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A BLOCK TO PREGNANCY IN THE MOUSE CAUSED BY PROXIMITY OF STRANGE MALES*

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Summary. Pregnancy and pseudopregnancy both failed in a high proportion of mice when the recently mated female was housed with or near strange males, particularly males of a different strain, or when she was housed with a castrated male. In these circumstances, the female returned to oestrus 4 to 5 days after the original mating and implantation did not take place.

The use of genetically marked test males of a different strain from the stud males showed that superfoetation did not occur.

By contrast, pregnancy was not blocked when the female was returned to her own stud male after being separated from him for 24 hr, or when she was kept in the presence of other females. The evidence suggests that the presence of other females may even help towards stabilizing a pregnancy.

The histology of the ovaries from females having blocked pregnancies showed a varying degree of pituitary-gonadotrophin stimulation.

The fate of the blastocyst from the first mating remains as yet undetermined.

INTRODUCTION

Experiments on the effects of feeding certain synthetic compounds to non-pregnant female mice (Bruce, 1958) were extended to include the pregnant animal. This involved placing the recently mated female with a different male on the day following the finding of the vaginal plug. Pregnancy failed in a number of the mice, particularly among the control groups receiving propylene glycol only and mating with the new male took place 3 to 6 days later. A further experiment showed that this effect was produced not only by dosage with inert material, but even without any treatment other than the introduction of a strange male (Table 1). About 40% of females failed to become pregnant or even pseudopregnant under these conditions, a far higher proportion than could be attributed to the expected incidence of anovular cycles or failure of corpus luteum development. Under the conditions of the experiment only eleven out of 141 control females (7·8%) returned to oestrus in 4 to 7 days,

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that is, without becoming pseudopregnant, after an infertile first mating. The infertility could not therefore be attributed to the stud males.

A scrutiny of the records of control groups in previous experiments supplied additional evidence. A total of twenty-three females, over a period of many months, had for one reason or another been housed with a strange male after mating; five mated with the second male within a few days. As isolated incidents, the significance of the second matings was not appreciated at the time, but it now became likely that the original pregnancy had been blocked by the presence of the strange males.

Experiments were immediately undertaken to explore the effect more fully. Two test situations were devised for study. Situation A was as already described:

TABLE 1

ANOMALOUS BEHAVIOUR OF RECENTLY MATED FEMALES IN CONTROL GROUPS

Treatment		*Females*			
		Total No.	*Pregnant*	*Pseudopregnant*	*Coitus with test ♂ or returned to oestrus at 4 to 5 days after initial coitus*
Original observation	Propylene glycol 0.04 ml/♀/day on days 1 to 6 of pregnancy	11	6	1	4
Repeat experiment	As above	25	13	3	9
	None	13	4	2	7
Totals		49	23	6	20

Females separated from stud males after coitus (Day 0), housed in pairs with a test male 24 hr later when treatment was started

the female was paired with a strange partner 24 hr after copulation with a stud male. Situation B was one of proximity without contact, the recently mated female being placed in a small cage inside a stock box containing other mice.

The results of these investigations, a summary of which has already appeared (Bruce, 1959), are reported here.

METHODS AND RESULTS

Young female mice, generally virgin, were placed with stud males and examined daily for matings. The females were removed from the male when the vaginal plug was found; they were housed together overnight and presented with the test situation the following day. Most of the females were tested in pairs, but a few were either housed alone or in threes depending on the number available. Daily vaginal smears were examined. The females were generally left in the test situation for 7 to 10 days. Pregnant females were isolated before parturition. Pseudopregnant females and those in which pregnancy had been blocked received a fertility test and the few infertile females were discarded. In all experiments, the males were left undisturbed and the females introduced into their boxes. This obviated handling the males which is sometimes resented

(Emmens, 1950). The experiment started on the day on which the vaginal plug was found, which was counted as Day 0. Pregnancy was considered to be blocked when the female returned to oestrus within 7 days — a longer interval, in the absence of pregnancy, is regarded as indicating a pseudopregnancy.

Mice

Most of the mice were albinos of the Parkes stock bred in this Institute. The colony is closed and randomly mated.

Black-eyed males of the inbred G sub-strain of CBA mice (Parrott & Parkes, 1960) were also used as test animals. They provided a genetic marker when paired with the albino females.

Only a few tests have been made with mice belonging to other strains as indicated.

Housing

The mouse boxes were all metal; sawdust was used as floor covering and wood-wool supplied for nesting to the pregnant females. The dimensions of the boxes were:

Small box	16″ × 6″ × 4″
Stock box	20″ × 15″ × 5″
Wire mesh cage (½″ mesh)	6″ × 4½″ × 4″

SITUATION A

The mated females were paired with the following partners:

(1) Strange normal male.
(2) Testosterone-treated albino male. Each male received 10 mg testosterone propionate in oil injected subcutaneously in four doses over a period of 2 weeks. The test was made 3 to 4 weeks after the last injection.
(3) Castrated albino male. Castration was carried out on the young adult. Tests were made at about 6 weeks and again at 18 weeks after the operation.
(4) Parous albino female.
(5) Ovariectomized albino female. The ovaries were removed from young parous females about 3 months old. The test was made about 4 weeks after operation.
(6) Original stud albino male.

The results are given in Table 2.

Pregnancy was regularly blocked in about one-third of the females by introducing them to a strange male within 24 hr of mating; it was blocked by an androgenized male but to no greater extent. It was also blocked by the presence of a castrated male. In view of the small numbers, the differences in the incidence of pregnancy block by normal, testosterone-treated or castrated albino males are not statistically significant even at the 5% level.

By contrast, pregnancy was carried to term when the female was returned to

her own stud male after separation from him for 24 hr; it was carried to term in the presence of other females whether parous or ovariectomized. In the latter groups, the few infertile matings (3/48, Table 2) were all followed by pseudopregnancy.

TABLE 2

SITUATION A

Partner given at 24 hr after mating with stud male	*No. of females*				
	Total No.	*Pregnant*	*Pseudopregnant*	*With blocked pregnancy*	
				No.	%
(1) Normal male { Albino	69	49	1	19	28
(1) Normal male { G strain	67	21	3	43	63
(2) Testosterone-treated male	18	10	1	7	40
(3) Castrated male	50	36	1	13	26
(4) Parous female	20	19	1	—	—
(5) Ovariectomized female	28	26	2	—	—
(6) Original stud male	46	42	4	—	—

SITUATION B

In this series of tests, in which the mated females or the test males were caged inside a stock box containing other mice, the animals could see, hear and smell, but not touch, one another. Pregnancy was blocked when the stock box contained males; it was not blocked when the mated female was surrounded by other females. In the latter circumstance, as when Situation A included another

TABLE 3

SITUATION B

Conditions of test	*No. of females*				
	Total No.	*Pregnant*	*Pseudopregnant*	*With blocked pregnancy*	
				No.	%
Females caged inside stock box containing: Albino males	32	21	3	8	25
Females caged inside stock box containing: G strain males	68	13	3	52	76
Females caged inside stock box containing: Virgin females	25	20	5	—	—
Females caged inside stock box containing: Parous females	24	20	4	—	—
Females free in stock box containing caged G strain males	27	3	3	21	78
Females caged inside stock box containing caged G strain males	26	10	4	12	46

female, the females which failed to become pregnant all became pseudopregnant (9/49, Table 3).

Similar results were obtained when the males were caged and the females were free in the stock box or even when both sexes were confined independently. In the latter test, the cages were touching one another in groups of nine in which females occupied the four corner cages and the centre cage, and the whole unit was enclosed in one of the stock boxes. The males were caged in

groups of three per cage. The number of females varied between five and ten according to whether they were caged individually or in pairs.

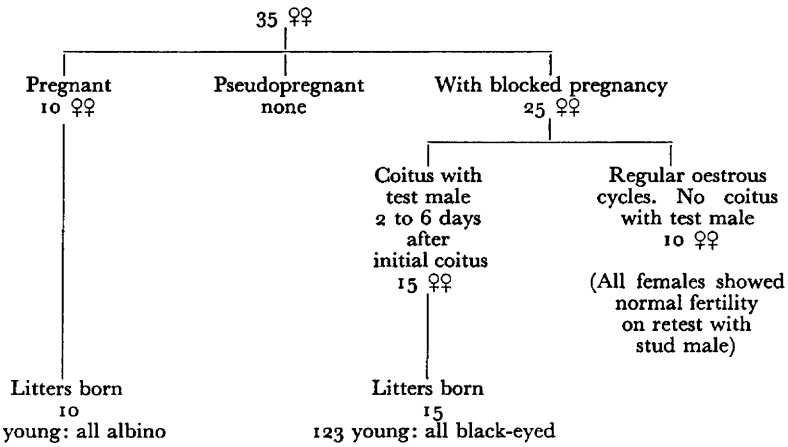
BLOCKED PREGNANCY

Non-occurrence of Superfoetation

The use of the genetically marked males in Situation A showed that superfoetation did not occur. Of thirty-five mated females paired with black-eyed males at 24 hr after mating with stud albino males, only ten remained pregnant (Table 4). These gave birth to a total of ninety-eight young, all pink-eyed. Of the twenty-five females in which pregnancy was blocked, fifteen mated with the

TABLE 4

PREGNANCY BLOCK IN THE MOUSE



Albino females housed with strange black-eyed test male 24 hr after coitus with stud albino male.

new test males and immediately became pregnant producing 123 black-eyed young. This includes one female whose litter of eight black-eyed young was born only 19 days after the introduction to the test male, 20 days after her original mating with the stud albino male. The remaining ten females continued to have regular cycles in the presence of the test male but no matings took place.

Unfortunately the stock to which the G strain males belonged was not vigorous. A number of individuals had poor sex drive or a high proportion of infertile matings. For this reason the stock were not used again in Situation A in spite of the advantage conferred by having a genetic marker within the experiment. The inferior males, however, continued to exhibit powerful blocking capacity and the strain was used extensively in Situation B.

Fate of the Blastocyst

The inhibition of implantation in blocked pregnancy raises the question of the fate of the blastocysts. Over fifty females were examined up to Day 4 of pregnancy. Segmenting eggs were recovered on Day 2, as from normal mice, but by Day 3 only two of the fifteen females examined yielded blastocysts, whereas, in

the normal mouse, blastocysts are regularly recovered from the uteri at this time. The exact fate of the blastocysts from a blocked pregnancy is not yet determined.

Histology of the Ovaries

The ovaries of the females with blocked pregnancies killed 8 or 10 days after the original vaginal plugs showed much variation in the numbers and size of their corpora lutea. Nine out of seventeen mice examined had enlarged corpora lutea of the pregnancy or pseudopregnancy type, indicating that the original mating stimulus had been effective. In the others, the corpora lutea were not appreciably different from those of the dioestrous cycle. Ovulation had taken place in both groups at the fresh oestrous periods and ruptured follicles or recently vascularized corpora lutea could be distinguished in the ovaries. Of two mice, both of which came on oestrus and ovulated after 4 and 8 days respectively, one had pseudopregnancy-type corpora lutea in the ovaries but the other did not. The appearance of the ovaries suggests a varying degree of pituitary gonadotrophin stimulation, such as might be expected.

THE MALES

Since the female is able to recognize her own stud male, after being separated from him and placed in the company of other mated females for 24 hr, discrimination must play a considerable part in the reaction. Nevertheless there was no evidence to suggest that pregnancy-blocking capacity was vested more strongly in some individual males than in others.

In both test situations, the superiority of males of the G strain over the albino males as pregnancy-blocking agents (Tables 2 and 3) was significant at $P = 0{\cdot}01$; this is probably not due to an inherent difference in pregnancy-blocking capacity between the strains but due rather to the fact that the males concerned belonged to different strains and that, in these tests, the stud male was always of the same strain as the female. As already stated, the G males could not be used as stud males because of poor sex drive. Strain differences are undoubtedly important. Males from six additional strains have been used successfully as blocking agents. Females from the only two of these strains tested have exhibited the block to pregnancy in Situation B. Thus, if a recently mated female is paired with a strange male of a different strain, or if she is within sight, sound and smell of such a male but without actual contact, there is failure of pregnancy in the majority of females and a prompt return to oestrus.

The attribute of the male which calls forth this reaction on the part of the female, and the pathways in the female through which it is achieved, are the subject of current study. Information is being sought about the role of the special senses in particular, about the importance of strain differences, and about the time relations involved.

THE FEMALES

Familiarity with either of the test situations before being paired with the stud male made no apparent difference to the reaction of the female when placed

subsequently with a strange male. It was noticed, however, that in the presence of a castrated male precisely the same modifications took place in the oestrous cycle of the virgin females as occur in the presence of a normal male. The oestrous cycle is shorter than when the male is absent, pseudopregnancy is rare and the incidence of abnormal cycles greatly reduced (Whitten, 1957a).

Usually the copulation plug is shed within 24 hr, but occasionally it remains in the vagina for 2 or even 3 days thus presumably supplying a more lasting stimulus. Forty-five mice in the series of tests here described still had the plug present in the vagina when presented with the test situation. Pregnancy was blocked in eighteen of these females. The extra stimulus therefore did not exert a protective action.

The block to pregnancy was not restricted to the less fecund females, since the litters born from stud matings (8·2 young per litter; mean of 122 litters) were no larger than those sired by the test male after a blocked pregnancy (8·6 young per litter; mean of forty-two litters).

When parous females were used, pregnancy appeared to be less readily blocked by the presence of a strange male but it broke down when the female was housed inside a male stock box (Table 5, cf. Table 2). This suggests that the latter situation supplies a stronger stimulus.

TABLE 5

PREGNANCY BLOCK IN THE PAROUS FEMALE

Test situation	*No. of females*				
	Total No.	*Pregnant*	*Pseudopregnant*	*With blocked pregnancy*	
				No.	%
Strange albino male at 24 hr after coitus	22	17	3	2	10
Strange black-eyed male at 24 hr after coitus	23	17	3	3	13
Caged in albino male stock box	29	19*	4	6	21
Caged in black-eyed G strain male stock box	15	2	1	12	80

* One animal aborted.

When the test situation concerns females only (see Tables 2 and 3) there is no mutual interference with pregnancy, but mutual interference between females is well known to occur as regards the oestrous cycle (van der Lee & Boot, 1955, 1956; Whitten, 1957b; Dewar, 1959) where the proximity of other females appears conducive to the formation of corpora lutea with the induction of pseudopregnancy. It seems probable that the absence of failed luteal function among the test groups containing only females is an expression of this reaction. The few mice in which both pregnancy and pseudopregnancy might have been expected to fail from random causes (about 8 %) all became pseudopregnant. The presence of other females may thus contribute towards stabilizing a pregnancy. Whether or not this influence is also effective among mated females exposed to the male in pairs cannot be decided from these experiments. Further,

it is of interest to note that mutual interference between females has also been observed in the incidence of mammary cancer (Mühlbock, 1958).

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