OVARIAN CHANGES DURING PREGNANCY AND PSEUDOPREGNANCY IN THE VOLE, *MICROTUS AGRESTIS*

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Summary. In the vole, *Microtus agrestis*, there was no significant change in the number of corpora lutea (cl) during either pregnancy or pseudopregnancy. In pregnant animals, two waves of luteal development took place and histological changes during the two phases of growth differed. In pseudopregnant animals, a similar increase occurred initially which was followed by a marked decrease after Day 8, at which time cl began to degenerate. Luteinized follicles were visible early in pregnancy and pseudopregnancy. Graafian follicles were present after Day 2 or 3. In pregnant animals, the number remained more or less constant, except for a slight increase on Day 19, but their maximum size was reduced at about mid-term, whereas, in pseudopregnant animals, a marked increase in size occurred between Days 8 and 10. Vaginal smears and histology indicated progesterone secretion up to Day 19 of pregnancy and for the 8 to 9 days of pseudopregnancy. There was, therefore, a good correlation between the presence of healthy cl and smears that indicated progesterone secretion.

INTRODUCTION

Voles (microtine rodents) occur throughout the Northern hemisphere, but many aspects of their reproductive physiology are insufficiently known or controversial (cf. Asdell, 1964). Amongst the latter are the degree of ovarian activity during pregnancy and, in particular, the relationship between the number of corpora lutea (cl) and the number of young.

According to one of the earliest studies (Brambell & Hall, 1939), the cl present in the British vole, *Microtus agrestis* increase slowly and steadily in size throughout gestation, and the number present is similar to the number of young born. By contrast, Greenwald (1956, 1957) found that in the Californian vole, *M. californicus* the number of cl markedly exceeds the number of young. Both these studies were carried out on wild populations of voles which precluded any deliberate experimental investigation. It was, therefore, decided to re-examine cl changes in both pregnant and pseudopregnant females of a laboratory-bred and maintained stock of *M. agrestis*. An attempt was also made

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to determine luteal function, as indicated by the appearance of the vaginal epithelium and vaginal smears, as well as ovarian follicular changes. In addition, the effect of progesterone on the vaginal epithelium was studied by investigating the effect of oestrogen and progesterone in spayed virgin females.

MATERIALS AND METHODS

Virgin females, 3 to 5 months old, derived from a laboratory stock, were maintained in this Department under conditions described by Breed (1969). Twenty-eight voles were mated with virile males and killed at various stages of pregnancy (four animals on each of Days 1, 3, 5, 7, 10, 14 and 19) and twenty-one mated with vasectomized males and killed at various stages of pseudopregnancy (three animals on each of Days 1, 2, 4, 6, 8, 10 and 12).

In order to determine changes within the first 6 days of mating, pairs of voles were left together for a maximum of 2 hr and then separated. The presence of a vaginal plug or of spermatozoa was taken as evidence of mating and designated as Day 0. Animals were killed by cervical dislocation on Day 1, 20 to 24 hr after pairing. For studying changes after Day 6, voles were paired at 19.00 hours and separated next morning at 09.30 to 10.00 hours (Day 1).

Vaginal smears, taken daily from most pregnant and all pseudopregnant animals, were air dried and stained by the method of Papanicolaou (1954) to ascertain the relative abundance of leucocytes, cornified and nucleated epithelial cells.

Uteri of pseudopregnant voles were weighed fresh, while the ovaries of both pregnant and pseudopregnant animals were weighed after fixation in Bouin's fluid. Ovaries and representative samples of vaginae were embedded in paraffin wax, serially sectioned at 7 μ and stained with Ehrlich's haematoxylin and eosin. Ovarian sections were scanned and diameters of CL and follicles measured as described by Breed (1969). Since initial observations showed that a healthy follicle with a diameter of 400 μ or more had a large antrum and a well developed discus proliferus, all such structures were recorded as Graafian follicles. Vesicular atretic follicles were also counted. When scanning sections containing luteal-like tissue, particular care was taken to determine whether an ovum was present. If found, such structures were recorded as luteinized follicles. Luteal cell and nuclear size was estimated by measuring two diameters at right angles and taking the mean.

Ovariectomy and treatment with steroids

Twelve virgin females (22 to 28 g) were anaesthetized with a 1:10 dilution of sodium pentobarbital (dose: 0·17 to 0·20 ml/vole—50 μg/g) given intraperitoneally, and ovariectomized. Complete removal of ovaries was confirmed by observing the excised material under a dissecting microscope. Daily subcutaneous injections (0·0004 μg/g) of 0·01 μg oestradiol-17-β dipropionate (Organon Laboratories) were started 3 days after the operation and from Day 14, eight animals also received 0·5 mg (20 μg/g) or 1 mg (40 μg/g) of progesterone (Organon)/day. A 1:9 mixture of benzyl alcohol and arachis oil was used as the vehicle for injection. Smears were taken daily from all females with
perforate vaginae. Animals were killed on Day 25 when uteri were removed and weighed. These, together with the vaginae, were subsequently prepared for histological examination as described above.

Numerical data were evaluated by analysis of variance where appropriate.

RESULTS

Luteal changes during pregnancy

Ovarian weights altered significantly during pregnancy ($F = 11.96, P<0.001$), reaching a maximum on Day 19 (Table 1); there was, however, no significant change in the number of CL ($F = 1.05; P>0.05$), the overall mean being $4.2\pm0.2$. A highly significant difference between the sizes of CL of females killed at the different stages was, however, evident ($F = 50.45; P<0.001$) (see Text-fig. 1). Between Days 1 and 3, considerable increase took place which was followed by little change until after Day 7. From then on, a steady increase occurred until Day 19. Luteinized follicles were present in females killed on Days 1 (one in one animal), 3 (one in two animals and five in a third) and 7 (three in one animal). These were smaller than the accompanying CL, except on Day 1.

The histological appearance of CL present in different females killed on the same day of pregnancy was similar. On Day 1, but not subsequently, mitoses were visible in some luteal cells and a central cavity was present. As pregnancy

![Text-fig. 1. Mean sizes (± S.E.) of corpora lutea during pregnancy (×) and pseudopregnancy (○).](image)
**Table 1**

Changes in Corpora Lutea and Follicles during Pregnancy

<table>
<thead>
<tr>
<th>Day of pregnancy</th>
<th>No. of animals</th>
<th>Ovarian wt (mg) Mean ± S.E.</th>
<th>Corpora lutea</th>
<th>Graafian follicles</th>
<th>Mean no. ± S.E.</th>
<th>Range of size of Graafian follicles (μ)</th>
<th>Atretic follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4.72 ± 0.71*</td>
<td>4.2 ± 0.6</td>
<td>0.2 ± 0.2†</td>
<td>0.2 ± 0.2†</td>
<td>0 to 600</td>
<td>601 to 650</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6.67 ± 0.71</td>
<td>4.0 ± 0.6</td>
<td>6.7 ± 1.1</td>
<td>2.2 ± 0.8 (3)</td>
<td>2.2 ± 0.6 (4)</td>
<td>0 to 600</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>5.40 ± 0.71</td>
<td>4.0 ± 0.6</td>
<td>4.6 ± 0.7</td>
<td>3.2 ± 1.2 (3)</td>
<td>1.2 ± 0.7 (2)</td>
<td>0 to 600</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>5.20 ± 0.71</td>
<td>3.5 ± 0.6</td>
<td>5.0 ± 0.7</td>
<td>6.5 ± 1.3 (4)</td>
<td>0 to 600</td>
<td>601 to 650</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>7.52 ± 0.71</td>
<td>5.0 ± 0.6</td>
<td>6.7 ± 1.1</td>
<td>2.7 ± 1.1 (3)</td>
<td>2.0 ± 0.7 (3)</td>
<td>0 to 600</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>7.00 ± 0.71</td>
<td>3.7 ± 0.6</td>
<td>4.7 ± 0.2</td>
<td>3.7 ± 1.1 (4)</td>
<td>4.2 ± 0.8 (4)</td>
<td>0 to 600</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>11.07 ± 0.71</td>
<td>5.7 ± 0.6</td>
<td>8.2 ± 0.9</td>
<td>3.7 ± 1.1 (4)</td>
<td>2.0 ± 0.2 (1)</td>
<td>0 to 600</td>
</tr>
</tbody>
</table>

*S.E. calculated from within group variance.
† S.E. calculated independently for each group.
( ) Total number of females with follicles in respective size range.
<table>
<thead>
<tr>
<th>Day of pseudo-pregnancy</th>
<th>No. of animals</th>
<th>Ovarian wt (mg) Mean ± S.E.</th>
<th>Corpora lutea</th>
<th>Graafian follicles</th>
<th>Range of size of Graafian follicles (μ)</th>
<th>Aretic follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>400 to 500</td>
<td>501 to 600</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>3.10 ± 1.37*</td>
<td>3.7 ± 0.7*</td>
<td>0.3 ± 0.3†</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>8.13 ± 1.37</td>
<td>4.7 ± 0.7</td>
<td>4.7 ± 2.0</td>
<td>4.0 ± 2.1 (2)</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>4.50 ± 1.37</td>
<td>4.7 ± 0.7</td>
<td>7.0 ± 0.6</td>
<td>5.7 ± 0.3 (3)</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>6.63 ± 1.37</td>
<td>5.0 ± 0.7</td>
<td>4.7 ± 1.3</td>
<td>0.3 ± 0.3 (1)</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>6.20 ± 1.37</td>
<td>4.3 ± 0.7</td>
<td>6.7 ± 1.4</td>
<td>3.0 ± 1.5 (3)</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>4.40 ± 1.37</td>
<td>4.3 ± 0.7</td>
<td>4.7 ± 0.3</td>
<td>0.3 ± 0.3 (1)</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>5.00 ± 1.37</td>
<td>3.7 ± 0.7</td>
<td>5.3 ± 0.9</td>
<td>0.7 ± 0.7 (1)</td>
<td>3.3 ± 0.9</td>
</tr>
</tbody>
</table>

* S.E. calculated from within group variance.
† S.E. calculated independently for each group.
( ) Total number of females with follicles in respective size range.
advanced, cells became more angular and their boundaries more distinct (see Pl. 1, Figs. 1 and 2). Diameters of luteal cells (17 to 20 µ) and nuclei (7 to 8 µ) changed little until Day 10, after which considerable increase occurred, cells on Day 19 being about 34 µ and nuclei about 10 µ (Pl. 1, Fig. 3). Luteal cells in luteinized follicles resembled those of the accompanying CL (Pl. 1, Fig. 4).

**Luteal changes during pseudopregnancy**

Neither ovarian weights (F = 1.49; P>0.05) nor numbers of CL (F =0.46; P>0.05) altered significantly after sterile matings (Table 2), although there was a highly significant change in CL size (F = 24.12; P<0.001). Between Days 1 and 2, the CL increased considerably, after which they remained constant until Day 8 when decline set in (Text-fig. 1). Up to Day 6, the CL histologically resembled the CL of females killed at about the same time of pregnancy. However, on Day 8, some luteal cells had patchy eosinophilia (Pl. 1, Fig. 5) and by Days 10 and 12, the cytoplasm stained little with eosin and many pyknotic nuclei were visible (Pl. 1, Fig. 6). Luteinized follicles were present in females killed on Days 1 (two in two animals), 2 (one in two animals), 6 (one in two animals and three in the third animal) and 8 (two in one animal) which histologically resembled the accompanying CL.

**Graafian and vesicular atretic follicles**

Graafian follicles were not usually present 1 day after mating but by Day 2 or 3, a full set had reappeared (see Tables 1 and 2). In pregnant animals, numbers did not subsequently change markedly, other than a slight increase on Day 19. Graafian follicles up to 600 µ usually occurred except on Day 10, when virtually none exceeded 500 µ. In pseudopregnant animals, Graafian follicles increased in size between Days 8 and 10 (Text-fig. 2 and Table 2). There was no statistically significant change in the numbers of vesicular atretic follicles during either pregnancy (F = 1.34; P>0.05) or pseudopregnancy (F = 1.30, P>0.05), although the figures indicate a slight increase at about mid-term which coincided with the reduction in Graafian-follicle size.

**Uterine weight during pseudopregnancy**

Uterine weights of pseudopregnant animals differed significantly between the different groups (F = 4.35, P<0.05) and Duncan's multiple range test (Steel & Torrie, 1960) showed that mean weights on Days 2 (52.6±5.8 mg), 10 (58.5±5.8 mg) and 12 (58.2±5.8 mg) were significantly greater than those on Days 1 (35.4±5.8 mg), 6 (38.6±5.8 mg) and 8 (27.4±5.8 mg).

**Vaginal epithelium**

The vaginae of females killed on Day 1 had a stratified squamous epithelium with an outer cornified layer up to 50 µ thick. Vaginal plugs were present on this day and also in two of the three pseudopregnant animals killed on Day 2 (Pl. 2, Fig. 7). On the latter day, desquamated epithelial cells and a mass of leucocytes were seen around the plugs (Pl. 2, Fig. 8). By Days 5 and 6, a mucified epithelium had developed within which leucocytes occurred (Pl. 2, Fig. 9). From Days 10 to 19 of pregnancy, the mucified layer became much more
Fig. 1. cl of female killed on Day 3 of pregnancy. \( \times 140 \).

Fig. 2. cl of female killed on Day 14 of pregnancy. \( \times 120 \).

Fig. 3. Luteal cells from female killed on Day 19 of pregnancy. Note cells tend to be angular. \( \times 280 \).

Fig. 4. Luteinized follicle adjacent to two cl (at bottom of plate) from female killed on Day 7 of pregnancy. Note similar cell structure of luteinized follicle to that of the cl. \( \times 35 \).

Fig. 5. cl of female killed on Day 8 of pseudopregnancy. Note patchy eosinophilia. \( \times 280 \).

Fig. 6. cl of female killed on Day 10 of pseudopregnancy. Note lack of eosinophilia and irregularly shaped nuclei. \( \times 280 \).

(Facing p. 452)
Fig. 7. Female killed on Day 1 of pregnancy. Plug visible in vaginal lumen and a cornified layer lining vaginal epithelium. × 15.

Fig. 8. Female killed on Day 2 of pseudopregnancy. A mass of leucocytes lies between the vaginal plug (bottom of plate) and epithelial wall. × 70.

Fig. 9. Female killed on Day 6 of pseudopregnancy. Mucified cells on outer edge of vaginal epithelium and a mass of leucocytes in lumen. × 120.

Fig. 10. Female killed on Day 19 of pregnancy. Note extensive mucification and lack of leucocytes in lumen. × 120.

(Facing p. 453)
Ovary of pregnant and pseudopregnant vole

extensive, being up to 200 \( \mu \) thick (Pl. 2, Fig. 10). Few leucocytes were present during this time. Contrasting with this, all females killed on Day 10 and 12 of pseudopregnancy had a well-developed stratified epithelium with an outer layer of nucleated epithelial or cornified cells.

**Vaginal smears**

Vaginal smears reflected the changes in the vaginal epithelium. An initial leucocytic invasion took place which lasted up to Day 6. In pregnant animals, thin mucified smears persisted until Day 19, whereas, in pseudopregnant animals, cornified or nucleated epithelial cells reappeared on Day 9 or 10. The vaginal opening at first became smaller and remained so throughout pregnancy, but, in pseudopregnant animals, an obvious enlargement occurred on Day 9 or 10.

**Text-fig. 2.** Mean sizes (± S.E.) of Graafian follicles during pregnancy (×) and pseudopregnancy (●).

**Uterine and vaginal changes following ovariectomy with replacement therapy**

In ovariectomized animals, the mean uterine weight for the four animals given oestrogen alone (35·60±2·50 mg) did not differ significantly (\( F = 1·40; P > 0·05 \)) from that of the females given progesterone in addition, either the four given 0·5 mg (34·75±2·16 mg) or the four given 1 mg (30·65±2·16 mg). The oestrogen-treated voles had cornified vaginal smears, whereas the addition
of progesterone resulted in an initial invasion of leucocytes after which the smears became thin. The progesterone-treated animals also had markedly smaller vaginal openings and mucified vaginal epithelia.

DISCUSSION

The marked increase in CL size during pregnancy no doubt resulted in the increase in ovarian weight. In pseudopregnant animals, the considerable variation in ovarian weights probably accounted for the failure of the presence of healthy CL to cause a significant increase. The growth and histological appearance of CL during the first 7 days is similar during both pregnancy and pseudopregnancy—an initial growth occurs for about 3 days, after which little change takes place. During the latter half of pregnancy, a second wave of growth sets in which is brought about by cell hypertrophy. No cell division was seen during this stage, in contrast to that during the initial phase of growth. Thus, the two waves of CL growth during pregnancy are similar to results obtained for other myomorph rodents, e.g. rats (Long & Evans, 1922), mice (Deanesly, 1930), and the bank vole (Clethrionomys glareolus) (Brambell & Rowlands, 1936). The maximum CL size was, however, much less than that of the bank vole and California field vole (Microtus californicus) (Greenwald, 1956). In the latter species, numerous CL were found during pregnancy, a number far exceeding the average litter size (Greenwald, 1956, 1957). This contrasts with the present study in which the number of CL remained constant throughout pregnancy (mean 4.2±0.2) and the mean number of young born in 301 first litters in the breeding colony (3.5±0.1) was slightly less than that of the CL, the difference between the two means presumably reflecting embryonic mortality. There was no indication of luteinization of follicles during pregnancy subsequent to that which occurred as a result of the coital stimulus.

In contrast to the finding during pregnancy, no second wave of luteal growth occurred during pseudopregnancy and degeneration of CL set in on Day 8 or 9 after mating. Regression of CL occurs more rapidly than in the mouse (Deanesly, 1930).

The initial leucocytic invasion followed by thin mucified smears that took place during the first few days of pregnancy and pseudopregnancy was also obtained after progesterone administration to ovariectomized oestrogen-maintained animals. It therefore appears that progesterone was secreted during both these states. Since smears of pregnant animals remained thin and mucified up to Day 19 and pregnancy lasts for 20 or 21 days (Ranson, 1934), continuous progesterone secretion probably occurs throughout this period. Sudden reversion to nucleated epithelial or cornified smears on Day 9 or 10 of pseudopregnancy suggests that progesterone secretion ceased at that time. Degeneration of CL coincided with the reappearance of epithelial cells in the vaginal smear.

The factor(s) necessary to maintain CL for the first 7 days appear to be present in both pregnant and pseudopregnant animals, whereas the second growth phase during pregnancy, starting between Days 7 and 10, occurs at about the time of luteolysis in pseudopregnant animals. Since implantation takes place
on about Day 5 in this species (Breed, 1968), it is suggested that, during pregnancy, a foeto-placental factor is secreted that induces the second wave of luteal growth.

Coitus results in almost complete disappearance of Graafian follicles (Breed & Clarke, 1970) but by Day 2 or 3, a full set has reappeared. The slight increase in size during the next few days was either followed by a reduction at mid-pregnancy, or by further enlargement on Days 10 and 12 of pseudopregnancy. The latter increase in size coincides with luteal regression. Since most adult virgin females have follicles that exceed 600 µ (Breed, 1968), whereas only five out of twenty-four pregnant animals had follicles over this size, it appears that functional or suppress full follicular development. The decline in Graafian-follicle size on Day 10 of pregnancy may indicate that the functional life of the wave of Graafian follicles that developed post coitum had terminated; a slight increase in vesicular atretic follicles on Days 10 to 14 further supports this suggestion. The small follicles on Day 10 were followed by larger Graafian follicles on Day 14, which may represent a second wave of follicular growth in preparation for the post-partum oestrus (Chitty, 1957; Breed, 1969). In some other species, rhythmical changes of follicular growth have been described during pregnancy (see Perry & Rowlands, 1962; Greenwald, 1966), whereas, in the rat, no such change occurs (Greenwald, 1967).

The increase in uterine weight at the end of pseudopregnancy agrees with similar results obtained for the rat (Yochim, Hiesterman & Keever, 1965; van Rees & de Groot, 1965), and may be due to the reduction of progesterone influence, although the uteri of ovariectomized animals treated with oestrogen and progesterone did not weigh significantly less than when the same dose of oestrogen was administered alone.

ACKNOWLEDGMENTS

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REFERENCES


