BRIEF COMMUNICATION

ENDOCRINE ASPECTS OF EGG IMPLANTATION
IN THE RAT

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Psychoyos (1961) examined the development of eggs which had been collected from donor rats, that were either normally pregnant or undergoing delayed implantation, and then transferred to the uteri of spayed and progesterone treated recipients. It was found that implantation of such eggs occurred only after the recipient had been treated with oestrogen. Later, he established the importance of the timing of oestrogen treatment in relation to egg transfer (Psychoyos, 1963). We have now examined the fate of blastocysts transferred to the uteri of rats, which were treated in several different ways, so as to obtain further information on the hormonal conditions affecting implantation.

A total of sixty-five adult hooded rats was used as recipients. Eggs were obtained from donors during the morning of Day 5 of pregnancy (Day 1 = day on which spermatozoa were found in the vagina) by flushing their uterine horns with physiological saline. The eggs thus obtained were mostly at the blastocyst stage, with the zona pellucida still present. For transfer a fine glass pipette was used, drawn out to 250 to 300 μ and controlled by a short length of polyethylene tubing; it was inserted into the uterine lumen through a small hole made with a needle at the tubal end of the uterus.

The recipients were divided into four groups as follows:

Group 1: Eight rats, 5 days pseudopregnant (control, synchronous transfer).

Group 2: Nineteen rats spayed on Day 3 of pseudopregnancy, and injected subcutaneously daily with 2 or 4 mg of progesterone, beginning on the day of spaying (delayed implantation condition); eggs were transferred to these animals 4 to 7 days after spaying.

Group 3: Eleven rats spayed at either pro-oestrus, oestrus or di-oestrus, and then treated daily with 2, 4 or 8 mg of progesterone; eggs were transferred to these animals on the day corresponding with the following oestrus, that is the one that would have occurred had they not been spayed, namely, 5 days after spaying at pro-oestrus, 4 days after spaying at oestrus, or 2 days after spaying at di-oestrus.

Group 4: Twenty-seven normally-cycling, intact rats, injected daily with 2 mg of progesterone for 7 days starting at pro-oestrus, oestrus, metoestrus, or

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TABLE 1
EGG TRANSFER IN PSEUDOPREGNANT AND SPAYED RATS TREATED WITH PROGESTERONE

Group No.	Condition of recipients	Progesterone dose from spaying to autopsy (mg/day)	Time of egg transfer	No. of recipients	No. of eggs transferred	4 days after transfer		8 days after transfer	
						No. of recipients with implants	No. of implants	No. of recipients with implants	No. of implants
1	Pseudopregnant	1	Day 5 of pseudopregnancy	8	91	8	60	0	0
2 ^a	Pseudopregnant, spayed on Day 3	2	Day 7, 8, 9, 10 or 11 of pseudopregnancy	7	74	0	0	7	31
b		4		12 ⁶ 12 ⁶	113 ⁵⁷ 56	0	0	6	32
3	Cyclic, spayed	2, 4 or 8	2 to 5 days after spaying	11	104	0	0	0	Deciduomata 55

All the rats were examined 4 days after transfer and those in which implantation had not occurred were given a single injection of 0.2 µg oestradiol and progesterone treatment was continued for another 4 days.

di-oestrus; eggs were transferred to these animals on the 4th day of progesterone treatment.

Recipients in all four groups were examined by laparotomy 4 days after egg transfer. At this time implantations, when present, were observed as swellings of about 5 mm diameter, the number of which was recorded. The differentiation of implantations and deciduomata were based on their external appearance and location, the deciduomata, which were few in number, occurring at the site of introduction of the transfer pipette. If implantations were not observed, as in Groups 2 and 3, a single injection of 0.2 µg of oestradiol was given and a second laparotomy was performed 4 days later. In Group 4, if implantations were observed at the first laparotomy, a second laparotomy was performed after an interval of 5 days, but whenever implantation had not occurred it was performed 3 days after the first laparotomy. The results pertaining to Groups 1, 2 and 3 are given in Table 1.

TABLE 2

FATE OF EGGS TRANSFERRED TO NORMALLY CYCLING RATS, TREATED WITH
PROGESTERONE (2 mg/DAY FOR 7 DAYS)

<i>Stage of cycle when progesterone treatment started</i>	<i>No. of eggs transferred</i>	<i>No. of implants 4 days after transfer</i>	<i>No. of embryos maintained to 9 days after transfer</i>	<i>No. of young born</i>
Pro-oestrus	59 (6)	5 (1)	1 (1)	1 (1)
Oestrus	78 (9)	38 (9)	7 (4)	6 (3)
Metoeustrus	36 (6)	18 (4)	8 (3)	3+ (3)
Di-oestrus	50 (6)	13 (2)	3 (1)	3 (1)

Numbers of rats in parentheses.

Implantation did not occur in spayed recipients treated with progesterone alone until oestrogen was given, regardless of the recipients' condition before spaying, i.e. whether they were pseudopregnant or cyclic. In Group 2, implantations were observed 4 days after oestrogen administration in six out of twelve rats; deciduomata were present in the remaining six rats.

Delayed implantation was not observed in Group 4 recipients which are included in Table 2. Of the total of seventy-four implants recorded in sixteen rats at the time of the first laparotomy (corresponding with Day 9 of pregnancy) fifty-five had undergone resorption before the time of the second laparotomy, that is 5 days later. Out of the nineteen surviving embryos, at least thirteen young were produced by eight females.

From the daily vaginal smear records it appears that, during the period of progesterone treatment, the oestrous cycle was generally depressed. In those rats in which progesterone treatment was started at pro-oestrus, cornified epithelial cells were observed on the following day but thereafter the cycle was suppressed.

When progesterone treatment was started at stages other than pro-oestrus, a pro-oestrous smear (all epithelial cells) was occasionally observed 3 to 4 days

after the previous oestrus. This pro-oestrus was, however, not followed by oestrus, but by di-oestrus with leucocytes dominant in the smear.

The delay in implantation that we observed following the transfer of normal eggs to spayed and progesterone treated recipients indicates that it is the condition of the uterus rather than that of the egg that determines whether or not implantation will occur: Psychoyos (1961) reached a similar conclusion. The same author reported that implantation was delayed when eggs were transferred to recipients which had been spayed and left untreated for 2 weeks in order to eliminate the ovarian hormones before commencing progesterone treatment. However, in the present experiment it was observed that delayed implantation occurred even in recipients spayed at oestrus and treated immediately with progesterone. A similar observation has been made by Dickmann (personal communication).

A high proportion of transferred eggs implanted in the cyclic rats which were treated with progesterone beginning at oestrus or metoestrus. As the eggs were transferred on the 4th day of progesterone treatment, the time of transfer in these recipients would correspond with pro-oestrus or oestrus of the following cycle, had they not been treated with progesterone.

It is suggested that a secretion of oestrogen coinciding with egg transfer created a uterine condition conducive to implantation. Further evidence of oestrogen secretion is provided by a change in the vaginal smear from 'di-oestrous' to 'pro-oestrous' in some of the rats. Psychoyos (1963) observed that a high proportion of eggs implanted in recipients which had been spayed and treated with progesterone before being given a single injection of oestrogen at the time of egg transfer. Thus, oestrogen, whether of endogenous or exogenous origin, acts synergistically with progesterone to induce implantation: the availability of oestrogen at the time of egg transfer appears important for implantation.

REFERENCES

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