OBSERVATIONS ON THE REPRODUCTIVE PHEROMONES OF MICE

II. NEURO-ENDOCRINE MECHANISMS INVOLVED IN THE OLFACTORY BLOCK TO PREGNANCY

C. J. DOMINIC*

Physiological Laboratory, University of Cambridge

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Summary. The neuro-endocrine mechanisms involved in the olfactory block to ovo-implantation in mice were studied by investigating methods of preventing the block from taking place. Daily administration of 50 i.u. of prolactin to females on Days 1 to 5 is fully effective in preventing the block to pregnancy. Pregnancy block can also be inhibited in normal females by the presence of a functioning ectopic pituitary homograft, which serves as an additional source of prolactin. Reserpine is also effective in inhibiting the block to pregnancy, thus providing a direct proof of hypothalamic intervention in the reaction. The inhibition of the pregnancy block by exogenous progesterone and the histological appearance of the corpora lutea give further support to the view that implantation failure is due to failed luteal function. The results reported in this study provide additional evidence in favour of the view that olfactory stimulation from males causes the failure of prolactin secretion and initiates the secretion of FSH and LH by stimulating the hypothalamus. This, in turn, causes the failure of corpus luteum development and the return of the female to oestrus in the ordinary way.

INTRODUCTION

The characteristics of the olfactory block to ovo-implantation as described by Bruce (1959, 1960), Bruce & Parrott (1960) and Bruce & Parkes (1961) indicate as the cause of the pregnancy failure an interference with pituitary function which in turn prevents the normal development of the corpora lutea. The administration of prolactin (Bruce & Parkes, 1960) or increased production of prolactin induced by suckling in lactating females pregnant from post-partum conception (Bruce & Parkes, 1961) would prevent the block, but progesterone appeared to be much less effective. The implication of the hypothalamus was taken for granted.

However, no further experiments directly concerned with the neuro-endocrine mechanisms involved have been reported although much information about other aspects of this reaction has accumulated in the meantime. Moreover only a brief description of the histology of the ovary after a blocked

* Present address: Department of Zoology, Banaras Hindu University, Varanasi 5, India.

pregnancy at 8 to 10 days after mating was given by Bruce (1960). It therefore seemed opportune to re-examine the question and to seek additional evidence of the neuro-endocrine background by earlier histological examination of the ovaries after pregnancy block.

The mechanism of the olfactory block was studied by investigating methods of preventing the block from taking place. It has been shown (Dominic, 1964, 1966) that exposure to the urine of alien males (i.e. belonging to a different strain from the stud male) is as effective in causing the pregnancy to fail in recently-mated females, as exposure to the males themselves. The former method was used to induce pregnancy block in the experiments described below.

MATERIALS AND TECHNIQUE

The animals used and the experimental procedures employed are fully described in the preceding paper (Dominic, 1966). As usual the female was exposed to urine from twelve alien (CBA) males in the arrangement described previously (i.e. the wire basket-funnel arrangement) for Days 1 to 3 after mating (vaginal plug Day 0).

TABLE 1
INHIBITION OF PREGNANCY BLOCK BY EXOGENOUS PROLACTIN

<i>Treatment</i>	<i>No. of females</i>	<i>No. of females remaining pregnant</i>	<i>No. of females pseudopregnant</i>	<i>No. of females returning to oestrus</i>
Fresh urine of alien males + prolactin	58	43	12	3 (5%)
Fresh urine of alien males + saline	58	5	3	50 (86%)
Undisturbed	26	25	—	1 (4%)

RESULTS

Effect of exogenous prolactin (Table 1)

Initial attempts to prevent the block to pregnancy caused by exposure to male urine, with prolactin, in the dose used by Parkes (1961), 10 i.u./female/day, on Days 1 to 3 after mating, were unsuccessful, and it was thought desirable to increase the dose to 50 i.u./female/day and to extend the period of administration to 5 days. A group of recently-mated females received intramuscular injections of prolactin (ovine, NIH-P-S4) at this dose level for Days 1 to 5 after mating, i.e. throughout the period of exposure to urine and for 2 further days. A control group, also exposed to urine, received a daily injection of saline (0.5 ml/female) for the same period.

In this dose, prolactin was fully effective in preventing the block to pregnancy in females exposed to fresh urine of alien males.

Effect of ectopic pituitary graft (Table 2)

Hypophysial autografts to an ectopic site continue to secrete luteotrophin (prolactin) in rats and mice (see Everett & Nikitovitch-Winer, 1963, for review),

but only small amounts of FSH and LH. Such grafts in normal mice can, therefore, be used as an additional source of prolactin. To examine the effect of pituitary grafts on pregnancy block, each P female received a homograft of the anterior pituitary from another female of the same age. The graft was placed in the subcapsular space of the right kidney and not more than 5 min elapsed between killing the donor and transplanting her pituitary to the renal capsule of the host. The commonest external manifestation of a functioning

TABLE 2
INHIBITION OF PREGNANCY BLOCK BY ECTOPIC PITUITARY HOMOGRAFT

<i>Treatment</i>	<i>No. of females</i>	<i>No. of females remaining pregnant</i>	<i>No. of females pseudopregnant</i>	<i>No. of females returning to oestrus</i>
Urine of alien males + ectopic pituitary homograft	64	53	—	11 (17%)
Urine of alien males	52	5	2	45 (87%)
Undisturbed	20	17	1	2 (10%)

ectopic graft in mice is a lengthening of the di-oestrous period which results in a series of pseudopregnancy-like cycles (Mühlbock & Boot, 1959). Vaginal smears from the graft-bearing females were examined and only those which showed this response were used. After a minimum of two pseudopregnancy-like cycles the graft-bearing females were paired with stud P males. On finding a vaginal plug, the female was separated from the male and tested in the usual way.

The presence of a functioning ectopic pituitary graft also prevented the block to pregnancy.

TABLE 3
INHIBITION OF PREGNANCY BLOCK BY RESERPINE

<i>Treatment</i>	<i>No. of females</i>	<i>No. of females remaining pregnant</i>	<i>No. of females pseudopregnant</i>	<i>No. of females returning to oestrus</i>
Urine of alien males + reserpine	54	38	8	8 (15%)
Urine of alien males + saline	54	7	3	44 (81%)
Undisturbed	21	17	1	3 (14%)

Effect of reserpine (Table 3)

In general, the production of pituitary hormones is stimulated by the central nervous system with the exception of prolactin, of which the secretion and release by the adenohypophysis is chronically inhibited by the hypothalamus (Meites, Nicoll & Talwalker, 1963). This implies that, in pregnancy block, olfactory stimulation from males causes the failure of prolactin secretion and

initiates the secretion of FSH and LH by stimulating the hypothalamus (Bruce, 1964). It was therefore of interest to examine the effect of reserpine on pregnancy block.

Reserpine (Serpasil, CIBA), 6.25 µg/female, was injected intramuscularly into recently-mated females exposed to male urine on Days 1 to 3 after mating. Reserpine was injected for 5 days. This dose of reserpine was found to be sufficient to cause the suppression of oestrus in unmated females without inducing any side-effects such as loss of body weight.

This treatment was also fully effective in preventing the block to pregnancy in females exposed to male urine.

Effect of progesterone (Table 4)

The experiments so far described have been concerned with the failure of pituitary function. The next experiment was designed to study the ovarian effects. As in the previous experiments hormonal treatment was given for

TABLE 4
INHIBITION OF PREGNANCY BLOCK BY EXOGENOUS PROGESTERONE

<i>Treatment</i>	<i>No. of females</i>	<i>No. of females remaining pregnant</i>	<i>No. of females pseudopregnant</i>	<i>No. of females returning to oestrus</i>
Urine of alien males + progesterone	54	49	3	2 (4%)
Urine of alien males + saline	54	5	—	49 (91%)
Undisturbed	51	45	—	6 (12%)

Days 1 to 5 after mating. The test females received progesterone (Protormone—Burroughs, Wellcome and Co.), 1 mg/female/day, intramuscularly. Control females received injections of saline (0.5 ml/female/day) over the same period.

Under these conditions progesterone proved just as effective as prolactin in preventing the block to pregnancy.

EXPLANATION OF PLATE 1

Fig. 1. A part of the corpus luteum on Day 7 of normal pregnancy. Note the luteal cells with well-defined outlines and abundant cytoplasm. Bouin fixation and haemalum staining. Compare with Fig. 3. $\times$ circa 1000.

Fig. 2. Corpus luteum on Day 7 of normal pregnancy. Frozen section stained in Sudan Black. Note the luteal cells with lightly staining and evenly distributed fat globules in the cytoplasm. Compare with Fig. 4. N, Nuclei. $\times$ circa 1200.

Fig. 3. Corpus luteum, on Day 7 after mating, from the ovary of a mouse whose pregnancy was blocked on Day 4. Bouin fixation and haemalum staining. Note the shrunken cells with ill-defined outlines. $\times$ circa 1200.

Fig. 4. Corpus luteum, on Day 7 after mating, from the ovary of a mouse whose pregnancy was blocked on Day 4. Frozen section stained in Sudan Black. Note the accumulation of coarse deeply staining fat globules in the luteal cells. F, Fat globules; N, nuclei. $\times$ circa 1200.

(Facing p. 418)

Histology of the corpora lutea in blocked pregnancy

Pregnant females were killed on Day 7 after mating. Females in which pregnancy was blocked were killed either on Day 7 after mating or on the day of return to oestrus. As a routine procedure, the right ovary was fixed in Bouin's fluid and the left one in 10% formol saline. Ovaries fixed in Bouin's fluid were embedded in paraffin, serially sectioned and stained in Mayer's haemalum for routine histological study. Ovaries fixed in formol saline were embedded in gelatine and frozen sections were taken from them. These sections were stained either with Sudan Black or Sudan IV for the demonstration of fat.

The histology of the corpora lutea of normal pregnancy has been described in detail by Deanesly (1930).

In contrast to the corpora lutea on Day 7 of normal pregnancy in which the cells are large with clearly defined cell boundaries and lightly staining diffused fat globules in the cytoplasm (Pl. 1, Figs. 1 and 2), the luteal cells after a blocked pregnancy are smaller, somewhat shrunken and with ill-defined cell boundaries (Pl. 1, Figs. 3 and 4). The fat granules appear coarser and stain very intensely with Sudan Black. In most cases there is considerable accumulation of fat and the luteal cells appear as darkly staining patches in the lumen of the corpus luteum. The corpora lutea, on the whole, appear to be poorly vascularized.

In females killed on the day of return to oestrus, i.e. the day of pregnancy failure, the histological appearance of the corpora lutea is essentially similar to that of animals killed on Day 7 after mating.

DISCUSSION

Prolactin, in doses previously found adequate to prevent pregnancy block in females exposed to the proximity of males in the original experiments of Bruce & Parkes (1960), proved inadequate when females were exposed to male urine. However, increasing the daily dose and extending the period of treatment to 5 days corrected this, so that pregnancy was maintained in spite of exposure to male urine. These observations suggest that exposure to male urine may provide a stronger stimulus than the proximity of males. The comparison, however, should be made with caution for it must be remembered that the experiments were not concurrent nor even carried out in the same laboratory.

The inhibition of the block to pregnancy in females bearing ectopic graft of the pituitary in the kidney capsule provides additional evidence in support of the view that in pregnancy block there is failure of the luteotrophic activity of the anterior pituitary.

Like prolactin, reserpine is also effective in inhibiting the olfactory block to pregnancy, thus providing direct proof of hypothalamic intervention in the reaction. Reserpine suppresses the inhibitory centre in the hypothalamus controlling the release of prolactin (see the review by Gaunt, Chart & Renzi, 1963). Reserpine inhibits the gonadotrophic cells and stimulates the luteotrophic cells of the hypophysis (Pasteels, 1961). It seems likely, therefore, that reserpine acts by removing the inhibitory influence of the hypothalamus on pituitary luteotrophin secretion.

The inhibition of pregnancy block by exogenous progesterone, and the histological appearance of the corpora lutea support the view that implantation failure is due to failed luteal function. Parkes (1961) obtained only 37% of inhibition of block to pregnancy in females receiving 1.5 mg progesterone daily for 3 days, presumably because the period of injection was not long enough to maintain pregnancy until the establishment of placental function. Treatment for 5 days in the experiments here reported enabled 90% of females to remain pregnant.

Signs of regression in the luteal cells of the corpora lutea of females killed on the day of return to oestrus presumably indicate that luteal function fails before the appearance of any external manifestation of pregnancy failure. Deanesly (1930) believes that corpus luteum degeneration in the mouse begins some time before the onset of next oestrus. The present findings are in agreement with her views.

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