HORMONAL INDUCTION OF PSEUDOPREGNANCY IN RATS

U. K. BANIK AND MELVIN M. KETCHEL

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

(Received 22nd December 1964)

Summary. Various hormonal methods are compared of obtaining pseudopregnant rats capable of implanting fertilized ova transferred to them. Injection of 20 µg of oestrone to rats on Day 1 of pregnancy, followed by injection of 1 mg/day of medroxyprogesterone on Days 2 to 4, resulted in a high rate of implantation of fertilized ova transferred on Day 5. Non-pregnant rats were able to implant ova transferred to them when injected with prolactin, but not with progesterone. Indirect evidence is presented which indicates that the ability to implant ova is not absolute but may vary in degree among rats receiving different treatments.

INTRODUCTION

Pseudopregnancy is ordinarily characterized by the presence, in non-pregnant animals, of corpora lutea which remain functional for periods longer than those in cycling animals. Pseudopregnancy in non-pregnant animals has been reported to result from the application, at the appropriate time in the oestrous cycle, of a number of agents and stimuli, including mechanical stimulation of the cervix (Berswordt-Wallrabe, Geller & Herlyn, 1964), electrical stimulation of the cervix (Shelesnyak, 1931; Greep & Hisaw, 1938), mating with vasectomized males (Bitman, Wrenn, Cecil & Sykes, 1960), injection of progesterone (Rothchild & Schubert, 1963; Everett, 1963), injection of oestrogen (Alloatteau, 1957) and injection of prolactin (Aschheim, 1954). These authors utilized various criteria for judging the establishment of pseudopregnancy, and obtained various degrees of success as judged by the percentage of treated animals in which pseudopregnancy was established.

The availability of pseudopregnant rats to receive and implant transferred fertilized ova has come to be of great importance to laboratories investigating many aspects of maternal and ovum physiology. We have observed that when pseudopregnancy is induced in rats by mechanical or electrical stimulation of the cervix, or by progesterone or oestrogen injections, the rats may stop cycling, and, in some cases, are also capable of developing decidual reactions characteristic of pregnant rats, but only rarely do such rats implant fertilized ova transferred to them. In contrast, almost all rats, in which pseudopregnancy is
induced by mating with vasectomized males, are made pregnant by the transfer to them of fertilized ova.

The usefulness of the technique of mating with vasectomized males to obtain pseudopregnant rats for egg transfer studies, however, is limited by its great inconvenience in terms of time, expense and variable numbers produced. The present report describes the results of attempts to develop hormonal methods of obtaining pseudopregnant rats suitable for studies involving the transfer of fertilized ova.

MATERIALS AND METHODS

To obtain pregnant rats, Sprague-Dawley females weighing 170 to 200 g were placed in cages overnight with males of proven fertility. Pregnancy was established by the finding of spermatozoa in the vaginal smear the following morning (Day 1 of pregnancy). Experiments with non-pregnant females utilized rats with normal oestrous cycles on the day of oestrus.

Specific doses of steroid hormones were contained in 0·1 ml of sesame oil and injected subcutaneously. Specific doses of prolactin (NIH-P-S-5 ovine) were contained in 0·1 ml of 0·85 % NaCl and injected intraperitoneally.

Ova transferred to pseudopregnant recipients were obtained from normal pregnant rats. Ringer's phosphate buffer (pH 7·2 to 7·3) was used for the collection and transfer of ova to the uterus of the recipient. The induction of pseudopregnancy was timed so that ova removed from a rat on Day 4 of pregnancy were transferred to a recipient rat on Day 4 of pseudopregnancy, or from a rat on Day 5 of pregnancy to a recipient rat on Day 5 of pseudopregnancy. All the fertilized ova recovered from a pregnant rat were transferred to the recipient, except in selected experiments involving recipients which were pregnant. In these latter cases, ova were transferred to only one uterine horn so that the other uterine horn could serve to indicate whether the recipient rat's own ova were implanting. On Day 9, rats which had ova transferred to them were killed and the number of implantation sites recorded.

RESULTS

Banik & Pincus (1964) have reviewed and confirmed the findings of earlier investigators which indicate that an injection of oestrone into a rat on Day 1 of pregnancy causes the expulsion of ova from the reproductive tract. The results of a series of experiments in which we have attempted to utilize such rats for ova transfer are recorded in Table 1. We have confirmed that implantation is prevented by a single injection of 20 μg of oestrone on Day 1 of pregnancy (Group II). An examination, on Day 3 or 4, of rats treated in the same manner as those in Group II revealed that no ova were present in the Fallopian tubes or uterus. However, when rats which had received oestrone on Day 1 of pregnancy had 5-day ova transferred to them on Day 5, an implantation rate of 24 % was observed (Group III). Injection of oestrone-treated rats with 5 mg of progesterone on Day 3 did not increase the implantation rate of transferred ova (Group IV), but when 1 mg of ovine prolactin was given daily on Days 2 to 4, the implantation rate was increased to 45 % (Group VI). Very low implant-
Hormonal induction of pseudopregnancy in rats

Ation rates were observed when 4-day ova were transferred on Day 4 (Groups V and VII).

Rats which received 20 μg of oestrone on Day 1 of pregnancy followed by 1 mg of medroxyprogesterone, 17α-acetoxy-6α-methylpregn-4-en-3,20-dione ('Provera') on Day 3 implanted 50% of the fertilized ova transferred to them on Day 5 (Group VIII). When the medroxyprogesterone treatment was extended to Days 2 to 4, an implantation rate of 80% was observed (Group IX). Groups X and XI consist of rats in which the oestrone treatment on Day 1 was increased to 40 μg. If the medroxyprogesterone dose on Days 2 to 4 was 1 mg/day, no implantation of transferred ova occurred. However, when the medroxyprogesterone dose was increased to 2 mg/day, an implantation rate of 58% was observed.

### TABLE 1

<table>
<thead>
<tr>
<th>Group and Treatment</th>
<th>Day of pseudopregnancy when ova transferred</th>
<th>No. rats</th>
<th>No. ova transferred</th>
<th>Implanted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. ova</td>
</tr>
<tr>
<td>I</td>
<td>Control—made pseudopregnant by mating with vasectomized males</td>
<td>5</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>II</td>
<td>Control—oestrone (20 μg) on Day 1 of pregnancy</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>Control—oestrone (20 μg) on Day 1 of pregnancy</td>
<td>5</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>IV</td>
<td>Oestrone (20 μg) on Day 1 progesterone (5 mg) on Day 3</td>
<td>5</td>
<td>5</td>
<td>63</td>
</tr>
<tr>
<td>V</td>
<td>Oestrone (20 μg) on Day 1 progesterone (5 mg) on Day 3</td>
<td>4</td>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>VI</td>
<td>Oestrone (20 μg) on Day 1 prolactin (1 mg) on Days 2, 3 and 4</td>
<td>5</td>
<td>5</td>
<td>49</td>
</tr>
<tr>
<td>VII</td>
<td>Oestrone (20 μg) on Day 1 progesterone (5 mg) on Days 2, 3 and 4</td>
<td>4</td>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td>VIII</td>
<td>Oestrone (20 μg) on Day 1 medroxyprogesterone* (1 mg) on Day 3</td>
<td>5</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>IX</td>
<td>Oestrone (20 μg) on Day 1 medroxyprogesterone* (1 mg) on Days 2, 3 and 4</td>
<td>5</td>
<td>9</td>
<td>55</td>
</tr>
<tr>
<td>X</td>
<td>Oestrone (40 μg) on Day 1 medroxyprogesterone* (1 mg) on Days 2, 3 and 4</td>
<td>5</td>
<td>5</td>
<td>37</td>
</tr>
<tr>
<td>XI</td>
<td>Oestrone (40 μg) on Day 1 medroxyprogesterone* (2 mg) on Days 2, 3 and 4</td>
<td>5</td>
<td>7</td>
<td>33</td>
</tr>
</tbody>
</table>

* 17α-acetoxy-6α-methylpregn-4-en-3,20-dione.
Rothchild & Schubert (1963) and Everett (1963) have reported that pseudopregnancy is induced in rats by a single injection of progesterone. In an effort to determine whether or not such rats would be useful as recipients of transferred ova, we injected each of seven rats on the day of oestrus with 10 mg of progesterone. Four days later we transferred a total of forty-eight fertilized ova to these seven rats. Although the rats did not return to cycle, none of the fertilized ova implanted. Further experiments on five rats treated with 10 mg of progesterone on the day of oestrus revealed that such rats did not respond with decidual reactions characteristic of pregnancy when a thread was implanted in the uterus.

It was reported by Aschheim (1954) that pseudopregnancy is induced in rats by treatment with prolactin. In order to determine whether or not prolactin-treated rats would be useful as recipients of transferred ova, groups of rats were injected with specific doses of prolactin (NIH-P-S-5 ovine) for 8 days beginning on the day of oestrus. On the 5th day fertilized ova were transferred to them. As seen in Table 2, 0·5 mg/day of prolactin interrupted oestrous cycles in two of six rats but no ova transferred to them implanted. Injection of 1 mg/day of prolactin caused three out of five rats to stop cycling, and 50% of twenty-six ova transferred to these three rats implanted. Five of six rats stopped oestrous cycles when the daily dose of prolactin was increased to 2 mg, but the implantation rate of transferred ova was not improved.

It was of interest to determine whether or not the decrease in the implantation rate in certain groups in the present study occurred because some rats failed to implant any ova, or whether there was a decrease in the percentage of ova implanting in each rat. A comparison of the percentage of transferred ova implanting in each rat made pseudopregnant by mating with a vasectomized male with the percentage of transferred ova implanting in groups with lower average implantation rates is shown in Text-fig. 1. The lowered average implantation rate is not explained by an increase in the number of rats which do not implant any ova.

**DISCUSSION**

In our experience an injection of 20 μg of oestrone on the day spermatozoa are found in the vaginal smear (Day 1), followed by injections of 1 mg of medroxy-

---

**Table 2**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>No. rats treated</th>
<th>No. rats made pseudopregnant</th>
<th>No. ova transferred (to pseudopregnant rats)</th>
<th>Implantations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>0·5 mg</td>
<td>6</td>
<td>2</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>1·0 mg</td>
<td>5</td>
<td>3</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td>2·0 mg</td>
<td>6</td>
<td>5</td>
<td>22</td>
<td>11</td>
</tr>
</tbody>
</table>

* Daily dose initiated on Day 2 counting oestrus as Day 1. Injections continued to Day 8.
progesterone acetate on Days 2 to 4, produces a rat in which 5-day ova may be transferred on Day 5 with satisfactory results. Both of the compounds used are readily available commercially. We have found that the problem of obtaining appropriate numbers of synchronized donor and recipient rats may be simplified by dividing the pregnant rats obtained on a given day into two groups. One group, which receives no treatment, is used to provide ova. The rats of the other group are treated with oestrone and medroxyprogesterone as described, and are thus available at the appropriate stage of pseudopregnancy for the transfer of ova from the donor group. To increase our assurance that all of the ova resulting from the pregnancy of the recipient rat have been expelled,

![Text-fig. 1](image)

**Text-fig. 1.** A comparison of implantation rates in individual rats within groups made pseudopregnant by different treatments. (a) Mated with vasectomized males; (b) given oestrone on Day 1; (c) given oestrone on Day 1 and progesterone on Day 3; (d) given oestrone on Day 1 and prolactin on Days 2 to 4. Reduction of average implantation rate in the groups made pseudopregnant by hormonal treatments is not accounted for primarily by an increase in the number of rats with no implantations, but rather by a general lowering of the implantation rate in many individuals.

we have utilized the practice of transferring ova only to one horn of the uterus. The presence, therefore, of any implantation sites in the other horn would indicate that the rat had not responded satisfactorily to the oestrone treatment.

It is interesting to note that animals receiving a single dose of oestrone on Day 1 of pregnancy not only expel their ova from the reproductive tract, but also have a lowered ability to implant ova transferred to them on Day 5. Although the oestrone prevents implantation of transferred ova, it may be counteracted by the injection of progestational compounds such as medroxyprogesterone or by prolactin.

The term pseudopregnancy does not appear adequately to differentiate between the several conditions which it is used to describe. Depending upon the aims of the investigator, the species used and the criteria for its establishment, the term has been used to describe (a) the condition following oestrus in which no pregnancy has occurred in such species as the dog, (b) the hormonal...
interruption of oestrous cycles in species which have oestrous cycles, (c) the ability of an animal to respond to uterine stimulation with a decidual reaction characteristic of pregnancy, and (d) the ability of an animal to become pregnant when fertilized ova are transferred to it. Although functional corpora lutea are assumed to be present in all of these conditions (see Turner, 1963), the actual endocrine condition must differ, for we have observed that rats which cease cycling because of progesterone injections are not always able to form decidual reactions characteristic of pregnancy, and that rats which are able to form decidual reactions characteristic of pregnancy are not necessarily able to implant fertilized ova transferred to them. We propose, therefore, that the term pseudopregnancy be restricted to include the condition following oestrus in which no mating has occurred in such animals as the dog, and the condition in other species in which the individual is capable of becoming pregnant when fertilized ova are transferred to it.

In the normal pregnant rat, a comparison of the number of corpora lutea with the number of implantation sites on Day 9 of pregnancy indicates an implantation rate of about 95% of the ova. Yet the implantation rate of ova transferred to rats made pseudopregnant by mating to vasectomized males was 57% in the present experiment (Group I), and in our laboratory usually ranges from 50% to 80%. The major loss of ova in these transfer experiments does not occur because some rats fail to implant any ova, but rather because some ova transferred to each rat fail to implant. It has not been possible to determine whether the procedure for transferring ova renders some of them incapable of implanting, or whether rats mated by vasectomized males have a lower ability to implant ova than do pregnant rats. While this question cannot be answered directly, the data in Text-fig. 1 provide evidence that the ability to implant ova is not absolute but may exist in varying degrees. This suggests, though by no means proves, that the ability of a rat mated to a vasectomized male to implant ova is not as good as that of a pregnant rat, and that further experimentation may produce a recipient capable of implanting as high a percentage of ova as does a pregnant rat.

ACKNOWLEDGMENTS

This investigation was supported by Grant HD-00624-02 from the National Institute of Child Health and Human Development, Department of Health, Education and Welfare. Valuable technical assistance was provided by Miss Ellen Babas. The prolactin used in this study was provided as a gift by the Endocrinology Study Section of the National Institutes of Health, United States Public Health Service.

REFERENCES

Hormonal induction of pseudopregnancy in rats


