IMPLANTATION AND EARLY PREGNANCY IN OVARIECTOMIZED GUINEA-PIGS

R. DEANESLY

Division of Experimental Biology, National Institute for Medical Research, London, N.W.7

(Received 4th February 1960)

Summary. Ovo-implantation in the guinea-pig on the 6th to 7th day post coitum is not affected by ovariectomy on days 3 to 7 p.c. If the ovaries are removed on the 2nd day after mating, however, implantation does not occur unless at least one injection of progesterone is given. In the ovariectomized guinea-pigs, the embryos develop normally for several days but may regress after day 14 unless progesterone is supplied, permitting further growth. No evidence was found of delayed implantation.

INTRODUCTION

The termination of pregnancy by ovariectomy in the early stages has been observed in rabbits, rats, mice, cats, opossums, ground squirrels and other mammals (refs. in Courrier, 1945; Mayer, 1953) and the importance of the corpus luteum secretion during implantation has been stressed in these and later reviews on the endocrinology of early pregnancy (Kehl, 1950; Boyd & Hamilton, 1952; Courrier & Baclesse, 1955; Mayer, 1959). Exogenous progesterone with or without oestrogen enables implantation to take place normally in ovariectomized mammals.

Loeb & Hesselberg (1917), however, in a paper on the mammary gland reported briefly that in four guinea-pigs ovariectomy at 3½ to 6½ days post coitum (p.c.) did not prevent the implantation which normally occurs at 6½ to 7½ days p.c. At 18 days p.c., when these animals were killed, pregnancy was terminating in three and in one horn of the fourth. Loeb & Hesselberg also stated that implantation occurred in half the guinea-pigs if the corpora lutea were cauterized at 3 to 4 days p.c., but this work was complicated by the occurrence of fresh ovulations (see also Loeb, 1923). Later workers such as Herrick (1928), Nelson (1934) and Artunkal & Colonge (1949), who examined the effects of ovariectomy on the pregnant guinea-pig, did not repeat Loeb & Hesselberg's experiments of removing the ovaries before implantation but found that ovariectomy at 14 to 27 days p.c. terminated pregnancy, and might do so up to about 40 days p.c.

Ovulation in the guinea-pig occurs about 12 hr after the onset of mating behaviour (Rowlands, 1957), but, as Blandau (1949a) and others have observed,
Implantation in Ovariectomized Guinea-Pigs

wide variations often exist in the early stages of development in a single uterine horn during the 6th day after mating. Squier (1932) found that the eggs reached the uterus at the end of the 3rd day after mating.

MATERIAL AND TECHNIQUE

The guinea-pigs used were 400- to 700-g albinos from the stock maintained at the National Institute for Medical Research, most of them young non-parous animals. Mating was dated from the discovery of the vaginal plug in the morning and this is recorded as the day of mating or day 0; day 1 p.c. is the day following that on which the plug is found and so on. Females were isolated after mating. Ovariectomy was carried out dorsally under ether anaesthesia combined with a subcutaneous injection of 100 mg atropine. No ligatures were applied to the Fallopian tube or uterus. The animals all made a good recovery. Progesterone and oestrone or oestradiol were dissolved in arachis oil and given as subcutaneous injections and the animals were killed 1 day after the last injection. Sixteen control guinea-pigs at similar stages of pregnancy were compared with the experimental animals. Measurements were made of uterine swellings and in later stages of the crown-rump lengths of the embryos. The entire reproductive tracts were fixed in Bouin’s fluid and serial sections were made of many ovaries and of early embryological stages.

RESULTS

PRE-IMPLANTATION OVARIECTOMY AT DAYS 3 TO 5 P.C.

In a preliminary test of the survival and response to hormones of the unimplanted guinea-pig blastocyst, well-developed living foetuses were found in guinea-pigs ovariectomized on days 3 and 5 p.c. and killed on day 24 and day 28, respectively (Table 2). For the last 11 days only, these had received 10 mg progesterone daily with the addition of 2 μg oestrone on the 4th and 5th days of injection. The appearance of the foetuses indicated that implantation must have
occurred without obvious delay, before any exogenous hormones were given (Pl. 1, Fig. 1). Further experiments (Table 1) showed that out of twenty-three guinea-pigs ovariectomized on days 3 to 5 p.c., eighteen had implantations when killed at 10 to 18 days p.c., although no hormones were given. Up to about 13 days p.c., the implantation sites appeared normal but from 14 to 18 days p.c. more than half of the sites were regressing. Apparently a hormone deficiency usually set in about a week after implantation although in the original experiment embryos had survived in one guinea-pig up to day 17 p.c. without hormone injections. The number of implantations was normal. Of the twelve guinea-pigs ovariectomized on day 5, only eight showed implantations, but the number is too small to show that this is a more sensitive stage than at 4 or 6 days. Presumably failure to implant may also have been due to a failure at fertilization. In two of these animals, plugs were found late in oestrus.

### Table 2

**Survival of Embryos in Thirty Guinea-Pigs Ovariectomized Days 3 to 5 P.C. and Given Hormones at Various Later Stages**

<table>
<thead>
<tr>
<th>Progesterone</th>
<th>Oestrogen</th>
<th>No. of guinea-pigs</th>
<th>No. with implantations</th>
<th>No. with live embryos</th>
<th>Killed (Day p.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg</td>
<td>1 µg oestradiol Days 11 to 16</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>10 mg</td>
<td>2 µg oestrone Days 16, 17</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>10 mg</td>
<td>2 µg oestrone Days 20, 21</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>10 mg</td>
<td>2 µg oestrone Days 22, 23</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>30</td>
<td>27</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. (a) 21-day normal control. (b) 21-day embryo from a guinea-pig ovariectomized on day 3 p.c. and given progesterone from days 11 to 20 p.c. and 1 µg oestradiol from days 11 to 16. (c) two 21-day embryos from a guinea-pig ovariectomized day 4 p.c. and given progesterone from days 11 to 20. The control embryo, the only one in the uterus, is a little larger than average.

Fig. 2. (a) 28-day normal control. (b) 28-day embryo from a guinea-pig ovariectomized day 5 p.c. and given progesterone 10 mg daily from days 17 to 27 and oestrone 2 µg days 16 and 17 (Table 2).

(Facing p. 244)
Implantation in Ovariectomized Guinea-Pigs

Table 2 shows the results of injecting progesterone and oestrogen from about day 11 p.c. into twenty-six similar ovariectomized guinea-pigs killed at 21 days p.c., 1 day after the last injection: implantations had occurred in twenty-three of these animals and live normal embryos were present in thirteen. Of the three animals with no implantations, one had mated only on the 6th day of vaginal opening. There was no evidence that the addition of oestrogen affected the results, but more embryos survived in the guinea-pigs receiving 10 mg progesterone instead of 5 mg. The last four animals in the Table, killed at 24 to 29 days p.c., are those of the original experiment. The weights of the fixed 21-day embryos of the experimental animals were variable and often rather less than those of the corresponding normals. The largest, however, were fully developed

Table 3
GUINEA-PIGS Ovariectomized on Day 2 p.c. and Receiving Progesterone at Varying Times

<table>
<thead>
<tr>
<th>Hormone injections</th>
<th>No. of guinea-pigs</th>
<th>No. with implantations</th>
<th>Killed (Day p.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 mg Day 2</td>
<td>2</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>3 mg Day 2</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2 mg Day 2</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>1 mg Day 2</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>0.5 mg Day 2</td>
<td>2</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 mg Day 3 to Day 11</td>
<td>4</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>10 mg Days 4 and 5</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg Days 11 to 20</td>
<td>2</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg Days 11 to 16</td>
<td>6</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg Days 7 to 14</td>
<td>2</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Oestradiol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 µg Days 7 and 8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Pl. 1, Figs. 1 and 2): eight such experimental embryos had an average weight of 107 mg (range 85 to 180 mg), while the corresponding eight embryos from four control 21- to 22-day pregnancies had an average weight of 118 mg (range 65 to 200 mg). Smaller embryos, about 50 to 60 mg, were found in both experimental and control groups. An increased number of surviving embryos and better growth could probably be obtained by more efficient hormone administration in the ovariectomized animals.

A preliminary note has been written on these experiments (Deanesly, 1960).

Pre-Implantation Ovariectomy at Day 2 p.c.

In the upper half of Table 3, the records show that normal implantation will occur after ovariectomy on day 2 if progesterone is given at the time of operation or as late as day 4. Single injections of 4, 2, 1 and 0.5 mg at ovariectomy
all permitted normal implantation, but after 0.5 mg only one swelling was found in two guinea-pigs. In the ten other guinea-pigs in which no progesterone was given till day 7 or day 11, only one implantation occurred which was regressing at autopsy. To these may be added the two guinea-pigs in Table 1 which also had no implantations. The guinea-pig which had a normal implantation, although no progesterone was given till day 4, shows that transport through the Fallopian tube can be independent of the ovarian hormones and that the absence of implantation in the other guinea-pigs was unlikely to be due to the eggs failing to enter the uterus from the tube.

Clearly the marked difference between the effects of ovariectomy on day 2 and on day 3 are due to the uterus having received an inadequate supply of progesterone at the earlier stage. Loeb (1908, 1932b) reports that deciduomata can be stimulated in the uterus of the guinea-pig only between the 3rd and 9th days after ovulation.

Not only do the blastocyst fail to develop in these experiments but they fail to survive to the 7th day after mating, otherwise the 10 mg of injected progesterone would presumably stimulate implantation. It is possible that the guinea-pig blastocyst only persists in the presence of the minimal amount of progesterone (or of a uterine secretion stimulated by progesterone) which is sufficient for its development.

OVARIECTOMY AT ABOUT THE TIME OF IMPLANTATION
(DAY 6 OR 7)

Mayer (1959), as the result of his experiments on the rat, stresses the susceptibility of the blastocyst to changes in the endocrine environment at the time of implantation. Six guinea-pigs were therefore ovariectomized on day 6 and two on day 7. Table 1 shows that all but one implanted normally though not receiving progesterone. One other guinea-pig, not in Table 1, was given progesterone from day 11 (5 days after ovariectomy) to day 19 and killed on day 20 and was found to have a normal living embryo.

DISCUSSION

These experiments show that, provided the uterus has received a minimum of progestational hormone, the whole process of ovo-implantation can occur in the guinea-pig in the absence of the ovaries or of exogenous hormones, and the embryos will continue to develop normally for several days. The results indicate early secretory activity by the developing corpus luteum which can be dispensed with before it is fully luteinized, and also an innate capacity for development by the guinea-pig blastocyst up to at least day 11 and sometimes longer. At this stage, the normal corpus luteum (Rowlands & Short, 1959) begins to show an increased concentration of progesterone which could imply increased production and secretion. Certainly the requirements of the embryos increase and in most cases they will fail to develop fully normally in the ovariectomized animals unless exogenous progesterone is supplied.

The guinea-pig therefore differs in an important respect from the other rodents including the hamster (Orsini & Meyer, 1959) in which ovariectomy
Implantation in Ovariectomized Guinea-Pigs

between mating and implantation of the eggs terminates the incipient pregnancy. Apart from the few recently mated guinea-pigs, ovariectomized by Loeb with results confirmed by the present work, the only records of implantation following ovariectomy are those of Buchanan, Enders & Talmage (1956) who found that implantation could occur in the armadillo 1 month after ovariectomy if the latter were carried out during the 4-month period when the eggs were normally delayed in the uterus.

All the evidence in these experiments suggests that the blastocyst of the guinea-pig either implants at about the normal time or disappears. There were no indications that, like that of the rat or mouse, it could remain for any length of time in a viable resting condition.

Loeb’s (1932a) report on occasional early-stage embryonic development in the ovaries of guinea-pigs shows that up to a point this process may be independent of the adjacent tissue. Ponse, Weihs, Libert & Dovaz (1954) and others have confirmed the sporadic occurrence of these trophoblastomas. Blandau’s (1949b) experiments on the culture of blastocysts indicated that those of the guinea-pig (6 to 8 days after fertilization) caused more proteolysis of the substrate than did rat blastocysts (5 to 6 days after fertilization) under similar conditions. On the other hand, Blandau found that the endometrium of the guinea-pig did not react to beads which stimulated deciduomata in rat uteri.

It is probable that important species differences exist in the actual implantation processes. Further work may indicate if oestrogen plays any part during nidation in the ovariectomized guinea-pig and if so from which tissues it is derived.

ACKNOWLEDGMENT

I am indebted to Miss N. Reid for valuable technical assistance in the course of this work.

REFERENCES


Loeb, L. (1932) The production of deciduomata and the relation between the ovaries and the formation of the decidua. J. Amer. med. Ass. 50, 1897.


Rowlands, I. W. & Short, R. V. (1959) The progesterone content of the guinea-pig corpus luteum during the reproductive cycle and after hysterectomy. J. Endocrin. 19, 81.