PREGNANCY BLOCK IN MICE: CHANGES IN PITUITARY LH AND LTH AND PLASMA PROGESTIN LEVELS

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Summary. A significant decrease in pituitary LH accompanied by an increase in blood plasma progestins was observed in pregnant females 24 hr after exposure to alien males (Day 2 post-mating). Conversely, no change in pituitary LTH was detectable until 48 hr after females were caged with alien males. These data suggest the LH release may play an important rôle in pregnancy block.

Pregnancy may be blocked in laboratory mice by isolating recently mated females from their stud males and exposing them to alien males (Bruce, 1959, 1960). Pregnancy can also be blocked by exposing recently mated females either to the urine of alien males (Dominic, 1964) or to a cage which has been soiled by alien males (Parkes & Bruce, 1962). However, after implantation has been accomplished, alien males no longer interfere with the maintenance of pregnancy. These findings suggest that the effect of the alien male is an interference with the endocrine maintenance of pregnancy sometime before implantation.

Studies on the mechanisms responsible for pregnancy block have demonstrated that the effects of exposure to alien males can be overridden by either injecting prolactin (LTH) (Parkes & Bruce, 1961) or by grafting pituitary tissue to the kidney capsule (Dominic, 1967). Furthermore, pregnancy block does not occur in lactating females where LTH levels are normally increased (Bruce & Parkes, 1961). On the basis of these data, Dominic (1967) suggested that the primary mechanism for pregnancy block was an inhibition of pituitary LTH release and the subsequent failure of the corpora lutea.

The present experiments were primarily designed to test the hypothesis that exposure of recently mated females to alien males would result in changes in pituitary LTH concentration. A secondary objective of this study was to determine whether or not changes in the levels of pituitary LH and plasma progestins coincided with implantation failure initiated by exposing pregnant mice to alien males. Our results show that changes in pituitary LH and plasma progestins occur within 24 hr of exposing mated females to alien males while

changes in pituitary LTH levels were not detected until 48 hr after male exposure. An alternative to LTH inhibition as the mechanism of pregnancy block is proposed.

Female SJL/J mice, between 6 and 18 weeks old, were reared in groups of four to six in $15 \times 15 \times 30$ cm stainless steel cages with 14 hr of light every 24 hr. At 18 weeks of age, the females were paired with SJL/J males of the same age. Females were examined each morning for vaginal plugs. After detecting a vaginal plug, a female was assigned at random to one of the following treatment groups: (1) Day 0, (2) Day 2-control, (3) Day 2-exposed to alien male, (4) Day 3-control, and (5) Day 3-exposed to alien male. Day-0 females were killed the morning a vaginal plug was observed. The Day-2 and Day-3 control females were left with their SJL/J stud male and killed either 2 or 3 days after the plug was observed. The Day-2 and Day-3 females exposed to an alien male were removed from their SJL/J stud male's cage 24 hr after the plug was observed, placed in a BALB/cJ male's cage and killed either 24 or 48 hr later. The BALB/cJ males were kept in a separate room from the SJL/J pairs to avoid mixing male odours in the same room which would lower the efficiency of the pregnancy block. All mice were killed by decapitation between 10.00 and 12.00 hours and blood was collected by drainage into heparinized tubes. The heparinized tubes were centrifuged at 10,000 *g* for 10 min at 5° C and the plasma was removed and stored at -25° C. Plasma progesterin concentrations were determined by the method of Murphy (1967).

Pituitary glands were removed and pooled by treatment groups and frozen at -25° C. At the time of assay, pituitaries were weighed, homogenized (10 mg/ml in 0.85% sodium chloride prepared with pyrogen-free water) centrifuged at 5000 *g* for 15 min at 5° C and the supernatant fluid assayed for LH and LTH activity.

Luteinizing hormone was measured for each treatment in duplicate by the 4-hr ovarian ascorbic acid depletion method of Parlow (1961), employing five rats (Sprague Dawley) for each dose level of unknown or standard. Pituitary LH potencies were measured with 0.2 and 0.8 mg-equivalents of fresh tissue and these were compared with 0.4 and 1.6 μ g of NIH-LH-s11.

Prolactin (LTH) was determined twice in each treatment group by the pigeon crop sac 'micro' method of Reece & Turner (1937). The pigeons (White-King) were 5 to 6 weeks old at the time of assay. Pituitary LTH concentrations were measured with 0.2 and 0.8 mg-equivalents of fresh tissue and these were compared with 1 and 4 μ g of NIH-P-s3. For each assay, eight pigeons were used at each dose level of the unknown or standard.

No significant changes were observed in the concentration of pituitary LTH on Days 0, 2, or 3 for the control animals (Table 1). Similarly, there were no measurable changes in the concentration of pituitary LTH between the Day-0 and Day-2 females that were exposed to alien males. However, the 3-day group exposed to males had a higher concentration of pituitary LTH than did the 3-day control females (1.47 versus 0.85 μ g/mg).

The pituitary LH concentration of control females ranged from 0.35 to 0.20 μ g/ml between Day 0 and Day 3 post-mating ($P > 0.25$, Table 1). In contrast to the relatively constant level of LH in the pituitaries of control females, the

pituitary LH concentration of females exposed to alien males for 24 hr was significantly less than that of females killed on the morning after copulation (0.06 versus 0.35 $\mu\text{g}/\text{mg}$; $P < 0.01$) and also less than that of the unexposed females (0.06 versus 0.25 $\mu\text{g}/\text{mg}$; $P < 0.05$). After 48 hr of exposure to alien males, however, the pituitary LH content reached a peak of 0.70 $\mu\text{g}/\text{mg}$ so that it was significantly higher than that of the 48-hr control animals (0.20 $\mu\text{g}/\text{mg}$) and of animals exposed for 24 hr (0.06 $\mu\text{g}/\text{mg}$; $P < 0.01$).

TABLE 1

CONCENTRATIONS OF PITUITARY LUTEINIZING HORMONE (LH), PROLACTIN (LTH), AND BLOOD PLASMA PROGESTINS IN PREGNANT FEMALE MICE EXPOSED TO ALIEN MALES

Treatment	No. of females	LH concentration ¹		LTH concentration ³		Progesterin concentration ⁵ (ng/ml) $\pm$ S.E.
		Potency	95% limits	Potency	95% limits	
Day 0, vaginal plug detected	15	0.41	0.28 to 0.66	0.93	0.41 to 1.84	5.1 $\pm$ 0.12
		0.32	0.23 to 0.47	0.78	0.32 to 1.28	
	Mean	0.35	0.24 to 0.52 ²	0.84	0.34 to 1.49 ⁴	
Day 2, control	16	0.22	0.16 to 0.30	0.82	0.45 to 1.62	8.5 $\pm$ 1.15
		0.29	0.20 to 0.46	0.75	0.30 to 1.47	
	Mean	0.25	0.19 to 0.35	0.76	0.35 to 1.42	
Day 2, exposed to alien male	13	0.04	0.01 to 0.17	0.59	0.34 to 1.20	13.7 $\pm$ 0.93
		0.08	0.03 to 0.18	0.79	0.30 to 1.40	
	Mean	0.06	0.02 to 0.13	0.68	0.30 to 1.24	
Day 3, control	15	0.23	0.10 to 0.38	0.91	0.40 to 1.80	23.5 $\pm$ 1.3
		0.19	0.08 to 0.37	0.80	0.33 to 1.51	
	Mean	0.20	0.10 to 0.34	0.85	0.34 to 1.58	
Day 3, exposed to alien male	15	0.63	0.52 to 1.09	1.26	0.89 to 2.40	5.7 $\pm$ 0.2
		0.77	0.58 to 1.32	1.91	1.12 to 3.62	
	Mean	0.70	0.55 to 1.20	1.47	1.00 to 2.87	

¹ Potency and 95% confidence limits expressed as μg -equivalents of NIH-LH-s11/mg of pituitary tissue (wet weight).

² Mean LH concentration expressed as the combined potency of two replicate assays and their 95% confidence intervals.

³ Potency and 95% confidence limits expressed as μg -equivalents of NIH-P-s3/mg of pituitary tissue (wet weight).

⁴ Mean prolactin concentration expressed as the combined potency of two replicate assays and their 95% confidence interval.

⁵ Mean progesterin concentration based upon duplicate measurements of three pools of plasma from each treatment. This method measures total progestins which include progesterone and 17 α -OH progesterone.

The plasma progesterin concentration increased from 5.1 to 8.5 ng/ml (Table 1) between Day 0 and Day 2 of pregnancy in the control females ($P < 0.01$), and between Days 2 and 3 of pregnancy, the plasma progesterin concentration increased three-fold to 23.5 ng/ml ($P < 0.01$; Table 1). At 24 hr following exposure to alien males, the females had higher blood plasma progesterin concentrations than control females, 8.5 versus 13.7 ng/ml ($P < 0.01$) but by Day 3 post-mating, the females exposed to the alien male had 5.7 ng/ml compared with 23.5 ng/ml blood progestins in control females ($P < 0.01$).

The observation that pituitary LTH concentration was increased on Day 3 in

females exposed to alien males suggests that LTH release may have been depressed by alien males. This interpretation is in agreement with the findings that pregnancy block can be prevented by exogenous LTH (Parkes & Bruce, 1962), lactation (Bruce & Parkes, 1961) and by ectopic pituitary grafts (Dominic, 1967). These data are consistent with the proposal by Dominic (1967) that interference with LTH secretion is the primary cause of pregnancy blocks. However, our data indicate that the mechanism of pregnancy block may also include altered secretion of other anterior pituitary hormones whose action may precede LTH blockage.

In this study, decreased pituitary LH and increased plasma progesterin concentrations were noted in females within 24 hr of exposure to alien males, during which time no comparable change in LTH was observed. Females with blocked pregnancies return to oestrus and typically show copulation plugs on Day 4 post-mating. However, in females exposed to alien males, the depletion of pituitary LH occurred at least 48 hr before ovulation could be expected. The ability of the female pituitary-gonadal axis to respond with pronounced changes after 24 hr of exposure to an alien male is not surprising since Bruce (1960) could obtain pregnancy block in females exposed to alien males for as little as 12 hr. Our findings suggest that pituitary LH release in post-insemination females is triggered by exposure to alien males, and it is tempting to speculate that this release may be a primary endocrine response in the sequence of events that leads to pregnancy block.

Additional evidence indirectly supports the possibility that LH release can be a primary factor in pregnancy block. Luteinizing hormone can modify the corpora lutea in pregnant rats either by depleting its secretory potential or by exerting a generalized luteolytic effect. Our findings of decreased pituitary LH and increased plasma progestins on Day 2 also correspond with the observations that progesterone administered on the day after mating induces luteal involution in mice (Burdick, 1942) and in guinea-pigs (Aldred, Sammelwitz & Nalbandov, 1961; Nalbandov, 1961).

The control females in this study exhibited a slight decline in pituitary LH between Day 0 and Day 3. These observations suggest that a continuing release of LH normally occurs in early pregnancy. The effect of the alien male may be an over-stimulation of the normal release. This would also explain why pregnancy block does not occur after 4 or 5 days post-mating when pituitary LH concentration is decreased and plasma progesterone levels are high. This study cannot exclude other explanations for pregnancy block; nevertheless, these data do indicate that the rôle of LH release should not be ignored in future studies of the physiological mechanism responsible for this phenomenon.

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