Pregnancy blocking in the vole, *Microtus agrestis* II. Ovarian, uterine and vaginal changes

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Summary. Pregnancy blocking in *M. agrestis* was associated with a rapid degeneration of CL, growth of follicles, a loss of embryos and return of the uterus to its non-pregnant state, and a return to cornified vaginal smears. These results are discussed in relation to the proposal that the immediate cause of pregnancy block is a failure of prolactin secretion resulting in a failure of CL function.

Introduction

Although the block to pregnancy induced by the exposure of newly mated females to a strange male has been demonstrated in *Peromyscus maniculatus* (Eleftheriou, Bronson & Zarrow, 1962), *Microtus agrestis* (Clulow & Clarke, 1968; Milligan, 1976) and *M. pennsylvanicus* (Clulow & Langford, 1971), most studies of the phenomenon have been performed on the mouse, *Mus musculus* (see Bruce, 1960; Parkes & Bruce, 1962; Dominic, 1966a, b). The characteristics of pregnancy blocking in the mouse suggest that its immediate cause is a failure of CL function, which in turn is responsible for a failure of implantation (Bruce, 1960; Parkes & Bruce, 1961; Dominic, 1966b). There is some evidence that this may also be the cause of the pregnancy block in the other species in which the effect has been demonstrated (Bronson, Eleftheriou & Dezell, 1969; Clarke & Clulow, 1973; Clulow & Mallory, 1974). In the present study, the ovarian, uterine and vaginal smear changes accompanying pregnancy blocking in *M. agrestis* were investigated to determine further the nature of the phenomenon in this species.

Materials and Methods

All animals were from the 'laboratory type' colony of voles (see Milligan, 1976). Virgin females were housed individually in wire-topped, plastic cages (29 × 19 × 10 cm) for 2 days, and were each paired with a stud male between 09.30 and 10.30 hours on the 3rd day. One hour after pairing, the females were examined for evidence of mating (spermatozoa in the vaginal smear, or a vaginal plug). Animals that had mated were left together for 48 hr. The female was then transferred to a clean cage and either the stud male was re-introduced for 24 hr into the female's cage (control females: Group 1), or a strange male (i.e. a male other than the stud) was introduced for 24 hr (Group 2). After this treatment, each female was transferred to a clean cage where she remained until the completion of the experiment. Vaginal smears were taken daily and assessed as described by Milligan (1974a). At 72, 96, 120 and 144 hr after the stud mating, 4 females from Group 1 and 4, 5, 6 and 6 females respectively from Group 2, were killed. Nine additional females were killed: three were virgin, three had mated 24 hr and three 48 hr before autopsy. The ovaries and left uterine horn of all females were examined histologically. Thirty measurements of the height of the luminal epithelium were made in each uterus. In most females, serial sections of the left uterine horn only were examined for embryos, but both horns of Group-2 females killed at 96 hr were searched. Student's *t* test was used in statistical analyses.

Results

Corpora lutea

All the control females (Group 1) possessed histologically healthy CL at death and were either

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pregnant or pseudopregnant. The histology of the CL of *M. agrestis* during early pregnancy has been described by Breed & Clarke (1970).

Only one of the Group 2 females killed at 72 hr showed luteal regression, but 12/17 killed at later times possessed degenerating CL and were deemed to have had their pregnancies blocked. The data from the 5 pregnant females were ignored. Luteal regression was characterized by a reduction in the vascularity of the CL, an invasion by polymorphonuclear leucocytes, cytoplasmic vacuolation, nuclear pyknosis and a rapid reduction in the diameter of the CL (Table 1).

Table 1. Mean ($\pm$ S.E.M.) luteal and follicular diameters (μ m) in unmated and pregnant females, and in females exposed to a strange male between 48 and 72 hr after the stud mating

Time after stud mating (hr)	CL		Follicles	
	Pregnant females	Females exposed to strange males	Pregnant females	Females exposed to strange males
0 (unmated)	—	—	523 $\pm$ 16 (3)	
24	589 $\pm$ 24 (3)	—	—	—
48	744 $\pm$ 5 (3)	—	448 $\pm$ 15 (3)	—
72	748 $\pm$ 26 (4)	750 $\pm$ 15 (4)	469 $\pm$ 11 (3)	499 $\pm$ 16 (4)
96	819 $\pm$ 27 (4)	380 $\pm$ 21 (4)	443 $\pm$ 10 (4)	511 $\pm$ 32 (4)
120	740 $\pm$ 36 (4)	277 $\pm$ 27 (4)	470 $\pm$ 1 (4)	574 $\pm$ 17 (4)
144	776 $\pm$ 16 (4)	127 $\pm$ 3 (4)	469 $\pm$ 7 (4)	629 $\pm$ 11 (4)

Follicles

Graafian follicles were found in all females except those killed at 24 hr. The mean diameter of the follicles was considerably smaller in pregnant than unmated females, but showed no marked changes over the first few days of pregnancy (Table 1). An increase in follicular diameter followed exposure of the females to a strange male. At 72 hr, the mean follicular diameter was larger in Group-2 than in Group-1 females, but this was not significant ($t = 1.54$; $P < 0.05$), unlike the difference at 96 hr ($t = 3.46$; $P < 0.02$).

Uterus

The uterus of virgin females was lined by a tall columnar epithelium (mean height $\pm$ S.E. = $30.4 \pm 2.6 \mu$ m). In the pregnant females, the epithelium became progressively lower as the time from mating increased (e.g. $13.3 \pm 0.8 \mu$ m at 96 hr) and the cells became increasingly cuboidal. The uterine epithelium of pregnant females killed at 96 hr was corrugated, but this was less marked at 120 hr and not apparent at 144 hr. In the virgin females and the mated females killed before 72 hr, mitoses were common in the luminal epithelium, but rare in the stroma. In Group-1 and Group-2 females killed at 72, hr, and in all pregnant females killed after this time, mitoses were rare in the epithelium, but common in the stroma. Oedema of the stroma was apparent in pregnant uteri at 96 hr and large areas of decidual tissue occurred at later times.

The histology of the uteri of Group-2 females killed at, or later than, 96 hr was similar to that of unmated females. The epithelium was tall and columnar ($27.4 \pm 3.2 \mu$ m at 96 hr) and was not corrugated. Mitoses were rare in the stroma and neither stromal oedema nor decidual tissue were observed.

Embryos

Morulae and blastocysts were found at the tubal end of the uterus in two Group-1 and two Group-2 females at 72 hr. In Group 1, blastocysts were found in 3 females killed at 96 hr and implantation was well advanced in those killed at 120 and 144 hr. Only one embryo, a morula, was found (at 96 hr) in Group 2.

Vaginal smears

Within 2 days of mating, the cornified cell vaginal smears typical of virgin females had been replaced by smears in which leucocytes predominated. Pregnant females maintained leucocytic or sparsely cellular smears until autopsy. Females in which pregnancy was blocked reverted to cornified cell vaginal smears within 3 days of exposure to the strange male.

Discussion

As in the mouse (Dominic, 1970) and *Microtus pennsylvanicus* (Clulow & Mallory, 1974), pregnancy blocking in *M. agrestis* was associated with a rapid degeneration of the CL. Growth of the Graafian follicles, which appeared to be suppressed during pregnancy, accompanied the luteal degeneration and vaginal smears reverted to the cornified cell type characteristic of oestrus.

Changes in uterine histology were also found. During early pregnancy, there was a reduction in luminal epithelial height and the distribution of mitoses changed from epithelial to stromal. A similar change in the mitotic distribution occurs during early pregnancy in the mouse (Finn & Martin, 1967). The corrugation of the epithelium at 96 hr after copulation is also characteristic of mice on Days 5 and 6 of pregnancy (Day 1 = day of finding vaginal plug) and is believed to reflect a close apposition of the luminal surfaces *in vivo* (Martin, Finn & Carter, 1970). Following exposure of the female voles to a strange male, however, the uterus rapidly reverted to the type characteristic of the non-pregnant animal in oestrus and attempts to induce a decidual reaction in such females have been unsuccessful (Milligan, 1974b). The fact that only a single embryo was found in the 12 females in Group 2 killed later than 72 hr suggests a loss or destruction of uterine embryos. Pregnancy blocking in voles may reflect, therefore, not only changes in the uterus which prevent implantation, but also a loss of available embryos. Bruce (1960) also observed an extreme reduction in the number of free embryos recovered from mice after exposure to strange males.

The present observations are consistent with the proposal that the immediate cause of pregnancy blocking in voles is the failure of CL function. Alterations in steroid levels accompanying the luteal and follicular events presumably account for the changes in the vaginal smears and uterus. The cause of luteal failure, however, remains uncertain. In the mouse, it has been suggested that it may be caused by increased LH and/or FSH secretion (Chapman, Desjardins & Whitten, 1970; Hoppe & Whitten, 1972; Bloch, 1973), but there is no direct supporting evidence. It seems unlikely that increased LH secretion is the immediate cause of CL failure in the vole as a single subcutaneous injection of ovine LH at 48 hr *post coitum* fails to exert any luteolytic effect, even when given in doses (up to 20 µg) sufficient to cause pregnant females to ovulate (Milligan, 1974b).

Members of three of the species in which pregnancy blocking has been demonstrated can exhibit a short luteal phase after spontaneous (mouse, deermouse) or induced ovulation (vole), and are dependent on mating to activate reflex mechanisms which stimulate the development of functional CL (Conaway, 1971; Milligan, 1975). Prolactin is probably an important luteotrophic hormone in these animals (Schwartz, 1973; Milligan, 1974b). A pregnancy-blocking effect also occurs in *Clethrionomys glareolus* (Clarke & Clulow, 1973), and *Microtus pennsylvanicus* (Clulow & Langford, 1971), which are closely related to *M. agrestis*. A suppression of prolactin secretion, as proposed for the mouse (Bruce & Parkes, 1960), could, therefore, account for the failure of the CL in all the animals in which pregnancy blocking has been demonstrated or implicated. Circumstantial evidence in support of this comes from the observation that the block does not occur in mice and deermice under experimental conditions that allow maintenance of the CL (e.g. prolactin administration and suckling: Bruce & Parkes, 1960, 1961; Dominic, 1966b; Bronson *et al.*, 1969). The observation of a rapid and sustained suppression of plasma prolactin levels in newly mated mice following exposure to a strange male provides more direct support (Beamer, 1972). The hormonal response of newly mated female voles to exposure to a strange male is under investigation.

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