UTERINE FLUID AND THE DURATION OF PSEUDO-PREGNANCY FOLLOWING TRANSECTION OF THE UTERUS IN THE RAT

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Summary. When the posterior ends of the uterine horns in cycling female Hooded Wistar rats were transected, fluid accumulated in the uterine lumen. During pseudopregnancy, the mean volume of uterine fluid in these rats fell from 0.97±0.18 ml on Day 2 to 0.06±0.07 ml on Day 8 (P<0.001). The volume remained low until the next onset of pro-oestrus.

Uterine fluid in normal rats at the time of oestrus contained 41.63±3.99 mequiv./l potassium, a higher level than that in blood plasma, and 2.07±0.88 mg/ml total protein. Following transection of the uterine horns, the level of potassium remained high. The mean total protein concentration rose to three to thirteen times the normal value.

Transection of the uterine horns led to an increase in the mean duration of pseudopregnancy from 13.4±0.20 days to 15.2±0.33 days (P<0.001). When uterine fluid was withdrawn from transected horns during pseudopregnancy, the duration of pseudopregnancy was reduced to 14.3±0.29 days (P<0.05). It is concluded that retention of uterine secretion is a causal factor in the prolongation of pseudopregnancy resulting from uterine transection.

INTRODUCTION

Under normal conditions, uterine secretion is present in significant quantities only during the pro-oestrous and oestrous phases of the cycle in the rat (Long & Evans, 1922). This secretion is released through the cervix towards the end of oestrus (Blandau, 1945) and throughout the remainder of the cycle the volume of fluid in the uterine lumen is very small (Warren, 1938). When the posterior parts of the uterus are ligated (Warren, 1938; Shih, Kennedy & Huggins, 1940; Ringler, 1961; Perrine, 1967) or transected (Bradbury, Brown & Gray, 1950; O'Shea, 1970), fluid accumulates in greater than normal quantities, is present throughout all stages of the cycle and shows a progressive increase in volume (Ringler, 1961; Perrine, 1967).

Neither ligation (Warren, 1938; Perrine, 1967) nor transection (O'Shea, 1971a) of the uterus appear to alter the duration or rhythm of the oestrous cycle. However, uterine transection does lead to a prolongation of pseudo-
pregnancy (Bradbury et al., 1950; O’Shea, 1970). There is evidence to suggest that this effect on pseudopregnancy is due to an interference with the normal uterine luteolytic mechanism (O’Shea, 1971b), but it has not been shown whether the accumulation of uterine fluid is a causal factor.

This paper describes experiments concerned with changes in the volume of fluid in transected rat uteri during and after pseudopregnancy, the nature and composition of fluid in transected and normal uteri and the effects of removal of uterine fluid on the duration of pseudopregnancy.

MATERIALS AND METHODS

Female, Hooded Wistar rats, aged 8 weeks, were used in two experiments. Lighting conditions were not strictly controlled, and the duration of daylight was subject to seasonal variation.

Experiment 1

The duration of pseudopregnancy was recorded in four groups of rats:

Group I. Control—ten normal, intact rats.

Group II. Posterior uterine section—fourteen rats in which bilateral transection of the posterior ends of the uterine horns was performed, using the technique described by O’Shea (1971b). Uterine fluid was collected on the first day of dioestrus following the end of pseudopregnancy.

Group III. Posterior uterine section and removal of uterine fluid—eighteen rats (one died before the end of pseudopregnancy). Uterine fluid was removed from four sub-groups each of four or five rats on Day 2 of the oestrous cycle or pseudopregnancy, and on Days 5, 8 and 11 of pseudopregnancy. It was not known by Day 2 whether pseudopregnancy had been induced: two of four rats in this sub-group which returned to oestrus within 5 days of collection of fluid were presumed not to have been pseudopregnant.

Group IV. Removal of uterine fluid—twelve normal rats which had not previously undergone any surgery were subjected to the same procedure of uterine fluid removal as that used in Group III. Three sub-groups each consisting of four rats were included on Day 2 of the oestrous cycle or pseudopregnancy, and on Days 8 and 11 of pseudopregnancy. Two rats from the Day-2 sub-group returned to oestrus within 4 days and were presumed not to be pseudopregnant.

Daily vaginal smears were used to determine the duration of pseudopregnancy, which was induced by sterile mating with vasectomized males. Mating did not take place until after the completion of at least one normal postoperative oestrous cycle in rats in Groups II and III. The first day of vaginal oestrus was designated Day 0, and the end of pseudopregnancy was recognized by the return of vaginal oestrus.

Hypodermic needles (25 or 23 gauge) inserted into the uterine lumen were used to collect uterine fluids as completely as possible, direct into 1-ml tuberculin syringes graduated to 0.01 ml. Collection was performed either by way of a midline ventral laparotomy under ether anaesthesia, or following an overdose of chloroform.
Analysis for total protein and potassium was performed on uterine fluids collected from four normal cycling rats on Day 0 of an oestrous cycle, and on uterine fluids of rats from Groups II and III. The pooled uterine fluid from the two horns of each rat was centrifuged for 10 min at 700 rev/min. The supernatant fluid was analysed for total protein, using the biuret method as described by Layne (1957), and for potassium, using a flame photometer (E.E.L. Flame Photometer Mark II, Evans Electroselectenium Ltd., Halstead, Essex).

The bacteriological examinations performed on eight samples of uterine fluid from rats in Group II were as follows:
(a) Gram stain of air-dried smears.
(b) 96-hr incubation on sheep blood agar plates under aerobic, microaerophilic and anaerobic conditions.
(c) 96-hr aerobic incubation in trypticase soy broth, followed by subculture to sheep blood agar plates incubated aerobically for 48 hr.

Experiment 2
The duration of pseudopregnancy was recorded in three groups of rats:
Group I. Control—twelve normal, intact rats.
Group II. Posterior uterine section and sham removal of uterine fluid—twelve rats. Following posterior uterine section, laparotomy was performed on Day 6 of pseudopregnancy when the uterus was exposed and manipulated as for fluid collection, with the omission of needle-puncture and fluid removal. The quantity of uterine fluid present on the first day of either the first (six rats) or second (six rats) dioestrus after the end of pseudopregnancy was measured. These rats did not mate at the end of pseudopregnancy and the second dioestrus followed a normal oestrous cycle.
Group III. Posterior uterine section and removal of uterine fluid—twelve rats. Uterine fluid was removed and measured on Day 6 of pseudopregnancy, and again on the first day of dioestrus following pseudopregnancy. One rat which developed pyometra was excluded.

RESULTS

Volume of uterine fluid
The volumes of uterine fluids in Exps 1 and 2 are shown in Tables 1 and 2, respectively.
No measurable quantity of uterine fluid was present in any rat from Group IV of Exp. 1, with the exception of one rat on Day 11. In this rat, which came into oestrus on the following day, 0.03 ml was present.
Following posterior uterine section, considerable quantities of uterine fluid were present up to Day 5 of pseudopregnancy (Group III, Table 1). All these rats had passed through two or three oestrous periods after operation. On Day 6, the amount of fluid was much reduced (Group III, Table 2) and on Day 8, the mean amount was very small (Group III, Table 1). The mean volume of 0.22 ml on Day 11 of pseudopregnancy was greatly influenced by one rat whose uterine horns contained a total of 0.66 ml of fluid. In two rats in this sub-group, no measurable amount of fluid was present. Following the oestrous period which
marked the end of pseudopregnancy, there was again a rise in uterine fluid, both in rats from which fluid had been withdrawn during pseudopregnancy (Group III, Table 2) and in rats from which fluid had not been withdrawn (Group II, Tables 1 and 2). Removal of uterine fluid on Day 6 of pseudopregnancy did not appear to influence the quantity of fluid present after the subsequent oestrous period (Table 2).

Table 1
VOLUME OF FLUID IN UTERINE HORMS FOLLOWING POSTERIOR UTERINE SECTION IN THE RAT

<table>
<thead>
<tr>
<th>Experimental group (Exp. 1)</th>
<th>Day of removal of fluid during the oestrous cycle or pseudopregnancy</th>
<th>No. of rats</th>
<th>Mean volume of uterine fluid (ml±S.D.)</th>
<th>P (Difference from Day 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>2, 5, 8, 11</td>
<td>4, 5, 4, 5</td>
<td>0·97±0·18, 0·88±0·36, 0·06±0·07, 0·22±0·30</td>
<td>N.S., &lt;0·001, &lt;0·01, N.S.</td>
</tr>
<tr>
<td>II</td>
<td>1st day of dioestrus after pseudopregnancy</td>
<td>4</td>
<td>0·49±0·16</td>
<td>&lt;0·01</td>
</tr>
</tbody>
</table>

N.S. = Not significant, P>0·05.

Table 2
VOLUME OF FLUID IN UTERINE HORMS FOLLOWING POSTERIOR UTERINE SECTION AND THE EFFECT OF REMOVAL OF FLUID DURING PSEUDOPREGNANCY IN THE RAT

<table>
<thead>
<tr>
<th>Experimental group (Exp. 2)</th>
<th>Day of removal of fluid</th>
<th>No. of rats</th>
<th>Mean volume of uterine fluid (ml±S.D.)</th>
<th>P (Difference from Day 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>Day 6 of pseudopregnancy</td>
<td>11*</td>
<td>0·22±0·18, 0·67±0·22</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td></td>
<td>1st day of dioestrus after pseudopregnancy</td>
<td>11*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1st day of dioestrus after pseudopregnancy</td>
<td>6</td>
<td>0·67±0·32</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td></td>
<td>1st day of 2nd dioestrus after pseudopregnancy</td>
<td>6</td>
<td>0·85±0·43</td>
<td>&lt;0·01</td>
</tr>
</tbody>
</table>

* Same group of rats.

The mean volume of uterine fluid present after the second oestrus following pseudopregnancy was greater than that present after the first oestrus, but this difference was not significant (Group II, Table 2).

Nature of uterine fluid
The fluid from normal cycling rats at oestrus was watery, almost colourless, and slightly opaque. Following posterior uterine section, similar, slightly yellowish, fluid was present on Day 2 in all rats, and in three of five rats on Day 5 of pseudopregnancy. In the remaining two rats, the fluid was somewhat more viscous and in one of the rats, it was distinctly brownish in colour.

In the later stages of pseudopregnancy, the fluid became more mucoid as it
became less abundant. By Day 6, the fluid increased in viscosity in many rats, and became more distinctly yellowish, features which were accentuated by Days 8 and 11.

After the oestrous period which marked the end of pseudopregnancy, at which time uterine fluid again became more abundant, the consistency had nearly returned to normal. However, of twenty-seven rats from which fluid was collected after pseudopregnancy, a distinct orange or brownish discolouration was present in eight, of which five contained small flecks of highly viscid mucus. All but four of these twenty-seven rats had undergone surgery, together with manipulation or puncture of the uterus, during pseudopregnancy.

Potassium. The mean values for potassium in uterine fluids are shown in Table 3. A significant \((P<0.01)\) difference from the normal uterine fluid present at oestrus was found only in the post-pseudopregnancy dioestrus group.

**Table 3**

<p>| Potassium and total protein in uterine fluid following posterior uterine section in the rat |
|---------------------------------------------|-----------------|---------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Day of removal of fluid during the oestrous cycle or pseudopregnancy</th>
<th>No. of rats</th>
<th>Mean potassium concentration (mequiv./l ± S.D.)</th>
<th>Mean total protein concentration (mg/ml ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>4</td>
<td>41.63 ± 3.99</td>
<td>2.07 ± 0.88</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>41.75 ± 3.80</td>
<td>7.80 ± 0.53</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>49.06 ± 11.75</td>
<td>7.65 ± 1.60</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>33.75 ± 4.95</td>
<td>26.80 ± 26.45</td>
</tr>
<tr>
<td>1st day of dioestrus after pregnancy</td>
<td>4</td>
<td>24.03 ± 5.95</td>
<td>14.12 ± 7.61</td>
</tr>
</tbody>
</table>

* Control, normal cycling rats.

**Total protein.** A marked increase in total protein was observed in fluids from rats in which posterior uterine section had been performed (Table 3). This difference was highly significant \((P<0.001)\) in the Day-2 and Day-5 sub-groups, and was significant \((P<0.05)\) on the first day of dioestrus following pseudopregnancy.

**Bacteriology.** The eight uterine fluids examined included three showing a distinct brownish colouration. In no case were any micro-organisms observed in smears or demonstrated by cultural examination.

**Effect of removal of uterine fluid on duration of pseudopregnancy**

The results of Exps 1 and 2 are shown in Table 4. The same trends were evident in the two experiments, and the means in comparable groups were similar: in these respects, there was no significant difference between the experiments.

In both experiments, posterior uterine section (Group II) resulted in a significant increase in the duration of pseudopregnancy relative to the control group (Group I), of 2.0 \((P<0.001)\) and 1.7 \((P<0.02)\) days, respectively.

In both experiments, the mean duration of pseudopregnancy in rats from which uterine fluid had been removed after posterior uterine section (Group
III) was less (0-9 and 1-0 days, respectively) than that in the equivalent groups (Group II) from which fluid had not been removed. When the results of the two experiments were combined by pooling the raw data, the mean shortening of pseudopregnancy was 0-9 days ($P<0-05$).

Removal of uterine fluid after posterior uterine section (Group III) did not bring the duration of pseudopregnancy back to the control level (Group I), and the differences of 1-1 days in Exp. 1 and 0-9 days in the combined results were significant at the 5% and 2% levels, respectively.

**Table 4**

**Effect of Removal of Uterine Fluid on Duration of Pseudopregnancy in the Rat**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th>Experiments 1 and 2 (pooled data)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of rats</td>
<td>Mean duration of pseudopregnancy (days ± S.E.M.)</td>
<td>No. of rats</td>
<td>Mean duration of pseudopregnancy (days ± S.E.M.)</td>
<td>No. of rats</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>13-3±0-26</td>
<td>12</td>
<td>13-4±0-31</td>
<td>22</td>
</tr>
<tr>
<td>II</td>
<td>14</td>
<td>13-3±0-41</td>
<td>12</td>
<td>13-1±0-55</td>
<td>26</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>14-4±0-36</td>
<td>11</td>
<td>14-1±0-49</td>
<td>26</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>13-2±0-33</td>
<td>11</td>
<td>14-1±0-49</td>
<td>26</td>
</tr>
</tbody>
</table>

The procedure of fluid removal had no detectable effect when applied to an additional group in which posterior uterine section had not been performed (Group IV) in Exp. 1. Nor did the sham operation on Group II in Exp. 2 produce any detectable effect on the duration of pseudopregnancy. The mean duration of pseudopregnancy in this group was not significantly different from that of Group II in Exp. 1.

**DISCUSSION**

It is clear from this study that the fluid which accumulates in the uterus of the rat after section of the posterior ends of the uterine horns is largely resorbed during pseudopregnancy. A similar phenomenon in the guinea-pig has been described by Ginther (1969). The resorption of uterine fluid during pseudopregnancy apparently took place fairly rapidly between Days 5 and 8 so that by Day 8, little or no fluid remained. Fluid quantity then remained low until the next onset of pro-oestrus.

Resorption probably accounts for the relatively small amount of fluid found in the transected uteri of rats which went through a series of pseudopregnancies in a previous experiment (O'Shea, 1970). Rats maintained for a comparable time after uterine ligation, but in which pseudopregnancy did not occur, appeared to show a progressive increase in the quantity of uterine fluid (Ringer, 1961; Perrine, 1967). Presumably this increase results from recurring periods of secretory activity at the oestrous phase of each cycle, without complete resorption in the intervening phases.
It is probable that both the secretion of uterine fluid at oestrus and its resorption during pseudopregnancy, are hormonally controlled. It has been shown that secretion and retention of uterine fluid can be induced by oestrogen administration (Shih et al., 1940; Ringler, 1961) and that progesterone administration causes release of the uterine fluid through the cervix (Armstrong, 1968). Progesterone given in conjunction with oestradiol prevents the accumulation of uterine fluid even in the ligated uterus (Armstrong, 1968), presumably either by depressing secretory activity or stimulating resorption. The hormonal basis for resorption of fluid from the transected rat uterus during pseudopregnancy is not known. However, resorption is first apparent on Day 6 of pseudopregnancy, coincident with a peak in the ovarian venous blood level of progesterone (Hashimoto, Henricks, Anderson & Melampy, 1968), suggesting that this hormone may be implicated.

The rise in protein concentration observed in the fluid in transected uterine horns was comparable to that found by Ringler (1961) after uterine ligation. The origin of this increased protein is not known, although it is possible that it was partly derived from cellular breakdown in the fluid. Preliminary observations (unpublished) on the cytology of the fluids in transected uteri in the present study indicated a rise in the total number of cells, and generally an increase in the proportion of large mononuclear cells, many of which possessed very large amounts of foamy cytoplasm. In normal uterine fluid from rats at oestrus, the cells were mostly neutrophils. Resorption of fluid during the latter part of pseudopregnancy may have had the effect of further concentrating the protein present in the remaining fluid.

The potassium levels in uterine fluids in rats in this study were similar to those reported by Ringler (1961). The retention of a high potassium concentration relative to the normal plasma level of approximately 4 mequiv./l (Levitt, Turner, Sweet & Pandiri, 1956) in all groups of rats with transected uteri suggests the continued functional integrity of the uterine epithelium. Histological observations on transected rat uteri (O'Shea, 1970) have shown that the lining epithelium can retain its structural integrity for long periods.

The duration of pseudopregnancy was significantly reduced after removal of fluid from the lumen of the transected uterus. From this, it is concluded that the retention of uterine secretion is responsible, at least in part, for the prolongation of pseudopregnancy which follows posterior uterine section. In view of the small numbers in individual sub-groups, it was not possible to determine whether fluid removal at different stages of pseudopregnancy produced different degrees of effect on the duration of pseudopregnancy.

The way in which accumulation of secretion in the uterine horns interferes with uterine luteolytic activity is not known. It was previously suggested (O'Shea, 1970) that uterine distension may have been responsible. The present demonstration that, due to resorption, very little fluid remains in the uterine horns beyond the early stages of pseudopregnancy makes it unlikely that physical distension is the major factor. Possibly, the effect on duration of pseudopregnancy is produced by some as yet unidentified constituent or constituents of the retained secretion.

There are evidently species differences in the effects of retention of uterine
secretion on the life-span of the corpus luteum. Ginther (1969) reported a shortening of the oestrous cycle in the guinea-pig following ligation of the uterus, although a small prolongation of the cycle during which operation was performed did occur. Little is known of the effects of retention of uterine secretion in other species. In cattle, Roberts (1956) stated that oestrous cycles are usually normal in cases of mucometra due to congenital abnormalities of the uterus. However, persistence of the corpus luteum has been reported as an exceptional accompaniment of mucometra in the cow (Roberts & Fox, 1968). A possible association between the accumulation of secretion in the uterus and prolongation of luteal life-span has also been suggested in the pig (Cooper, Scofield & Brooks, 1970), though such effects in these and other species await experimental confirmation.

ACKNOWLEDGMENTS

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REFERENCES


