EMBRYONIC SURVIVAL AND DEVELOPMENT IN UNILATERALLY OVARIECTOMIZED RATS

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(Received 23rd April 1970, revised 8th December 1970)

Summary. Unilateral ovariectomy on the 3rd day of pregnancy in the rat resulted in arrested embryonic development and decreased implantation in the ipsilateral uterine horn. These degenerative changes were apparent 48 hr, but not 24 hr, after operation. A single subcutaneous injection of either oestrone (1.0 µg) or progesterone (2.0 mg) at 08.00 hours on the 5th day of pregnancy induced implantation and foetal maintenance in the ipsilateral horn. Treatment with progesterone was consistently more effective than oestrogen.

These observations suggest that unilateral ovariectomy induces a hormonal deficiency in the adjacent uterine horn which is incompatible with optimum preimplantation embryonic development.

INTRODUCTION

Unilateral ovariectomy performed early in pregnancy in the rat (Lamming & Little, 1968) and mouse (Bruce, Renwick & Finn, 1968) results in significantly higher embryonic mortality in the ipsilateral compared to the contralateral uterine horn. These data are interpreted as evidence for local control of the uterus by the adjacent ovary. The local effect is observed only if ovariectomy is performed within 86 to 88 hr *post coitum* (*p.c.*) in the rat (Lamming & Little, 1968) and within 60 hr *p.c.* in the mouse (Bruce *et al.*, 1968). Pregnancy is not terminated in rats unilaterally ovariectomized later than 110 hr *p.c.* (Little, Gray & Lamming, 1969) or after implantation (Krehbiel, 1941; Nuti, Ward & Meyer, unpublished data).

Previous workers have reported embryo survival in terms of the number of viable foetuses 13 days *p.c.* (Lamming & Little, 1968) and 8 to 14 days *p.c.* (Bruce *et al.*, 1968), or the number of animals in which unimplanted blastocysts could be recovered from the horn on the same side as the ovariectomy 5 to 7 days *p.c.* (Bruce *et al.*, 1968). Neither the time at which embryonic development is affected by unilateral ovariectomy nor the mechanisms involved are clearly understood. Little *et al.* (1969) proposed that the oviduct may mediate the local effect of the ovary on the uterus and that this effect may be primarily hormonal.

The objectives of this study were to determine: (1) the time at which

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embryonic development is altered by unilateral ovariectomy, and (2) the effect of exogenous oestrone and progesterone on foetal implantation and survival in the ipsilateral horn of unilaterally ovariectomized rats.

MATERIALS AND METHODS

Mature virgin female rats of the Sprague-Dawley strain (Badger Research Corporation, Madison, Wisconsin) weighing 175 to 200 g were maintained on Rockland Mouse/Rat Laboratory Diet (Teklab, Inc., Monmouth, Ill.) and unrestricted water, at a temperature of 72 to 78° F and under a light regimen of 14 hr of light per day (05.00 to 19.00 hours CST). Females were placed with males in the evening and vaginal smears examined daily for the presence of spermatozoa. The day on which spermatozoa were found was designated the 1st day of pregnancy. The animals were ovariectomized according to the method of Cochrane & Meyer (1957).

In an effort to determine the relative importance of vascular trauma associated with ovariectomy, the mesovarium of eight animals was unilaterally ligated on the 3rd day of pregnancy (ligated horn). The opposite ovary was exposed for a comparable period of time but was not ligated (intact horn). The animals were killed on the 20th day of pregnancy and the numbers of viable, dead and resorbed fetuses were recorded as well as foetal, placental and ovarian weights.

Experiment 1

Fifty-seven animals were unilaterally ovariectomized on the 3rd day of pregnancy between 10.00 and 14.00 hours CST. Sham operations, in which the ovary was exposed for a comparable period of time, were performed on the contralateral side. These animals were killed at various times between the 4th and 8th days and the number of implantation sites in the contralateral and ipsilateral horns was counted. All uteri without implantation sites and oviducts from animals killed on the 4th day were flushed with Medium 199 (Microbiological Associates, Inc., Bethesda, Maryland) and the number of embryos and their stage of development were recorded.

Twenty intact pregnant animals (controls) were killed on the 5th day of pregnancy. A single uterine horn from each animal was flushed with Medium 199 and the number of embryos and their stage of development were recorded.

Experiment 2

Unilateral ovariectomy was performed on fifty animals between 10.00 and 14.00 hours CST on the 3rd day of pregnancy. Subcutaneous (s.c.) injections of either progesterone (Upjohn Co., Kalamazoo, Michigan, Lot HR 855) or oestrone (USP, International Hormones, Hicksville, L.I., New York, Control Number 5274) were administered in 0.2 ml corn oil (USP, Magnus, Mabee and Reynard, Inc., New York) at various times after unilateral ovariectomy. Five treatments were employed. Oestrone (0.5 µg) was injected on the 4th day at 16.00 hours or the 5th day at 08.00 hours, or 1.0 µg oestrone was injected at 08.00 hours on the 5th day. Progesterone (2 mg) was given as a single injection

on the 5th day at 08.00 hours or daily at 08.00 hours on the 5th to 20th days inclusive. The control group was injected daily with 0.2 ml corn oil on the 5th to the 20th days at 08.00 hours. Animals were subjected to laparotomy on the 10th day through a mid-ventral abdominal incision and the occurrence of implantation sites in both the contralateral and ipsilateral horns was noted. At autopsy on the 20th day, foetal, placental and ovarian weights, and the numbers of live and dead fetuses and resorption sites were recorded.

RESULTS

At autopsy on the 20th day of pregnancy, six of the eight unilaterally ligated animals were pregnant in both horns, with means of 4.3 ± 1.0 and 5.5 ± 0.7 fetuses per ligated and intact horn, respectively; these were not significantly different ($P < 0.10$). Visual examination revealed that the ovary on the same side as the ligation was considerably smaller in three of the animals. Two of these

TABLE 1
EMBRYO SURVIVAL ON THE 3RD DAY OF PREGNANCY IN
UNILATERALLY OVARECTOMIZED RATS

Day of autopsy	No. of animals	Animals with embryos in:		
		Ipsilateral horn only	Contralateral horn only	Both horns*
4	9	0	1	8 (89%)
5 a.m.	12	1	4	7
5 p.m.	12	0	3	9 } (67%)
6	8	0	3	5 (62%)
7	8	0	6	2 (25%)
8	9	0	7	2 (22%)

* Number in parentheses expresses percentage of pregnant rats with embryos in both horns.

animals had three and five fetuses in the ligated horn while there were no fetuses in the corresponding horn in the third animal. There was a significant difference ($P < 0.05$) in the mean number of corpora lutea on the intact (5.7 ± 0.6) and ligated (2.2 ± 1.0) sides.

Experiment 1

On the afternoon of the 4th day, approximately 24 hr after unilateral ovariectomy, embryos were recovered from both the ipsilateral and contralateral uterine horns and/or oviducts in eight out of nine animals (Table 1). The average number of embryos recovered from the ipsilateral and contralateral horns was quite similar, with 70 to 75 % either in the eight-cell stage or morulae (Table 2). At this time, there was no difference in either the stage of development or the incidence of degeneration in embryos as a result of unilateral ovariectomy. An embryo was considered to have degenerated if all the cells within the zona pellucida were aggregated into an amorphous mass.

On the morning of the 5th day, embryos were recovered from the ipsilateral horn in eight out of twelve animals (Table 1). Sixty-one per cent of the embryos from the side of the ovariectomy were normal blastocysts, with or without zonae pellucidae, in contrast to 89% in the contralateral horn and control animals (Table 3). Furthermore, the incidence of retarded development and degenerated embryos in the horn ipsilateral to the ovariectomy was greater than that observed in either the contralateral horn or in horns from intact animals (control). An average of 2.9 ± 0.4 embryos per ipsilateral horn was significantly different ($P < 0.05$) from 4.8 ± 0.4 per contralateral horn.

TABLE 2
EMBRYO DEVELOPMENT ON THE 4TH DAY OF PREGNANCY
IN UNILATERALLY OVARECTOMIZED RATS

Stage of development	No. of embryos in:	
	Ipsilateral horn (8)*	Contralateral horn (9)*
Two to six cells	8	6
Eight cells	13	10
Morulae	13	18
Blastocyst	0	1
Degenerated embryos	2	3
Total no. of embryos	36 (4.5 ± 0.2)†	38 (4.2 ± 0.2)

* No. of horns.

† Average number of embryos/horn $\pm$ S.E.

Embryos were recovered from the ipsilateral horn in nine out of twelve animals killed on the afternoon of the 5th day, approximately 48 hr after unilateral ovariectomy (Table 1). There was a significant difference ($P < 0.05$) in the average number of embryos recovered from the ipsilateral (2.6 ± 0.6) and contralateral (4.0 ± 0.4) horns. Only 43% of the embryos from the ipsilateral horn were normal blastocysts compared to 96% in both the contralateral and control horns. In the ipsilateral horn, 48% of the embryos were degenerated and 9% were retarded in development.

When autopsy was performed 3 days after unilateral ovariectomy on the 6th day of pregnancy, five out of eight animals had embryos in the ipsilateral horn (Table 1). Means of 2.8 ± 0.9 and 5.1 ± 0.3 embryos in the ipsilateral and contralateral horns, respectively, were not statistically different ($P < 0.10$) (Table 4). Fewer degenerated embryos were recovered from both horns. Embryos from the ipsilateral side appeared to be normal although development was retarded, with four out of fourteen embryos (29%) implanted in the ipsilateral side and twenty-five out of forty-one (61%) in the contralateral horn.

There was a marked reduction in the number of animals with implants in the ipsilateral horn on the 7th and 8th days (four out of eighteen). The implants in the ipsilateral horns on the 8th day were much smaller than those in the

TABLE 3
EMBRYO DEVELOPMENT ON THE 5TH DAY OF PREGNANCY IN UNILATERALLY OVARECTOMIZED RATS

Stage of development	a.m.		p.m.	
	Ipsilateral horn (8)*	No. of embryos in: Contralateral horn (11)	Ipsilateral horn (9)	No. of embryos in: Contralateral horn (12)
Four-cell to morulae	5 (22%)†	6 (11%)	2 (9%)	0
Normal blastocysts with zona without zona implanted	6 8 0	41 6 0	4 6 0	17 24 6
Total	14 (61%)	47 (89%)	10 (43%)	37 (96%)
Degenerated embryos	4 (17%)	0	11 (48%)	2 (4%)
Total no. of embryos	23 (2.9 ± 0.4)†	53 (4.8 ± 0.4)	23 (2.6 ± 0.6)	49 (4.0 ± 0.4)
				27 (3.4 ± 0.6)

* No. of horns.

† % of total embryos in group.

‡ Average number of embryos/horn ± S.E.

TABLE 4
EMBRYO DEVELOPMENT ON THE 6TH DAY OF PREGNANCY IN UNILATERALLY OVARECTOMIZED RATS

Stage of development	No. of embryos in:	
	Ipsilateral horn (5)*	Contralateral horn (8)
Normal blastocysts with zona	0	4
without zona	1	0
elongated	8	9
implanted	4	25
Degenerated embryos	1	3
Total no. of embryos	14 (2.8 ± 0.9)†	41 (5.1 ± 0.3)

* No. of horns.

† Average number of embryos/horn ± S.E.

TABLE 5
IMPLANTATION IN UNILATERALLY OVARECTOMIZED RATS TREATED WITH OESTRONE OR PROGESTERONE

Treatment	No. of animals	Animals with implants in:			
		Ipsilateral horn only	Contralateral horn only	Both horns	Neither horn
E* 0.5 µg, 4th day p.m.	7	1	6	0	0
E 0.5 µg, 5th day a.m.	7	1	6	0	0
E 1.0 µg, 5th day a.m.	7	3	4	0	0
P† 2.0 mg, 5th day a.m.	10	6	2	0	2
P 2.0 mg, daily	10	4	3	0	3
Corn oil (control)	9	2	6	0	1

* Oestrone (s.c.) in 0.2 ml corn oil.

† Progesterone (s.c.) in 0.2 ml corn oil.

TABLE 6
POSTIMPLANTATION FOETAL SURVIVAL IN UNILATERALLY OVARECTOMIZED RATS TREATED WITH OESTRONE OR PROGESTERONE

Treatment	No. of animals*	Total no. of foetuses					
		Ipsilateral horn			Contralateral horn		
		Alive	Dead	Resorbed	Alive	Dead	Resorbed
E† 0.5 µg, 4th day p.m.	1	3	0	1	5	0	0
E 0.5 µg, 5th day a.m.	1	3	1	0	5	0	0
E 1.0 µg, 5th day a.m.	3	9	0	0	18	0	0
P‡ 2.0 mg, 5th day a.m.	6	25	0	2	36	0	1
P 2.0 mg, daily	4	20	1	1	18	0	1
Corn oil (control)	2	8	0	1	4	1	0

* Results include only the females with implants in both uterine horns.

† Oestrone (s.c.) in 0.2 ml corn oil.

‡ Progesterone (s.c.) in 0.2 ml corn oil.

contralateral side, suggesting that either asynchronous implantation had occurred or the embryos had begun to resorb in the ipsilateral horn.

Experiment 2

The results of the hormone replacement studies are summarized in Tables 5 and 6. Embryos implanted in the ipsilateral horn in two out of nine unilaterally ovariectomized control animals given corn oil alone. A single implant in the ipsilateral horn of one of these animals had resorbed. The other animal had eight foetuses in the ipsilateral horn. We cannot offer an explanation for this single instance of successful pregnancy.

Both oestrone and progesterone induced implantation in the ipsilateral horn of unilaterally ovariectomized rats, although the latter was consistently more effective (Table 5). Oestrone ($0.5 \mu\text{g}$) administered either on the afternoon of the 4th day or the morning of the 5th day, was no more effective in inducing implantation than corn oil given alone ($P < 0.10$). In contrast, a single injection of $1.0 \mu\text{g}$ oestrone on the morning of the 5th day induced both implantation and foetal maintenance in three out of seven animals. Progesterone (2 mg) was equally effective in inducing implantation and foetal maintenance in the ipsilateral horn whether administered as a single injection on the 5th day or daily from the 5th to the 20th days ($P < 0.10$). However, the single dose of progesterone (2.0 mg on the 5th day) was the only treatment which produced a significant difference in implantation when compared with the corn oil controls ($P < 0.01$).

There was no evidence of any real difference in postimplantation embryonic survival between treatment groups ($P < 0.10$) (Table 6). However, the mean number of viable foetuses in the ipsilateral horn following progesterone treatment (4.5 ± 0.7) was significantly different ($P < 0.01$) when compared with rats given oestrone (3.0 ± 0.02). Although there was no significant difference ($P < 0.10$) in foetal survival in the ipsilateral (4.5 ± 0.7 foetuses) and contralateral (5.4 ± 0.6 foetuses) horns of animals treated with progesterone, the average number of foetuses in the ipsilateral horn of animals receiving oestrone (3.0 ± 0.02) was significantly different when compared with the contralateral horn (5.6 ± 0.3).

There was no apparent correlation between the number of corpora lutea in the contralateral ovary and the frequency of implantation and foetal maintenance in the ipsilateral horn of hormone-treated rats. The mean number of corpora lutea per contralateral ovary was 5.8 ± 0.6 for animals treated with progesterone and 5.8 ± 0.1 for animals receiving oestrone. The close similarity between foetal weights in the ipsilateral ($2.5 \pm 0.1 \text{ g}$) and contralateral ($2.7 \pm 0.1 \text{ g}$) horns as well as placental weights (ipsilateral: $0.7 \pm 0.03 \text{ g}$; contralateral: $0.75 \pm 0.03 \text{ g}$) indicates that nidation was synchronized in the two horns. Compensatory hypertrophy of the contralateral ovary may have occurred during the pregnancy although hypertrophy was not apparent at the time of autopsy.

DISCUSSION

The results of these studies indicate that distinct differences in embryonic

survival and development occur in the ipsilateral as compared to the contralateral uterine horns of unilaterally ovariectomized rats. Embryonic development was normal for approximately 24 hr after unilateral ovariectomy on the 3rd day of pregnancy. By the morning of the 5th day, however, the incidence of embryonic degeneration and developmental retardation in the ipsilateral horn was increased over that found in the contralateral horn; by the afternoon of the 5th day, 48 hr after unilateral ovariectomy, these differences were marked. Very few embryos survived for 72 hr in the ipsilateral horn. These observations suggest a local effect of the ovary on the uterus.

Operative procedures associated with ovariectomy may alter the vascular supply to the oviduct and uterus and thus affect embryo transport and survival (Senger, Lose & Ulberg, 1967; Bazer, Ulberg & LeMunyan, 1969). Although ligation of the ovarian vessels may be one factor contributing to increased embryonic mortality after unilateral ovariectomy, it is not the major factor, since it did not eliminate the influence of the ovary on the uterus in six out of eight animals.

These data suggest that local transport between ovary and uterus is accomplished by some mechanism which does not directly involve the ovarian vein and artery. Communicating vessels between the uterus and ovary which were not ligated and/or small blood vessels which have regenerated following ligation may provide the means whereby ovarian substances reach the uterus. Although lymphatic circulation between ovary and uterus has not been described in the rat, one cannot rule out the possibility that lymphatic vessels may also participate in a local transport mechanism. Little *et al.* (1969) postulated that the local effect is mediated by the oviduct, since a significant reduction in embryonic survival was noted in the ipsilateral horn following unilateral salpingemphraxis. It is not clear how oviducal intervention is accomplished, i.e. whether it is an inherent property of the oviduct and its secretions or whether it is associated with the vascular relationship between ovary, oviduct and uterus.

Perhaps the most tenable explanation for reduced foetal survival is that unilateral ovariectomy induces a local deficiency in ovarian hormones in the adjacent uterine horn such that the hormonal environment is incompatible with optimum preimplantation embryonic development. This interpretation is consistent with the recent observation that normal development from morula to blastocyst can occur in the complete absence of ovarian hormones (Dickman, 1969); if, however, ovarian hormones are present, any deviation from the hormonal conditions present on the 4th day of pregnancy is lethal to the morula (Dickman & Noyes, 1960).

Exogenous systemic administration of oestrone and progesterone was utilized to determine the hormonal requirements for implantation and foetal maintenance in the ipsilateral horn. In bilaterally ovariectomized rats, exogenous progesterone is required for the maintenance of viable blastocysts; nidation will not occur until a small quantity of oestrogen is administered (Cochrane & Meyer, 1957; Psychoyos, 1960; Meyer & Nutting, 1964; Nutting & Meyer, 1964). Progesterone alone, whether administered daily in large amounts (Canivenc, Laffargue & Mayer, 1956) or applied directly to the uterus (Psychoyos, 1962) will not induce implantation in bilaterally ovariectomized rats.

Our studies show that preimplantation embryonic development is retarded in the ipsilateral horn when rats are unilaterally ovariectomized early in pregnancy (on the 3rd day) and that either progesterone or oestrone administered on the 5th day of pregnancy induces implantation and foetal maintenance in the ipsilateral horn. Implantation in response to progesterone alone was not due to the natural oestrogenicity of the corn oil used as the progesterone vehicle (Booth, Bickoff & Kohler, 1960), since corn oil alone induced successful implantation in only one out of eight control animals.

The apparent paradox of progesterone-induced implantation in the unilaterally ovariectomized rat may be resolved by postulating that the intact ovary, through systemic circulation, supplies small quantities of hormones to the ipsilateral horn. Sufficient progesterone may be available to the ipsilateral horn so that optimal endometrial sensitivity (Psychoyos, 1966) is achieved, although there is not enough progesterone to maintain viable blastocysts. There may also be an oestrogen deficiency which is compensated for by the administration of adequate amounts of progesterone. Thus, exogenous progesterone not only ensures embryonic survival but may also facilitate implantation by synergy with subliminal amounts of oestrogen.

Oestrone (1 µg s.c. on the 5th day) may both initiate implantation and enhance embryo survival by potentiating the sub-optimal amounts of progesterone produced by the intact ovary.

It is evident from the data in Exp. 1 that most embryos in the ipsilateral horn degenerated within 48 hr after unilateral ovariectomy. The results of hormone replacement experiments suggest that there may be a critical period following unilateral ovariectomy when additional progesterone is required if viable embryos are to be maintained. If hormone treatment was withheld until late on the 5th day, embryo degeneration might progress beyond the point at which reversal could be achieved by hormone therapy. Compensatory hypertrophy of the intact ovary within a few days after unilateral ovariectomy (Benson, Sorrentino & Evans, 1969) or extra-ovarian sources (Resko, 1969) may provide a sufficient quantity of progesterone and/or oestrogen to maintain pregnancy in both horns. This hypothesis would explain the observation that a single injection of progesterone on the 5th day was as effective in inducing implantation and foetal maintenance as a series of injections from the 5th to the 20th days.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service Training Grant No. 5 T01 HD00104-04 from the National Institute of Child Health and Human Development.

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