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Comparative Effects of Antiserum to Luteinizing Hormone on Pseudopregnancy and Pregnancy Induced in the Same Rat

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The comparative role of luteinizing hormone (LH) in maintaining pregnancy and histamine-induced decidualization in the rat was studied with the help of a new system, wherein the above two states could be brought about simultaneously in the same animal, but in different uterine horns. Specific and well-characterized LH antiserum, administered daily, both during the pre-trauma (days 1–4) and post-trauma (days 5–8) periods, resulted in the termination of pregnancy and inhibition of decidualization. This antiserum effect could be reversed by suitable steroid therapy. Results suggest that the antiserum blockade of ovarian steroidogenesis continued even after cessation of its treatment.

Early pregnancy and decidualization seem directly comparable in that both are dependent upon LH to stimulate the ovarian synthesis of much-needed progesterone and estrogen.

Previous investigations from this laboratory have shown that selective neutralization of endogenous luteinizing hormone (LH), using a specific and well-characterized antiserum (A/S), resulted in the inhibition of implantation and the termination of pregnancy in the rat (Madhwa Raj, Sairam and Moudgal, 1967, 1968; Madhwa Raj and Moudgal, 1970). These effects of the antiserum were shown to be counteracted by estrogen and progesterone, indicating that LH is involved in the synthesis of these two steroids in the intact rat.

In an attempt to compare the relative need for LH to induce decidualization and maintain pregnancy, a new system has been described here, wherein, in the same animal, but in different uterine horns, the above two phenomena could be studied. A preliminary account of this was reported by us recently (Moudgal *et al.*, 1970).

MATERIALS AND METHODS

Immunization of rabbits with ovine LH, collection of antisera and its characterization have been described earlier in detail (Madhwa Raj and Moudgal, 1970). The rabbit anti-ovine LH used here has been shown to be free of antibodies to serum- and organ-specific proteins. Both agar gel diffusion test and a sensitive radiolabeled hormone binding technique have been used to detect the presence of antibodies in the LH antiserum to other possible contaminating hypophyseal hormones, such as follicle-stimulating hormone, prolactin or growth hormone. Quantitative precipitin and biological neutralization tests were used to determine the binding capacity of both ovine and murine LH to the antiserum (Madhwa Raj and Moudgal, 1971).

Adult, virgin, female albino rats of our Institute colony weighing about 160–200 g were mated with males of proven fertility, and the day on which sperms were observed in the vaginal smear was considered as day 1 of pregnancy. On this day, the left uterine horn was ligated at the uterotubal junction, to ensure that no ova would pass into this uterine horn. Decidualization was induced in the ligated horn by an intraluminal injection of 20 μg histamine (histamine dihydrochloride, Sigma Chemical Co.) in 0.1 ml of distilled water, at 10 h on day 5 of pregnancy, the time of maximal uterine sensitivity (Varavudhi *et al.*, 1966; Shelesnyak and Kraicer, 1961). Fertilized ova were allowed to implant normally in the contralateral horn. The rats were separated into different groups, with suitable controls, for treatment with LH antiserum and steroid hormones.

LH antiserum (0.2 ml/rat) was administered by subcutaneous route at 10 h on different days, both during the "pre-trauma" (days 1–4) and the "post-trauma" (days 5–8) phases. Steroids, when supplemented, were injected subcutaneously, using refined peanut oil as the vehicle. The control rats were traumatized on day 5, and injected with 0.9% saline instead of antiserum. The animals were autopsied on day 10. At autopsy, the ovarian and uterine weights, the number of implantation sites, and the extent of decidualization were noted. Differences between groups were analyzed with Student's "t" test.

The uterine tissues were processed for histology and stained with haematoxylin and eosin.

RESULTS

Characterization of antiserum by methods described earlier has showed it to be specific for LH. One milliliter of this antiserum bound 400 μg of ovine LH in the quantitative precipitin test, and this antiserum cross-reacted with rat pituitary LH to the extent of 50% in the above test (Madhwa Raj and Moudgal, 1970, 1971). Twenty-five microliters of this antiserum was able to neutralize the LH activity contained in one rat pituitary, as assayed by the ovarian ascorbic acid depletion test of Parlow (1961).

Effects of LH antiserum in the "pre-trauma" period. The control animals, as a result of fertile mating, followed by unilateral ligation of the tube, showed implantation sites only in the unligated horn. Traumatization of the ligated horn on day 5 led to a massive decidual cell response (DCR) in this horn, as seen on day 10 (Table 1, Group I). Daily administration of 0.2 ml LH antiserum from days 1 to 4, in addition to inhibiting implantation, also brought about a suppression of the DCR significantly ($P < 0.001$, Table 1, Groups I:II). Supplementation was estradiol-17β on day 4 only, either at the 2-μg or 0.01-μg level, did not reverse the effects of the antiserum, as seen at autopsy on day 10. While daily supplementation with 4 mg progesterone could partially reverse the LH antiserum effects (Table 1, Group V), the degree of DCR and the number of implantation sites seen were greater when 0.01 μg of estradiol-17β was also included with progesterone ($P < 0.001$), Table 1, Groups II:VI). However, a higher

TABLE 1

EFFECTS OF LH ANTISERUM IN THE PRE-TRAUMA PERIOD (DAYS 1–4)[a]

Group	Treatment	No. of rats	Uterine weight (mean ± SE)		Average No. of sites/rat in the non-traumatized horn	P value (traumatized horn)
			Traumatized horn	Non-traumatized horn		
I	Saline control	9	1182 ± 167.8	645 ± 124.8	5	
II	LH antiserum (days 1–4)	7	102 ± 5.13	80 ± 7.2	—	<0.001 (I:II)
III	LH antiserum + 2 μg estradiol-17β (days 1–4)	8	110 ± 13.02	97 ± 8.35	—	<0.001 (I:III) >0.5 (II:III)
IV	LH antiserum + 0.01 μg estradiol-17β (days 1–4)	5	265 ± 49.5	140 ± 14.15	—	>0.02 (I:IV) <0.001 (II:IV)
V	LH antiserum + 4 mg progesterone (days 1–4)	8	322 ± 80.72	122 ± 22.65	2	<0.001 (I:V) >0.02 (II:V)
VI	LH antiserum + 4 mg progesterone (days 1–4) 0.01 μg estradiol-17β (days 1–4)	7	596 ± 52.11	212 ± 43.54	4	0.001–0.01 (I:VI) <0.001 (II:VI)
VII	LH antiserum + 4 mg progesterone (days 1–4) + 2 μg estradiol-17β (day 4)	6	107 ± 11.14	107 ± 20.8	—	<0.001 (I:VII) >0.5 (II:VII)

[a] The day sperms were seen in the vaginal smear was considered as day 1 of pregnancy. The left uterine horn was ligated at the uterotubal junction on day 1. 0.2 ml of LH antiserum was given per rat per day, from days 1–4, by the subcutaneous route. Steroid hormones dissolved in refined peanut oil were also given by subcutaneous route. The left uterine horn was traumatized with 20 μg histamine on day 5. All the rats were autopsied on day 10. Control animals received 0.9 % saline instead of antiserum.

TABLE 2

EFFECTS OF LH ANTISERUM IN THE POST-TRAUMA PERIOD (DAYS 5–8)[a]

Group	Treatment	No. of rats	Uterine weight (mean ± SE)		Average No. of sites/rat in the non-traumatized horn	P value (traumatized horn)
			Traumatized horn	Non-traumatized horn		
I	Saline control	9	1182 ± 167.8	645 ± 124.8	5	
II	LH antiserum (days 5–8)	9	410 ± 79.69	184 ± 33.6	—[b]	<0.001 (I:II)
III	LH antiserum + 4 mg progesterone (days 5–8)	8	712 ± 93.8	280 ± 34.32	3	>0.02 (I:III) >0.02 (II:III)
IV	LH antiserum + 2 μg estradiol-17β (days 5–8)	8	113 ± 14.12	133 ± 20.18	—	<0.001 (I:IV) 0.001–0.01 (II:IV)
V	LH antiserum + 0.01 μg estradiol-17β (days 5–8)	5	234 ± 74.47	186 ± 41.36	—	0.01–0.02 (I:V) 0.1–0.5 (II:V)
VI	LH antiserum + 4 mg progesterone (days 5–8) + 0.01 μg estradiol-17β (days 5–8)	5	863 ± 100.0	585 ± 21.34	5	0.05–0.1 (I:VI) <0.001 (II:VI)

[a] 0.2 ml of LH antiserum was given per rat per day, from days 5–8, by the subcutaneous route. The left uterine horn was traumatized with 20 μg histamine on day 5. All rats were autopsied on day 10.

[b] Some had 2–3 resorbing sites.

dose of estradiol-17β, e.g., 2 μg, was inhibitory even in conjunction with progesterone to both DCR and implantation. These results are summarized in Table 1.

Effects of LH antiserum in the "post-trauma" period. LH antiserum affected the DCR and the survival of the blastocyst, in a similar manner, when administered to the rats from days 5 to 8. This inhibitory effect could be reversed by the daily supplementation of 4 mg progesterone alone ($P < 0.05$, Table 2, Groups II:III). Inclusion of 0.01 μg of estradiol-17β with progesterone brought about a further increase in the DCR ($P < 0.001$, Table 2, Groups II:VI) and also increased the number and the size of the implantation sites seen at autopsy on day 10. This potentiation effect of estradiol-17β, however, was not statistically significant when compared to the effect of progesterone alone ($P > 0.5$, Table 2, Groups III and VI). Estradiol-17β alone, either at the 0.01-μg or the 2-μg level, was ineffective in overcoming the LH antiserum inhibition (refer Table 2).

No significant change in the ovarian weights or number of corpora lutea could be observed following treatment with the antiserum both in the pre- and post-trauma period.

The histological examination of the uteri of rats from different groups essentially confirmed the inhibitory effect of LH antiserum on the DCR and the ability of a combination of progesterone and estrogen to reverse it. The inability of estrogen alone to overcome the effect of the antiserum on the DCR was also confirmed by histology.

Effect of continued steroid supplementation on the LH antiserum inhibition of decidual cell response (DCR). In the previous experiments it was observed that if the steroid supplementation was restricted only to days of the LH antiserum treatment, their ability to overcome the inhibitory effect was only partial. These results could probably be explained as due to continued inhibition by LH antiserum of steroid synthesis beyond day 4 (Table 1) or day 8 (Table 2), the period during which no exogenous steroids were administered. In contrast, it can be seen from results presented in Table 3, that continued supplementation with steroids will completely overcome the effects of the antiserum inhibition. It can also be observed that when the antiserum is given from days 1 to 9, administration of estradiol-17β on day 4 only with continuous progesterone admin-

TABLE 3

EFFECT OF CONTINUED STEROID SUPPLEMENTATION ON THE LH ANTISERUM INHIBITION OF THE DCR[a]

Group	Treatment	No. of rats	Uterine weight (mean ± SE)		Average no. of sites/rat in the non-traumatized horn	*P* value (traumatized horn)
			Traumatized horn	Non-traumatized horn		
I	Saline control	9	1182 ± 167.8	645 ± 124.8	5	
II	LH antiserum + 4 mg progesterone (days 1–9)	4	755 ± 32.9	232 ± 41.9	3	>0.10 (I:II)
III	LH antiserum + 4 mg progesterone (days 1–9) + 0.01 μg estradiol-17β (day 4)	4	1110 ± 104.9	281 ± 37.5	4	>0.50 (I:III)
IV	LH antiserum + 4 mg progesterone + 0.01 μg estradiol-17β (days 1–9)	4	116 ± 9.0	308 ± 78.3	4	<0.001 (I:IV)
V	LH antiserum + 4 mg progesterone (days 5–9)	4	1256 ± 123.3	335 ± 25.3	4	>0.50 (I:V)
VI	LH antiserum + 4 mg progesterone + 0.01 μg estradiol-17β (days 5–9)	4	945 ± 24.7	370 ± 33.5	5	>0.10 (I:VI) >0.10 (V:VI)

[a] 0.2 ml of LH antiserum was given per rat per day, either from days 1–9 or days 5–9, by the subcutaneous route. The left uterine horn was traumatized with 20 μg histamine on day 5. All rats were autopsied on day 10.

istration will bring about a more complete reversal, when compared to that brought about by progesterone alone.

DISCUSSION

Pseudopregnancy in the rat has been studied extensively by several investigators, and since it bears a great resemblance to the events occurring in early pregnancy, it has been used as an experimental model (see review, Shelesnyak, 1957). Implantation has been directly compared to the trauma induced either by mechanical or chemical means (Shelesnyak, 1957). There seems, however, to be a difference in the relative need for progesterone to sustain pregnancy and pseudopregnancy. It has been shown that while the plasma progesterone level reaches maximal concentration by day 6 in pseudopregnancy, the same is attained in pregnancy by day 12 (Wiest, 1970). Thus, there seems to be a direct correlation between the life span of the corpus luteum and the duration of pseudopregnancy or pregnancy.

In the present investigation, making a direct comparision between pseudopregnancy and pregnancy, it has been possible to show that both react to the lack of LH in a similar manner. The observation that the antiserum effects, both in the pre- and post-trauma or pre- and post-implantation period could be overcome by a combination of progesterone and estrogen and not by estrogen alone, agrees with our earlier observation on the LH antiserum effects in pregnant rats. The results of Kiracofe *et al.* (1969), however, seem to suggest that of the two steroids, progesterone and estrogen, estrogen could reverse the LH antiserum effects on DCR to a greater extent. Even though it is not possible at the present time to understand the reasons for the disparity between Kiracofe's and our observation, it could be attributed generally to the higher titre of antiserum used by us, resulting, probably, in a more complete neutralization of endogenous LH, leading to a greater degree of inhibition of DCR. Thus the ability of estrogen to overcome the antiserum effect observed by Kiracofe *et al.*, could be compared to the facilitatory effect of estrogen on progesterone action observed here (Table 1, Groups V and VI; Table 2, Groups III and VI; Table 3, Groups II and III). It was generally observed, in confirmation of the work of Yochim and De Feo (1962, 1963), that while the estrogen in

small quantities potentiated progesterone effect, inclusion at high doses was inhibitory.

It is possible to conclude from these investigations that both pregnancy and pseudopregnancy are dependent on LH to maintain the synthesis of progesterone and estrogen at optimal concentrations. This confirms the recent observations of Moudgal, Behrman and Greep (1972) and Behrman, Moudgal and Greep (1972), that LH antiserum injected into either pregnant or pseudopregnant rats, brings about a drastic reduction in the progesterone content of ovarian venous effluents.

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