

NIDATION NUMBERS IN UNILATERALLY OVARIECTOMIZED AND CONTROL LONG-EVANS RATS: WITH ONE CASE OF TRANSUTERINE MIGRATION

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ABSTRACT

Implantation sites were counted 2 - 5 days after first litter delivery in 30 untreated and 46 unilaterally ovariectomized (ULO) Long-Evans rats. No significant difference between the means (10.7 vs. 9.6) of the left and right horns of control rats was found. A negative correlation between these two horns is evidence for compensatory ovulation. Left-sided ULO rats (26) had a mean of 10.5 sites and right-sided ULO rats (20) had a mean of 9.8. These two sides are not significantly different from each other or the control rats. In contrast to some previous reports, no evidence for a sinistral or dextral bias was evidenced. One case of blastocyst migration from a right horn to a left was found in which two areas of haemosiderin pigment were found in the left horn of a left ULO rat.

INTRODUCTION

In the process of investigating hormonally-induced superovulation in unilaterally ovariectomized (ULO) Long-Evans rats, a search of the literature failed to locate the needed base-line data on numbers of nidation (implantation) sites for this strain. Furthermore while numbers of implantation sites have been reported for

several other strains of rats (see Table 1), these studies have utilized a variety of methods to obtain their values. Reported procedures include counting numbers of ova in oviducts (Long and Evans, 1922), counting implantation sites 6 - 7 days post-coitum (p.c.) using the Psychoyos (1965) "blue reaction" (O'Grady and Heald, 1969), and counting litter sizes in a cage designed to prevent cannibalism (Long and Evans, 1922).

Several workers have observed compensatory ovulation for various strains of rats. Barr, Jensch and Brant (1970) noted that in albino rats as the number of fetuses in one horn increases, the number in the other horn decreases, resulting in a fairly constant total mean for the two horns. Compensatory ovulation in ULO Wistar rats was reported by Johnson (1970) when he counted implantation sites 7 - 17 days p.c. and obtained a mean of 10.4 ± 0.51 fetuses in untreated rats and 9.1 ± 0.88 in ULO rats. He found the number of implantation site differences between the two groups (untreated vs. ULO rats) not to be statistically significant, thus confirming earlier reports as cited by Danforth and de Aberle (1928). In hemicastrated Holtzman rats Peppler (1971) reported compensatory ovulation in young individuals but not older ones, which added the factor

TABLE 1: Summary of literature concerning egg implantation counts or litter sizes in normal and ULO rats of various strains.

STRAIN	COUNT MADE ON	MEAN	RANGE	SOURCE
<i>Normal</i>				
Long-Evans	Litter size	6.9	1-14	Long & Evans (1922)
Long-Evans	Oviduct eggs	9.6	—	Long & Evans (1922)
Wistar albino	Implantations	—	4-18	Frazier (1955)
Sprague-Dawley (Holtzman strain)	Implantations 6 days p. c.	14.3	—	O'Grady & Heald (1969)
DFE	—	15 (mode)	6-21	Buchanan (1974)
Wistar	decidual enlargement 7-17 days p. c.	10.4	—	Johnson (1970)
<i>Unilaterally ovariectomized</i>				
Wistar	7 days p.c.	9.1		Johnson (1970)
Holtzman	pre-ovulatory ova young-5 day cycle	10.2		Peppler (1971)
Holtzman	pre-ovulatory ova young-4 day cycle	9.6		Peppler (1971)
Holtzman	pre-ovulatory ova 257-262 days old	8.0		Peppler (1971)
Holtzman	pre-ovulatory ova 465 days old	7.5		Peppler (1971)

of age to compensatory ovulation. Utilizing ova flushed from the oviduct, he also noted a distinct difference in ovulation totals per cycle in 4 and 5 days cycle rats. He further demonstrated a decrease in mean ova counts with age in these ULO animals. Pepler attributed this progressive decrease in ova to fewer follicles maturing with increased age rather than through atresia.

In addition workers have reported a distinct dextral implantation bias in normal rats using Wistar albinos (Barr, Jensch and Brant, 1970). They reported a mean difference of 3.15 fetuses and a maximum difference of 10. Buchanan (1974) also reported a dextral bias, using DFE rats. Buchanan feels that the right ovary has an initially larger population of competent follicles and that is the cause of the dextral bias.

Transuterine migration of blastocysts in the rat is considered by many authors to be very unlikely or impossible because of the duplex cervical structure (Long and Evans) 1922, (Boyd, Hamilton and Hammond, 1944) (Johnson, 1970). On the other hand, Young (1953) states that albino rats have a common lower cervical canal and that the uterus is therefore intermediate between the typical "duplex" and "bicornuate" uterus. His data indicate a high percentage (69%) of transuterine migrations following unilateral ligation of an oviduct. To date further work to substantiate Young's conclusions has not been reported.

The lack of a consistent reporting format by previous studies ruled out accepting results of implantation site numbers from other workers and other strains for Long-Evans rats. Thus, our objectives in this study, using Long-Evans rats, were to: (1) determine the range, mean and mode for each horn as well as for the total implantations in untreated first-litter rats, (2) acquire these same data for both right and left ULO rats, (3) investigate the extent of compensatory ovulations between sides of controls and to compare total implantations between controls and ULO rats, (4) to determine whether or not there exists a dextral (or sinistral) bias regarding numbers of nidation sites in these rats and (5) determine the frequency, if any, of transuterine migration in ULO rats.

METHODS AND MATERIALS

Virgin Long-Evans hooded female rats 60 - 90 days old were kept at 24°C with the light controlled to provide 14h light (6 am to 8 pm EST) and 10h dark. Food (Purina Chow or Wayne Lab Blox) and water were provided *ad lib*.

Unilateral ovariectomies, with right or left sides chosen randomly, were performed in 46 experimental animals. These were done by way of midventral incisions in animals under chloral hydrate anesthesia (40 mg/100g) administered intraperitoneally. The ovary, periovarian sac and a small portion of fallopian tube were removed simultaneously. In these young animals the ovarian blood vessels are quite small and blood loss is minimal. Twenty-six left and twenty right ovaries were removed in this fashion.

One week following ovariectomies the females were individually caged with proven males and subsequent matings were detected by the presence of vaginal sperm

plugs under the cage. Thirty normal females (not ovariectomized) were individually caged and treated as described above.

Two to five days after delivery laparotomies were performed in order to count nidation sites. These sites were identified as residual swellings along the uterine horns, typically with haemosiderin pigment present (Warwick, 1956). This method of counting was chosen because it allowed for the recovery of the mother and subsequent nursing of the litter. Furthermore the number of implantations was required rather than number of live births.

RESULTS AND DISCUSSION

The distribution of nidation sites between the left and right cornua of the 30 control rats are presented in Figure 1. The total number of sites was 321 of which 152 were in the left horn and 169 in the right. The range for the left horn was 0-10 with a mean of 5.1 ± 2.24 while the range for the right was 2-9 with a mean of 5.6 ± 1.50 . Statistically, as determined by Model I Anova, the difference between these means is not significant. The mean number of sites per rat was 10.7 ± 2.15 .

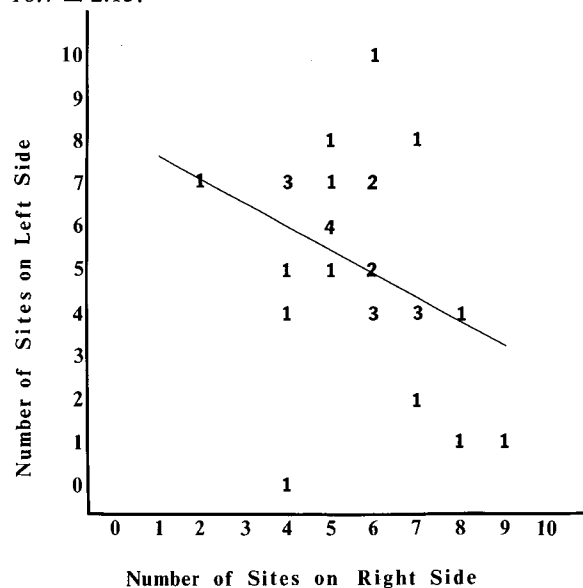


FIG. 1. Relationship of nidation sites between the left and right cornua of 30 non-ovariectomized rats. The negative slope ($Y = 8.3850 - .5890X$) is taken as evidence for compensatory ovulation. Right side $\bar{x} = 5.6 \pm 1.50$ (2 - 9); Left side $\bar{x} = 5.1 \pm 2.24$ (0-10); Total $\bar{x} = 10.7 \pm 2.15$ (4 - 6).

These control rat sites (as well as all others) were counted two to five days after delivery of the litters. As a consequence the counts include all sites that were maintained long enough to develop the typical haemosiderin pigment, even if the embryos were subsequently absorbed. The numbers obtained from the control animals, as might be expected, are not significantly different (mean 10.7 vs. 9.6) from the oviduct counts of Long and Evans (1922). However, nidation counts are

relatively easy and do not interfere with the survival of the young. Neither do they preclude further matings. Both of these problems occur with ova counts. Our mean of 10.7 is considerably greater than the mean litter size of 6.9 reported by Long and Evans. Again, that is to be expected since the data reported here include absorptions as well as young that were delivered while the data of Long and Evans include only those delivered. Litter counts were maintained in our study, but are not dependable since special caging techniques were not used to separate the newborn from their mothers and thus prevent cannibalism (Long and Evans, 1922). Secondly our objectives for subsequent studies required implantation site data rather than litter sizes.



FIG. 2. Photograph of left side ULO rat uterus 5 days after delivery showing a total of 15 haemosiderin sites, 13 on the right and 2 on the left. The latter two are considered to have migrated from the right side. Litter size was 11. No definite evidence is available as to which 11 sites of the 15 went full term.

A comparison of the number of nidation sites of the left cornu to that number in the right in each control rat used in this study reveals a negative slope (Fig. 1) with a negative correlation ($r = -.303095$) significant at $P < 0.05$, and is evidence for compensatory ovulation between the two sides.

The 26 left-sided ULO rats had a total of 285 nidation sites on the right side, with a mean of 10.5 ± 2.16 . However, since one of these animals also had two sites on the left (Fig. 2), the actual total number noted in these rats was 287. The 20 right-side ULO rats had a total of 196 on the left with a mean of 9.8 ± 2.31 . Again, these two sides (right ULO rats compared to left ULO rats) are not statistically different as determined by a Model I Anova.

Table 2 is a summary of all cornua, including the ULO rats. When the ULO rat cornua on the untreated sides were compared with the total numbers in the control rats, no statistical differences were demonstrated. This is taken as further evidence for compensatory evulation.

In contrast to some previous reports neither a dextral nor sinistral bias was evident from the results of this study.

A model which could explain the compensatory ovulation in either ULO or control rats was suggested by Danforth and de Aberle (1928). They hypothesized that there is available, in a variable but limited amount, material in an animal that is necessary for the maturation of ovarian follicles. They further assumed that as one ovary appropriated some of this material then the remaining amount available for the other ovary was reduced. Since this conclusion occurred relatively early with regard to the development of our understanding of endocrinology, the lack of specific hormone reference is quite understandable and their deduction all the more remarkable.

TABLE 2. Summary of nidation sites in control and ULO rats. All counts made after litters born.

	No. of Cornua	Mean	Std. Dev.	Range	Median	Mode
CONTROLS						
Left Cornu	30	5.10	2.24	0-10	5	4
Right Cornu	30	5.60	1.50	2-9	6	6
Both Cornu	60	10.70	2.15	4-16	11	11
ULO'S						
Left Ovary Remaining	20	9.8	2.31	4-13	10	9.5
Right Ovary Remaining	26	10.5	2.16	6-14	11	11

Updating this model a bit one might refer to a given quantity of F.S.H. present in the body fluids for a limited period. Some minimal percentage of this F.S.H. will be picked up by receptor sites on potential follicles. Chance favors an approximate 50/50 distribution between the two ovaries, but this division might be altered for a variety of reasons, chief of which would seem to be numbers of pre-follicular cells in each ovary having the appropriate number of receptor sites present. Unequal blood supplies to the two ovaries might also be a factor that would upset an equivalent distribution. After some minimum length of time the necessary threshold quantity of F.S.H. is no longer met due to the attachment to receptor molecules and the natural degradation processes of the hormones. Ultimately, negative feed-back to the hypothalamus by estrogens from follicular cells set in motion would serve to cut off the supply of F.S.H.

Peppler (1971) attributes the compensatory follicular maturation to an increased exposure time to a constant level of gonadotropins for the duration of the estrous cycle rather than because of a higher level of gonadotropins.

Finally, as mentioned earlier, evidence for one case of migration from a right horn to the left was found in a left side ULO rat when two areas of haemosiderin pigment were found in the left cornu and 13 in the right