gonadotropin is very carefully timed. While in the rat (Niswender *et al.* 1968) and hamster (Goldman and Porter 1970) it occurs between 4–6 p.m. of proestrus, in the rabbit (Scaramuzzi *et al.* 1972) it occurs 2 hr after cervical stimulation and in the primate (Monroe *et al.* 1970) it occurs over a 24 hr period at mid-cycle (Table I).

Though physiologists for a considerable period have accepted the idea of the presence of an 'ovulation-inducing hormone' they have failed to spell out clearly what this could be. This is despite the fact that earlier investigators (Catchpole 1964) had succeeded in either inducing ovulation with LH or blocking ovulation in regularly cycling animals by the use of LH antibody (Kelly *et al.* 1963; Schwartz 1969)

TABLE I

*Approximate time and duration of gonadotropin (FSH and LH) surge as it occurs in some experimental animals*

| Species | Time of surge | Duration of surge (hr) |
|---|---|---|
| Rat | 2 to 6 p.m. of proestrus | 2 |
| Hamster | 2 to 6 p.m. of proestrus | 2 |
| Sheep | 4 to 14 hr of estrus | 10 |
| Monkey | On day 11 or 12 | 2–4 |
| Human | On day 13 or 14 | 16–20 |
| Rabbit | Within 1 hr of coitus | 4–6 |

The reservation of physiologists seemed justified by the following later observations: (i) the judicious use of radioimmunoassay (RIA) showed that at proestrus or midcycle there is a release in surge-form of both FSH and LH (Fig. 1); (*ii*) hypophysectomized immature rats primed with PMS ovulated in response to FSH preparations contaminated from an apparent nil to small amounts of LH (Lostroh and Johnson 1966; Harrington *et al.* 1970), and (*iii*) the reduction in the ability of a general purpose gonadotropin antibody, after it has been absorbed free of FSH antibody (Goldman and Mahesh 1969) to block ovulation. Since the conclusions drawn from experiments described in (*ii*) and (*iii*) above appeared questionable to us it was considered worthwhile to reinvestigate the relative role of FSH and LH in inducing ovulation by using antisera which were characterized by a variety of methods for their specificity of action.

The characterization of the LH antiserum prepared in our laboratory has been described in detail in several of our earlier publications (Moudgal and Li 1961; Madhwa Raj *et al.* 1968; Madhwa Raj and Moudgal 1970) and it may suffice here to state that it does not appear to react with FSH as shown by the sensitive radio-labelled binding test (Table II). In contrast, unabsorbed FSH antiserum appears to be contaminated with antibodies to LH (Table II). The interfering LH antibodies were removed by a new technique which consists of treating the FSH antiserum with solid LH immunosorbent (Jagannadha Rao *et al.* 1973). This method ensures total removal of contaminating antibodies without it in any way altering the antibody titer of the principle immunogen and it also prevents introduction of a new contaminant to the antiserum in the way of excess of absorbing material (Table II).

Three different experimental models using rats and hamsters were employed for studying the effect of (a) antiserum 'cleansed' FSH, and (b) specific neutralization of FSH on the ovulation process. As is evident from data presented in Fig. 2, FSH did not appear to influence ovulation. In contrast, neutralization of LH in every case led to a blockade of ovulation (Jagannadha Rao *et al.* 1973). Similarly the use of HCG antiserum, whose cross-reactivity with monkey LH was earlier established, in cycling monkeys (Macaca fascicularis) resulted, as a consequence of neutralization of endogenous LH surge, in blockade of ovulation (Moudgal *et al.*

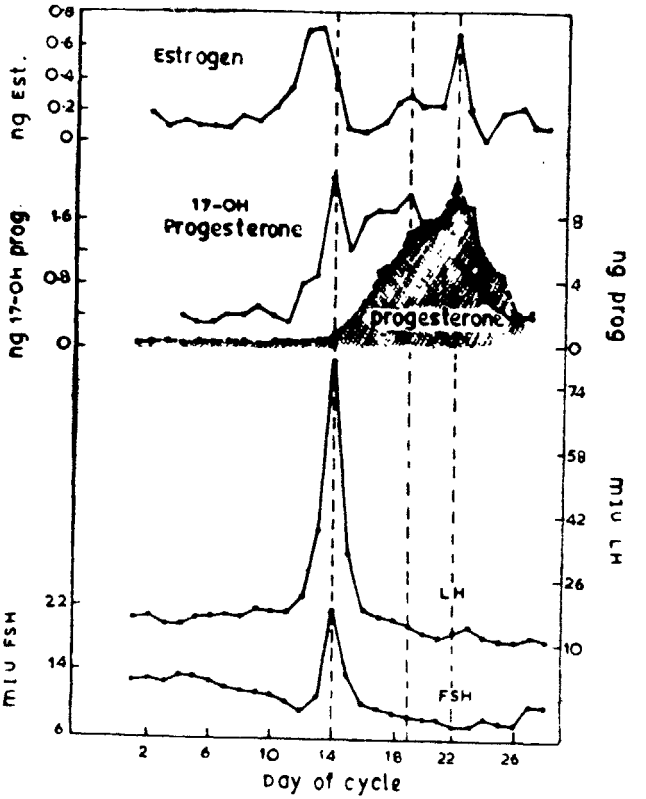



FIG. 1. Typical changes in levels of plasma gonadotropins LH and FSH, and steroids-estrogen, 17 $\alpha$ OH progesterone and progesterone that occurs throughout the menstrual cycle of primates. (*Based on the results of Vande Wiele et al.* 1970)

1971). Three independent parameters—a periodic laparoscopic examination of the surface of the ovary for any ovulation points, foreshortening of the cycle length and failure of plasma progesterone to raise (an indication of non-formation of a corpus luteum) were used as criteria of successful blockade of ovulation (Fig. 3).

The above experiments done in three different species of animals clearly point to LH being the true physiological trigger behind ovulation. The functional significance of FSH surge appearing at the same time as the pre-ovulatory LH surge is not clear and remains to be investigated. It appears essential that LH be present in a surge form for it to be effective in inducing ovulation. In keeping with this is the observation that women who show a chronic high or low level of plasma LH but not in a surge form do not seem to ovulate.

The work of Sasamoto in mice (1969) and Madhwa Raj and Moudgal in rats (1970) has very clearly shown that even though it is necessary for LH to appear in surge form to be effective as an ovulation inducer, its critical need is apparently confined to the first 60–120 min (Table III). LH must be, during this initial period, triggering a primary event which perhaps by bringing about a cascade of

TABLE II

*Characterization of gonadotropic antisera by $^{125}$I-labelled hormone-binding studies*

| Antiserum to | $^{125}$I–Hormone | % specific binding |
|---|---|---|
| Ovine FSH (UA) | FSH | 58 |
| Ovine FSH (UA) | LH | 52 |
| Ovine FSH (UA) | HCG | 13 |
| Ovine FSH (A) | FSH | 51 |
| Ovine FSH (A) | LH | 6 |
| Ovine FSH (A) | HCG | 1.5 |
| Ovine LH (A) | LH | 82 |
| Ovine LH (A) | FSH | 2 |

UA, Unabsorbed antiserum; A, Absorbed antiserum

*Note :* The details of the method are as described by Madhwa Raj and Moudgal (1970) and Jagannadha Rao *et al.* (1973). The hormones (ovine LH and FSH) used for iodination were highly purified substances obtained through the courtesy of Dr. H. Papkoff University, of California, USA. HCG used had a unitage of 18,000 μ/mg. Per cent specific binding refers to net binding after deducting non-specific binding.

TABLE III

*Minimal time exposure to LH needed for the graafian follicle of rats and mice to ovulate*

(Based on the studies of Sasamoto 1969; and Madhwa Raj and Moudgal 1970)

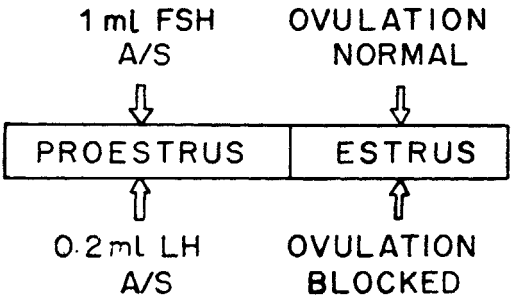
| Duration of gonadotropin neutralization | NO/N | |
|---|---|---|
| | Rats | Mice |
| Untreated (60 min) | 8/8 | 22/23 |
| 10 min | 0/4 | 3/15 |
| 30 min | 0/7 | 3/12 |
| 120 min | 7/9 | 14/15 |

No = Number of animals in which ovulation was observed

N = Total number of animals in each group

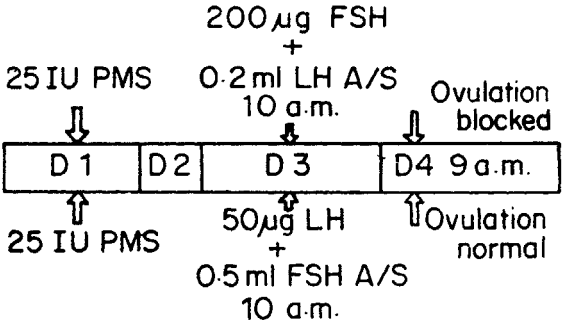
*Note :* For inducing follicular maturation in immature animals, PMS was used and LH (in rats) or HCG (in mice) were given 48 hr later. Exposure to gonadotropin action was controlled by giving appropriate antisera at specific time intervals.

events eventually leads to the physiological process of ovulation. What this primary event could be is presently ill-understood. The fact that actinomycin D and puromycin block LH induced ovulation has been taken to suggest that new nucleic acid and protein synthesis are involved in LH action (Pool and Lipner 1966). Similarly, by using cyanoketone, an inhibitor of steroid hormone synthesis, steroids



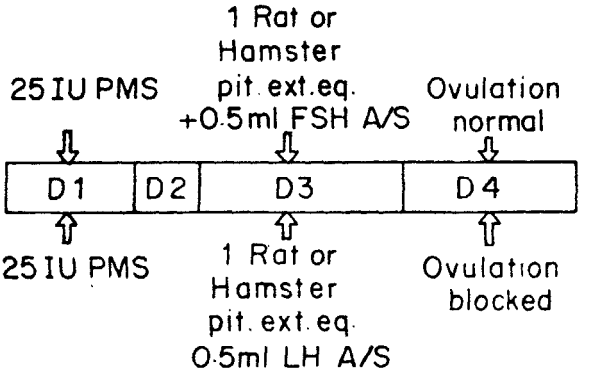

| | RAT | HAMSTER |
|---|---|---|
| | 6/6 | 5/5 |
| | 0/6 | 0/5 |

(A)




| | RAT | HAMSTER |
|---|---|---|
| | 0/4 | 0/4 |
| | 3/3 | 6/6 |

(B)




| | RAT. | HAMSTER |
|---|---|---|
| | 12/12 | 4/4 |
| | 1/7 | 4/4 |

(C)

are implicated in translating LH action on ovulation (Lipner and Greep 1971). Cyclic AMP, a well known mediator of LH action, however, does not appear to induce ovulation when injected direct into the follicle (Le Maire *et al.* 1972). The use of aspirin and indomethacin, two powerful inhibitors of prostaglandin synthesis, in ovulation experiments appears to suggest that prostaglandins are probably involved in the release of LH from the pituitary and also in translating LH action on the ovulable follicle (Orezyk and Behrman 1972; Armstrong and Grinwich 1972). Additional proof is necessary before this suggestion is universally accepted. The mechanics of ovulation itself according to Rondeli (1970) involves the synthesis of hydrolysing (collagenolytic ) enzymes which bring about an increase in stretchability and perhaps consequently weakness of a particular area of the follicle wall. Even here further experimentation appears necessary before the physical process of bursting of ova from the follicle becomes clear.

*Gonadotropins and their influence on corpus luteal function*

Ovulation is followed by luteinization, a de-differentiation process which brings about the conversion of preponderantly estrogen-secreting follicular granulosa and thecal cells into progesterone-secreting luteal cells. While Channing (1969) using *in vitro* granulosa cell cultures feels that LH is necessary for luteinization, El Fouly *et al.* (1970) and Stoklosowa and Nalbandov (1972), using the technique of ovectomy, observe that luteinization is brought about by just the expulsion of the ova itself, and that perhaps ova has an inhibitor of luteinization. Though these luteal cells possess a minimal capacity to synthesize progesterone, their ability of continuing to do so, apparently, is greatly increased in the presence of exogenous LH in the medium (Stoklosowa and Nalbandov 1972).

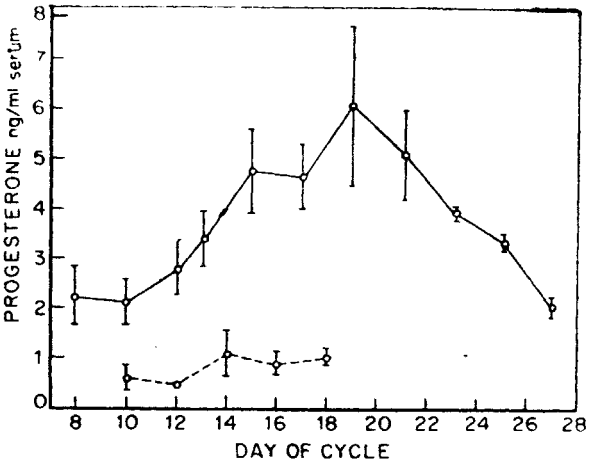
The question that has been raised several times over the last 10–15 years, is whether the luteal cells once they are formed require further gonadotropin stimulus to fulfil their physiological function—that of secreting optimal amounts of progesterone. It has been agreed that species exhibit a great degree of variation in their dependence upon gonadotropin support, the corpus luteum of the pig appearing to be at one end of the spectrum in becoming autonomous soon after it is formed, while the corpus luteum of the rat and hamster are placed at the other end of the spectrum being dependent upon a continuous gonadotropin support.

Most of the classical studies on luteal function have been done using rodents as the experimental animals of choice. Earlier studies essentially used two physio-

FIG. 2. A-C. Studies on ovulation in adult and immature rats and hamsters. A, adult cycling rats and hamsters were used in the present study; antiserum was given at 10 a.m. of proestrus, the quantity administered being more than sufficient to neutralize endogenous FSH or LH activity ; B, immature rats of 25 days and hamsters of 28 days were used in this study—FSH and LH used were of ovine origin and gifts of NTH, Bethesda. (The figures No/N shown against rats and hamsters refer to the number of animals ovulated/number of animals used); C, this study also made use of immature rats and hamsters similar to that detailed above. The experimental protocol is indicated in the figure. The amount of LH and FSH antisera used was sufficient, as determined in a separate experiment, to neutralize the LH and FSH activity, respectively present in one rat or hamster pituitary.

*(Based ou the results of Jagannadha Rao et al.* 1973)

logical end-points, namely, (a) continued maintenance of vaginal diestrus smear in the phase of constant estrogen challenge, and (b) obtaining a good decidual cell reaction in pseudopregnant rats traumatised on day 5 as indices of luteal functionality. Using the above two parameters, prolactin has been shown (Astwood and Greep 1938; Malven and Sawyer 1966; Macdonald and Greep 1968; Macdonald *et al.* (1970) repeatedly to be the luteotropin in rodents. The suggestion that this



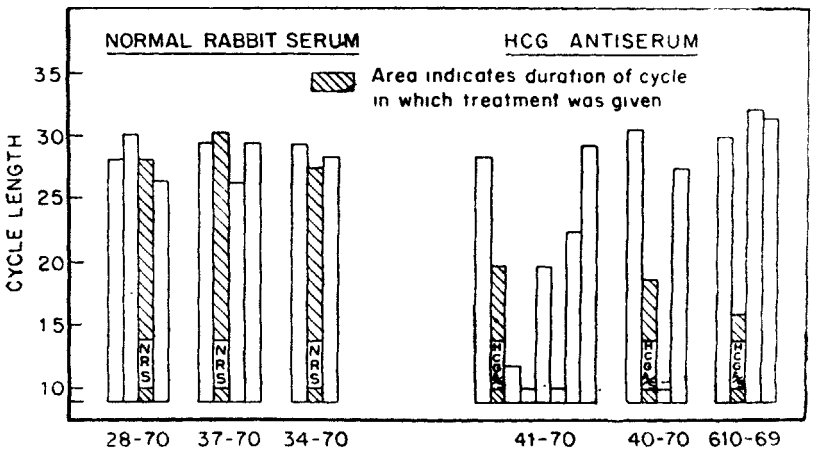




FIG. 3. Effect of HCG antiserum on ovulation in *Macaca fascicularis*. The upper figure refers to plasma progesterone levels (mean ± S. E) in these macaques following NRS (——) and antiserum injection (. . . .) The lower figure refers to the effect of antiserum or normal rabbit Serum (NRS) injection on cycle lengths of experimental and controls respectively. The numbers in the X-axis refer to the individual number of macaques used in the investigation. The antiserum and NRS were injected in volumes of 2 ml/day, from days 10-13 of the cycle. (*After Moudgal et al.* 1971)

should be so in all species of animals has been put forward by Greenwald (1968). This preference to prolactin is despite the fact that it is unable to increase progesterone output, in *in vivo* and*vitro* studies, In contrast to this is LH, which has been held responsible only for estrogen synthesis while in actual fact it has been observed to increase luteal progestin production in most of the species tested (Armstrong *et al.* 1964; Hillard *et al.* 1963 and Rice *et al.* 1964).

Onset of pregnancy or implantation ensures the lengthening of the functional life-span of the corpus luteum. This would mean that the corpus luteum would receive as a consequence of nidation stimuli which puts it on the road to full development. Thus the corpus luteum of pregnancy secretes maximal amounts of progesterone. In the rat progesterone production reaches a maximum by day 12-14, it thereafter reducing gradually to reach minimal values in time for parturition (Fig. 4 a) (Hashimoto *et al.* 1968). One should expect, in keeping with the above, that there should be a differential threshold for progesterone to maintain different physiological end-points. The data presented in Fig. 4 b show very clearly that this assumption is correct, and that maintenance of diestrus smear in the vagina and obtaining optimal decidualization are both dependent upon minimal

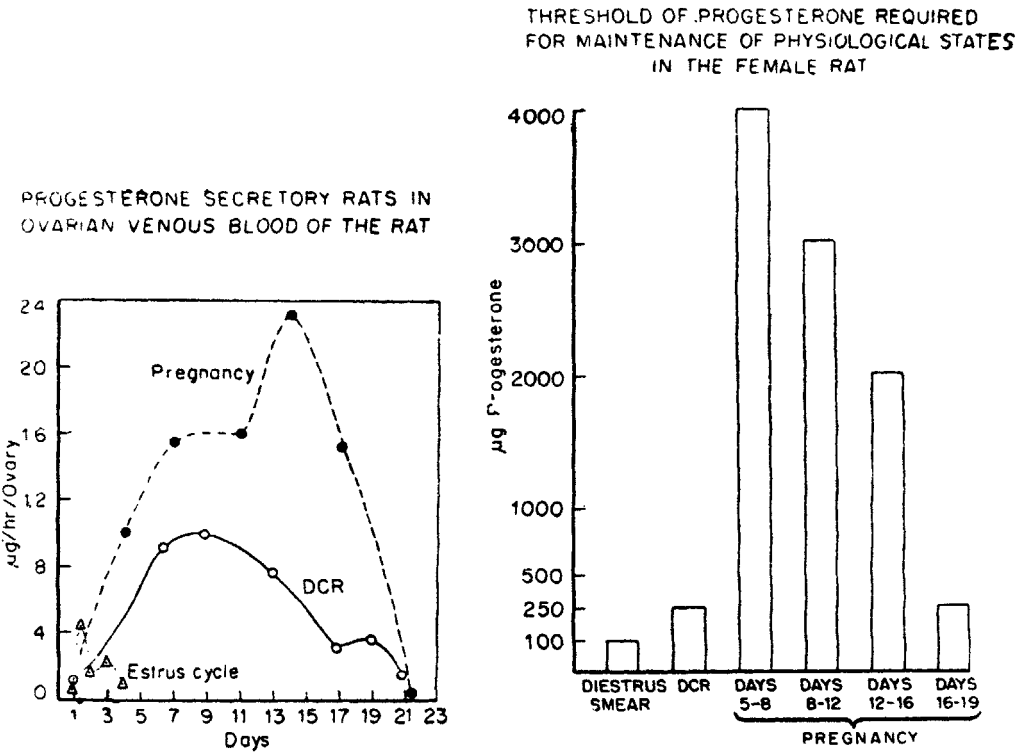



FIG. 4. A comparison between the endogenous ovarian progesterone secretory rats during different physiological states of the rat and the threshold of progesterone required for maintenance of these physiological end-points. The volumes for ovarian progesterone secretory rate (Fig A) were compiled from the data of Hashamoto *et al.* (1968). The progesterone threshold values (Fig B) were from the data of Maneckjee and Moudgal (1973).

amounts of progesterone (Maneckjee and Moudgal 1973). Even in pregnancy, the period when progesterone requirement is high is restricted only for the first 12 days, the need for progesterone dropping sharply thereafter. If maintenance of pregnancy is a measure of luteal functionality, then prolactin on its own is unable to support gestation when given to hypophysectomized rats (Greenwald and Johnson 1968; Ahmad and Lyons 1969) and hamsters (Greenwald 1967). Addition of FSH and LH to the prolactin supplementation, however, has been shown to support pregnancy in hypophysectomized rat and hamster. It is believed that prolactin's inability to support pregnancy in hypophysectomized animals is not due to its lack of luteotropic activity but due to the absence of much needed estrogen support made available by the combined action of FSH and LH (Greenwald and Johnson 1968). The reason for disbelieving the role of LH as a luterotropin is perhaps due to its ability to (a) break the continuous vaginal diestrus pattern (may be by increasing estrogen output), (b) its ability to cause luteolysis when given in relatively large doses in saline over a period of time, and (c) finally the fact that plasma LH level is at its lowest during the luteal phase, a period when maximal amount of progesterone is being produced.

Administration of HCG antiserum, which has been characterized earlier for its cross-reactivity with monkey LH (Moudgal *et al.* 1971) to cycling monkeys in their luteal phase results, as shown in Fig. 5, in a reduction in plasma progesterone level and consequently in termination of the cycle (Moudgal *et al.* 1972). This experiment thus clearly shows that eventhough the plasma levels of LH during the luteal phase are very low, its neutralization can significantly alter luteal functionality. Vande Wiele *et al.* (1970) reporting on induced ovulation in the human observe that HCG given as a ovulation inducer is not able to ensure continued maintenance of progesterone production from the corpus luteum for the entire luteal phase. In order to obtain normal luteal lentgh of 14 days, it appears essential to give 2 to 3 more injections of HCG. Since LH appears to have a much shorter tissue half-life than HCG, in normal menstrual cycle, the continuous presence of LH, eventhough in small amounts, may be necessary to obtain full luteal phase of 14 days.

Pregnancy in the rat and hamster can be disrupted by administering a single injection of LH antiserum on any day before day 11 (Fig. 6) (Madhwa Raj *et al.* 1967, 1968; Moudgal *et al.* 1969; Madhwa Raj and Moudgal 1970; Jagannadha Rao *et al.* 1970, 1972). This effect can be overcome only by simultaneous administration of progesterone or LH but not by prolactin, FSH or estrogen. Similar to the cycling monkey in its luteal phase. the LH levels in the pregnant rat and hamster are at a minimum throughout gestation.

Luteal functionality perhaps is best judged, instead of by using physiological end-points by actually determining its ability to secrete progesterone. Thus, it should be evident from Fig. 7 that lack of LH effects progesterone output from the corpus luteum of the pseudopregnant, pregnant or lactating rat (Behrman *et al.* 1972; Yoshinaga *et al.* 1971 and Moudgal *et al.* 1972). LH deprival further appears to influence cholesterol turnover in the corpus luteum. Due to pronounced inhibition in the activity of the enzyme cholesterol esterase and an activation of the enzyme cholesterol ester synthetase, there is an accumulation of cholesterol

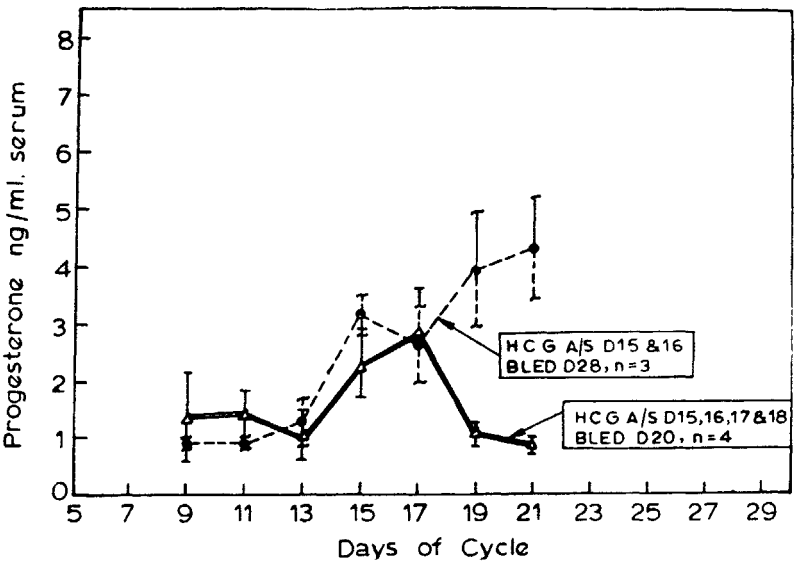



FIG. 5. Effect of HCG antiserum on the luteal phase of cycling macaques (*Macaca fascicularis*). Antiserum was given for either 2 or 4 days and plasma was withdrawn on specific days during the experiment for determination of progesterone concentration, an indication of luteal functionality. (*After Moudgal et al.* 1972)

MAINTENANCE OF PREGNANCY AS A CRITERION OF LUTEAL FUNCTIONALITY IN RATS & HAMSTERS

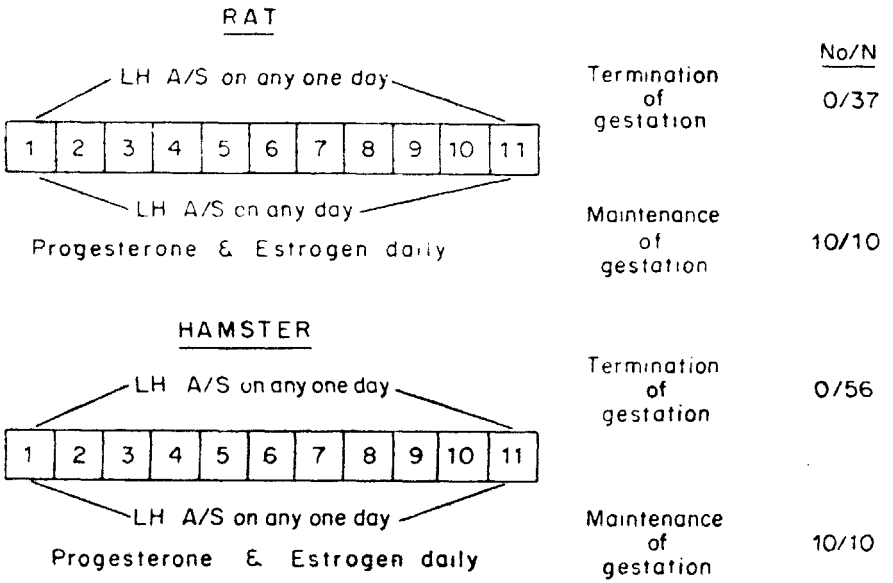



FIG. 6. Effect of LH deprival on luteal functionality as judged by the ability of LH antiserum to disrupt pregnancy in rats and hamsters. The experimental protocol is given in the Figure (*After Madhwa Raj and Moudgal* 1970; *and Jagannadha Rao et al.* 1972).

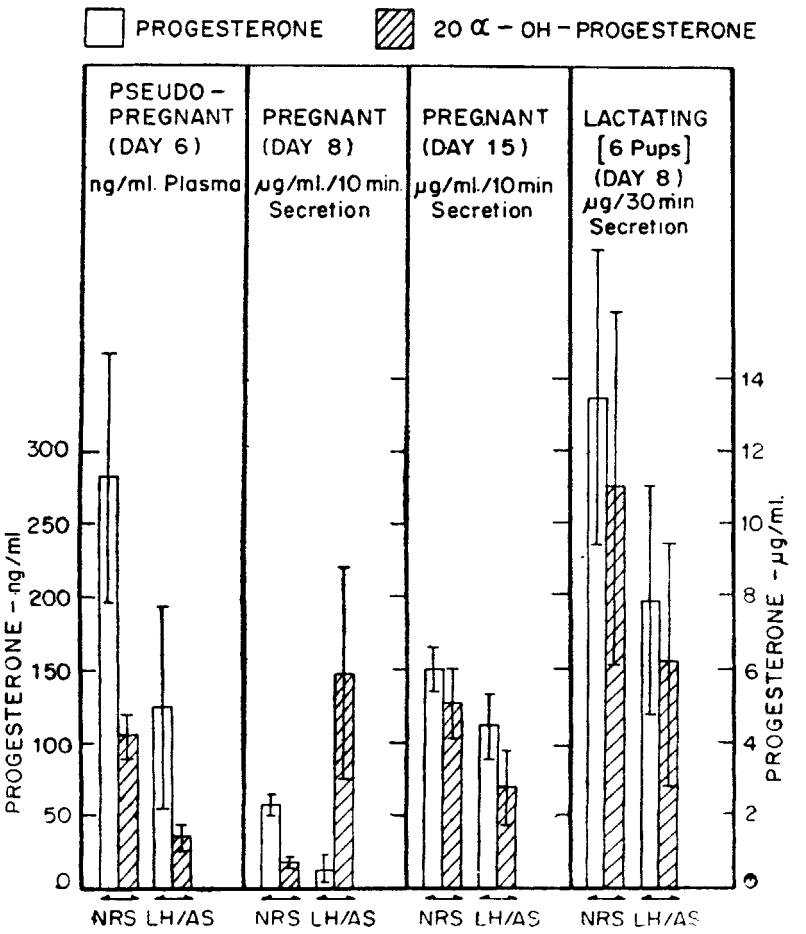



FIG. 7. Effect of LH antiserum injection on plasma progesterone levels of rats in different physiologieal states. Experimental details are described in the individual publications. (*Based on the results of Yoshinaga et al.* 1970; *Moudgal et al.* 1972; *and Behrman et al.* 1972).

ester stores in the ovary (Table IV). LH could thus very well control progesterone secretion from the corpus luteum by influencing the rate of cholesterol turnover or synthesis in this tissue. Earlier evidence (Armstrong *et al.* 1969) points to LH regulating another key-step in steroidogenesis, namely, the activity of the side chain cleaving enzyme desmolase. It is suggested that LH exercises this control by making available optimal amount of reducing equivalents (NADH) essential for the expression of desmolase activity. It is also known that LH increases the intracellular level of cyclic AMP (Marsh *et al.* 1966), which generally accelerates the conversion of pregnenalone to progesterone (Marsh and Savard 1966).

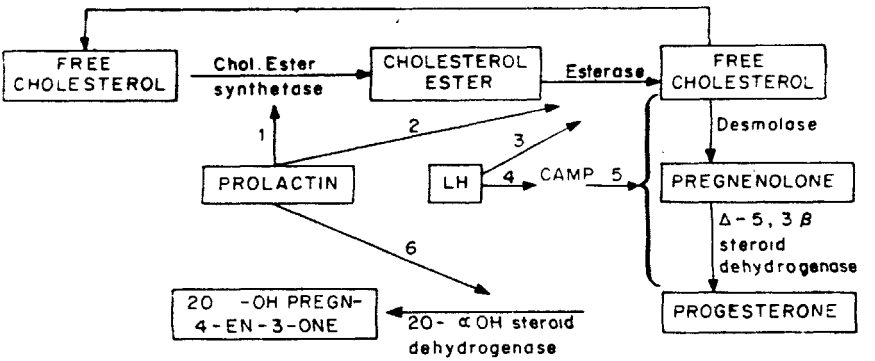
Prolactin is also known to influence the activity of the enzymes that control cholesterol turnover. Thus, while hypophysectomy reduces the level of cholesterol ester synthetase and esterase, the supplementation with prolactin permits maintenance

TABLE IV

*Effect of LH deprival on cholesterol turnover in the ovary of the rat*
(Based on the results of Moudgal *et al.* 1972 and Behrman *et al.* 1972)

| Treatment | Cholesterol mg/g | Cholesterol ester mg/g | Cholesterol ester synthetase cpm/min/mg protein | Cholesterol esterase % hydrolysis min/mg protein |
|---|---|---|---|---|
| | | Day 8 pregnant rats | | |
| *NRS | 4.25±0.25 | 11.1±1.6 | — | — |
| LH A/S | 5.35±0.29 | 24.3±2.5 | — | — |
| | | Day 5 pseudopregnant rats | | |
| *NRS | 1.8±0.2 | 4.4±0.7 | 651±78 | 1.19±0.18 |
| LH A/S | 2.5±0.9 | 10.6±1.0 | 1013±109 | 0.10±0.02 |

*Control rats which received normal rabbit serum instead of LH antiserum




1, 2 Maintenance function ; 3 Activates enzyme ; 4 Enhances level of CAMP, 5 Accelerates steroidogenesis, and 6 Inhibits enzyme.

FIG. 8, Attempts at integrating the available knowledge on LH and prolactin action on luteal function. The depicted model perhaps is mostly true for the rodent and its applicability to other higher species needs to be confirmed.

of enzyme activity at pre-hypophysectomy levels (Behrman *et al.* 1972). In addition, prolactin has been shown to have a very important regulating effect on the type of progestin leaving the ovary of the rat. Thus, prolactin by inhibiting the activity of the enzyme 20α–hydroxy steroid dehydrogenase prevents conversion of physiologically active progesterone to metabolically inactive 20α–OH dihydro-progesterone (Wiest *et al.* 1968). Perhaps this role of prolactin as a regulator of the type of progestin leaving the ovary is true for other species also.

These newer evidences are incorporated into the overall scheme of progesterone synthesis depicted in Fig. 8. It thus appeais that both prolactin and LH have to synergise to produce progesterone at optimal rates. While LH could be regarded as the pace setter or amplifier of the signal, prolactin in addition to providing the substrate appears to regulate the type of progestational steroid leaving the ovary. This integrated scheme presupposes that the luteal cell once it is formed is endowed with all the necessary enzymes involved in steroidogenesis and what the tropic stimulus would do is only to increase the rate of turnover or synthesis of progestins. This would also allow us to understand how prolactin alone could maintain luteal functionality as judged by physiological end-point like diestrus smear and decidualization reactions, dependent upon low progesterone threshold. In contrast, under situations where progesterone threshold is high, like in pregnancy, the dependence on amplification of signal by LH appears to become critical.

## References

Ahmed, N., Lyons, W. R., and Papkoff, H. (1969). Maintenance of gestation in hypophysectomized rats with highly purified pituitary hormones. *Anat. Rec.*, **164**, 291–304.

Armstrong, D. T., and Grinwich, D. L. (1972). Blockade of spontaneous and LH-induced ovulation in rats by indomethacin, an inhibitor of prostaglandin biosynthesis. *Prostaglandins*, **1**, 21–26.

Armstrong, D. T., Jackanicz, T. M., and Keyes, P. L. (1969). Regulation of steroidogenesis in the rabbit ovary. *In :* 'The Gonads' pp. 3–24, *ed. by* K. W. Mckerns. Apleton Century Crofts, New York.

Armstrong, D. T., O'Brien, J., and Greep, R. O. (1964). Effects of luteinizing hormone on progestin biosynthesis in the luteinized rat ovary. *Endocrinology*, **75**, 488–500.

Astwood, E. B., and Greep, R. O. (1938). A corpus luteum stimulation substance in the rat placenta. *Proc. Soc. exp. Biol. Med.*, **38**, 713–716.

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. Endocr.*, **52**, 419–426.

Catchpole, H. R. (1964). Physiology of the gonadotropic Hormones. *In :* Gonadotropins, their Chemical and Biological Properties and Secretory Control, pp 40–70, *ed. by* H. H. Cole. W. H. Freeman & Co., San Francisco.

Channing, C. P. (1969). The use of tissue culture of granulosa cells as a method of studying the mechanism of luteinization. *In :* The Gonads, pp 246–276. *ed. by* K. W. McKerns. Appleton Century Crofts, New York.

El Fouly, M. A., Cook, B., NeKola, and Nalbandov, A. V. (1970). Role of ovum in follicular maturation. *Endocrinology*, **87**, 288–293.

Ferin, M., Zimmering, P. E., and Vande Wiele, R. L. (1969). Effects of antibodies to estradiol 17β on PMS induced ovulation in immature rats. *Endocrinology*, **84**, 893–900.

Goldman, B. D., and Mahesh, V. B. (1969). Possible role of acute FSH release in ovulation in the hamster as demonstrated by utilization of antibodies to LH & FSH. *Endocrinology*, **84**, 236–245.

Goldman, B. D., and Porter, J. C. (1970). Serum LH levels in intact and castrated Golden Hamsters. *Endocrinology*, **87**, 676–679.

Greenwald, G. S., and Johnson, D. C. (1968). Gonadotropin requirements for the maintenance of pregnancy in hypophysectomized rat. *Endocrinology*, **83**, 1052–1064.

Greenwald, G. S. (1967). Luteotropic complex of the hamster. *Endocrinology*, **80**, 118–130.

————(1968). Formation and maintenance of corpora lutea in laboratory animals. *J. Anim. Sci.*, **27**, 139–162.

Harrington, F. E., Bex, F. J., Elton, R. L., and Roach, J. B. (1970). The ovulatory effects of follicle stimulating hormone treated with chymotrypsin in chlorpromazine blocked rats. *Acta endocr.*, **65**, 222–228.

Hashimoto, I., Henricks, D. M., Anderson, L. L., and Melampy, R. M. (1968). Progesterone and pregn-4-en-20α-o1-3-one in ovarian venous blood during various reproductive states in the rat. *Endocrinology*, **82**, 333–341.

Hillard, J., Archibald, D., and Sawyer, C. H. (1963). Gonadotropic activation of preovulatory synthesis and release of progestin in the rabbit. *Endocrinology*, **72**, 59–66.

Jagannadha Rao, A., Madhwa Raj, H. G., and Moudgal, N. R. (1970). Need of luteinizing hormone for early pregnancy in the golden hamster (*Mesocricetus auratus*). *J. Reprod. Fert.*, **23**, 353–355.

————(1972). Effect of LH, FSH and their antisera on gestation in the hamster *Mesocricetus auratus*. *J. Reprod. Fert.*, **29**, 239–249.

Jagannadha Rao, A., Moudgal, N. R., Madhwa Raj, H. G., Lipner, H., and Greep, R. O. (1973). Role of FSH and LH in initiation of ovulation in rats and hamsters : A study using rabbit antisera to ovine FSH and LH (Communicated to *J. Reprod. Fert.*).

Kelly, W. A., Robertson H. A., and Stansfield D. A. (1963). The suppression of ovulation in the rat by rabbit anti ovine LH serum. *J. Endocr.*, **27**, 127–128.

Le Maire, W. J., Mills, T., Yutaka Ito, and Marsh, J. M. (1972). Inhibition by 3′–5′ cyclic AMP of luteinization induced by intra follicular injection of luteinizing hormone. *Biol. Reprod.*, **6**, 109–116.

Lipner, H. and Greep, R. O (1971) Inhibition of steroidogenesis at various sites in the biosynthetic pathway in relation to induced ovulation. *Endocrinology*, **88**, 602–607.

Lostroh, A. J., and Johnson, R. E. (1966). Amounts of interstitial cell stimulating hormone and follicle stimulating hormone required for follicular development, uterine growth and ovulation in hypophysectomized rat. *Endocrinology*, **79**, 991–996.

Macdonald G. J., and Greep R. O. (1968). Maintenance of progestin secretion from rat corpora lutea. *Perspect. Biol. Med.*, **11** 490–497.

Macdonald G. J. Tashjian A. H. Jr., and Greep R. O. (1970). Influence of exogenous gonadotropins antibody formation and hysterectomy on the duration of luteal function in hypophysectomized rats. *Biol. Reprod.*, **2**, 202–208.

Madhwa Raj, H. G., Sairam, M. R., and Moudgal, N. R. (1967). Role of gonadotropins in implantation—A study using specific antigonadotropins. *Indian J. exp. Biol.*, **5**, 123–124.

Madhwa Raj, H. G., Sairam, M. R., and Moudgal, N. R. (1968). Involvement of luteinizing hormone in the implantation process of the rat. *J. Reprod. Fert.*, **17**, 335–341.

Madhwa Raj, H. G., and Moudgal, N. R. (1970). Hormonal control of gestation in the intact rat. *Endocrinology*, **86**, 874–889.

Madhwa Raj, H. G., and Moudgal, N. R. (1970). Effect of anti-lutinizing hormone serum on the ovulation of rats. *Nature, Lond.*, **227**, 1344–1345.

Malven, P. V., and Sawyer, C. H. (1966). Formation of new corpora lutea in mature hypophysectomised rats. *Endocrinology*, **78**, 1259–1263.

Moneckjee, R. and Moudgal, N. R. (1973). Differential threshold of progesterone required for maintenance of diestrus smear pseudopregnancy and pregnancy in rats. *Proc. Soc. exp. Biol. Med.*, **143**, 69–72.

Marsh, J. M., Butcher, R. W., Savard, K., and Sutherland E. W. (1966). The stimulatory effect of luteinizing hormone on adenosine 3′, 5′ monophosphate accumulation in corpus luteum slices. *J. Biol. Chem.*, **241**, 5436–5440.

Marsh, J. M., and Savard, K. (1966). The stimulation of progesterone synthesis in bovine corpora lutea of adenosine 3′, 5′ monophosphate. *Steroids*, **8**, 133–148.

Monroe S. F., Atkinson, L. E., and Knobil, E. (1970). Patterns of circulating luteinizing hormone and their relation to plasma progesterone levels during the menstrual cycle of the monkey. *Endocrinology*, **87**, 453–455.

Moudgal, N. R., and Li, C. H. (1961). An immunochemical study of sheep pituitary interstitial cell stimulating hormone. *Arch. Biochem. Biophys.*. **95**, 93–98.

Moudgal, N. R., Macdonald, G. J., and Greep, R. O. (1971). Effect of HCG antiserum on ovulation and corpus luteum formation in the monkey (*Macaca fascicularis*). *J. Clin. Endo. Metab.*, **32**, 579–581.

Moudgal, N. R., Macdonald, G. J., and Greep, R. O. (1972). Role of endogenous primate LH in maintaining corpus luteum function of the monkey., *J. clin. Endocr., Metab.*, **35**, 113–116.

Moudgal, N. R., Madhwa Raj, H. G., Jagannadha Rao, A., and Sairam, M. R. (1969). The need of luteinizing hormone for maintaining early pregnancy in the rat. *Indian J. epx. Biol.*, **7**, 45–46.

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. Endocr.*, **52**, 413–418.

Niswender, G. D., Midgley, A. R., Monroe, S. E., and Reichert, L. E., Jr., (1968). Radioimmunoassay for rat luteinizing hormone with anti ovine serum and ovine LH 1311. *Proc. Soc. exp. Biol. Med.*, **128**, 807–811.

Orczyk, G. P., and Behrman, H. R. (1972). Ovulation blockade by aspirin or indomethacin—*in vitro* evidence for a role of prostaglandin in gonadotropin secretion. *Prostaglandins*, **1**, 3–13.

Rice, B. F., Hammerstein J., and Savard K. (1964). Steroid hormone formation in the human ovary II Action of gonadotropins in vitro in the corpus luteum. *J. clin. Endocr., Metab.*, **24**, 606–615.

Rondell, P. (1970). Follicular process in ovulation. *Fedn. Proc. Fedn. Am. Soc. exp. Biol.*, **29**, 1875–1879.

Sasamoto, S. (1969). Inhibition of HCG induced ovulation by anti HCG serum in immature mice pre-treated with PMSG. *J. Reprod. Fert.*, **20**, 271–277.

Sawyer, C. H. (1964). Control of secretion of gonadotropins in: Gonadotropins — their chemical and Biological Properties and Secretory Control, pp. 113–159, *ed. by* H. H. Cole, W. H. Freeman & Co., San Francisco.

Scaramuzzi, R J , Blake, C. A., Papkoff, H., Hillard, J., and Sawyer, C. H. (1972). Radioimmunoassay of rabbit luteinizing hormone serum levels during various reproductive states. *Endocrinology*, **96**, 1285–1291.

Schwartz, N. B. (1969). A model for the regulation of ovulation in the rat. *Recent Prog. Horm. Res.*, **25**, 1–43.

Stocklosowa, S., and Nalbandov, A. V (1972). Luteinization and steroidogenic activity of rat ovarian follicles cultured in vitro. *Endocrinology*, **91**, 25–32.

Vande Wiele, R. L., Bogumil, J., Dyrenfurth, I., Ferin, M., Jewelowicz, R., Warren, M., Rizkallah, T., and Mikhail, G. (1970). Mechanisms regulating the menstrual cycle in women. *Recent Prog. Horm. Res.*, **26**, 63–95.

Wiest, W. G., Kidwell, W. R., and Balough, K., Jr., (1968). Progesterone catabolism in the rat ovary: A regulatory mechanism for progestational potency during pregnancy. *Endocrinology*, **82**, 844–859.

Yoshinaga, K., Moudgal, N. R., and Greep, R. O. (1971). Progesterone secretion by the ovary in lactating rats. Effect of LH antiserum, LH and prolactin. *Endocrinology*, **88**, 1126–1130.