THE EFFECTS OF INTROMISSION FREQUENCY ON SUCCESSFUL PREGNANCY IN THE FEMALE RAT

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The copulatory pattern of several species of rodents including the rat, mouse, hamster, and deermouse (Peromyscus) includes a series of mounts during which the male briefly inserts the penis into the vagina but withdraws without ejaculating. Ejaculation occurs during the last insertion of a series, when intromission is maintained for a longer period of time.

The function of pre-ejaculatory intromissions has previously been investigated from the standpoint of the male. Beach postulated that each intromission of the male rat contributed an increment toward the attainment of an ejaculatory threshold; a series of studies developing this model have subsequently been carried out.

Another function of the multiple intromission pattern, however, may apply to the female. A variety of neurogenic factors are known to trigger endocrine events connected with reproductive functions. For example, females of some species (rabbit, cat, ferret, and mink) are induced ovulators: their ovaries do not release eggs unless there has been copulation by the male, glass rod insertion, or some other form of stimulation to the vagina and cervix. The neurogenic stimuli activate the hypothalamo-hypophyseal system causing the release of luteinizing hormone (LH), which in turn leads to ovulation.

In addition, there are species in which the female normally produces nonfunctional corpora lutea but upon appropriate stimulation will maintain a prolonged luteal phase, pregnancy, or pseudopregnancy. This prolongation induces progestational changes in the uterine endometrium and delays the subsequent estrus. Stimulation in this case leads to a change in the female's endocrine status—presumably an extended secretion of prolactin (LTH). Thus, in both induced ovulation and pseudopregnancy, vaginal stimulation can play a decisive role in initiating a neuroendocrine reflex. Finally, Beach has proposed the hypothesis that the female rat's secretion of progesterone, and hence the probability that the progestational changes necessary for implantation will take place after mating, is dependent upon stimulation derived from repeated insertions by the male during copulation. Based on this hypothesis, one of the present authors, Wilson, performed a pilot study. He found that 5 of 6 females receiving 4 or more intromissions prior to ejaculation became pregnant, whereas only 1 of 6 receiving less than 4 intromissions became pregnant. The purpose of the present study was to confirm and extend these findings demonstrating a correlation between the multiple penile intromissions of the male rat and the maintenance of a successful pregnancy in the female.

Materials and Methods.—Twenty male and 20 female Long-Evans rats obtained from Diablo laboratories served as S's. They were 130 days old at the beginning of the experimental tests. All animals had continuous access to food and water.

Observation cages were cylindrical, with 24-inch glass sides and 30-inch diameter wooden floors covered with wood shavings. Responses were scored with an Esterline-Angus events recorder.
The animals were obtained when 60 days old and were maintained on a 12-hr light (6 AM-6 PM)-12-hr dark cycle. Each female was paired in a cage with a male until a litter was born. This constituted the fertility test which ended 9 weeks later when every female had delivered at least one litter. For one week during the fertility test (after most S’s had delivered), vaginal smears were taken. These data are not reported.

After each female had proved fertile, she was housed with 4 or 5 others and tested 3 times daily (7 PM, 9 PM, and 11 PM) for behavioral estrus. Males were caged individually. In initial tests for estrus, androgenized females were used as stimulus partners, but these animals mounted only infrequently, and active males were subsequently employed.

When experimental females came into estrus, they were mated with either an experimental or a control male. The experimental males were allowed several preliminary intromissions with a stimulus female brought into behavioral estrus by means of hormone injections. When the observer considered the male ready to ejaculate, the stimulus female was replaced by an experimental female (5–30 sec delay), and the male was allowed to continue until he ejaculated, usually on the first or second intromission with the experimental female. For 9 females the male’s ejaculation occurred soon enough so that the female received a total of 3 or fewer intromissions, counting the ejaculatory intromission and any prior intromissions she may have received during the tests for estrus; these females constitute the Low Intromission group (LI). Five of the 10 females in the High Intromission or control group (HI) underwent regular mating series with untreated males, i.e., no preliminary intromissions were given, and they received a mean of 7.4 intromissions. The other 5 members of the HI group were females who had been given an experimental male who was considered ready to ejaculate, but who did not do so quickly enough for our purposes; these females received a total of 4 or more intromissions prior to or with the ejaculate (mean intromissions = 9.4). One female did not come into estrus during any test session and is not included in the results. A mating pair was left undisturbed for 3 min after ejaculation, then the female was examined for the presence of a vaginal plug. Mated females were housed individually for 20 days, at which time they were palpated to diagnose pregnancy. Those judged pregnant were sacrificed and subjected to laparotomy. Counts were made of viable fetuses, implantation sites, and corpora lutea. Nonpregnant females were returned to colony cages and given daily tests for behavioral estrus. When each became receptive, another mating session was held using the same male that had been used in the experimental test. In this postexperimental test, the male experienced no preliminary intromissions with a stimulus female and delivered his full complement of intromissions to the experimental female (mean = 11, range = 8–14). Each female was again caged individually and examined for pregnancy on the 20th day.

Results.—The main results are presented in Table 1. The probability that the reduction in pregnancies in the LI group is due to chance is 0.0046, by Fisher’s exact probability test. Vaginal plugs were found in all S’s of the LI group but were not seen in 4 of the S’s in the HI group. Despite this discrepancy, 3 of the latter became pregnant.

Considering those pregnancies which were successful, no relation was found between number of intromissions received and number of viable or resorbed fetuses, or between intromissions and corpora lutea.

HI S’s were with a male for a median time of 5 min 27 sec during the mating tests; LI S’s were with a male for a median time of 4 min. This difference is statistically significant (p < 0.02, Mann-Whitney U test).

Discussion.—In female Long-Evans rats of demonstrated fertility, successful pregnancy depends in part on the number of intromissions delivered by the male.

<table>
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<tr>
<th>Group</th>
<th>Number Pregnant</th>
<th>Fertility test</th>
<th>Experimental test</th>
<th>Posttest</th>
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<tr>
<td>Low intromission (LI)</td>
<td>9 of 9</td>
<td>2 of 9</td>
<td>7 of 7</td>
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<tr>
<td>High intromission (HI)</td>
<td>10 of 10</td>
<td>9 of 10</td>
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prior to ejaculation. When 3 or fewer intromissions were permitted (LI group), 7 of 9 females failed to initiate and/or maintain a normal pregnancy. The presence of a plug in all LI S’s after mating indicates that insemination was highly probable, and argues against the likelihood of nonfertilization as the primary cause of the failure of pregnancy. Moreover, since all 7 of the nonpregnant, LI S’s did become pregnant during posttest fertility checks with the same male, we conclude that intromission frequency is a crucial factor influencing nidation. However, other variables such as olfactory stimuli, time between successive intromissions, effects of mounts, and state of receptivity may also be relevant. Indeed, the HI females not only received more intromissions than the LI S’s, but also spent more time in the presence of the male.

Ball\textsuperscript{14} reported a quantitative relationship between an aspect of the male’s sexual pattern and the production of pseudopregnancy in female rats. In one experiment, delayed estrus always occurred after 2 vaginal plugs, sometimes after one plug, and rarely after intromissions without ejaculation. Our results might therefore be due to differences in LTH-progesterone production (pseudopregnancy). Without the requisite number of intromissions, the female’s pituitary LTH is not released, the secretion of progesterone is not maintained, and implantation of the fertilized ovum (dependent on a progestational uterus) does not occur.

An alternative explanation might be provided by recent work of Shelesnyak and co-workers.\textsuperscript{15, 16} In addition to the requisite progestational uterus, implantation of the rat blastocyst depends on an estrogen surge which occurs on the third day after mating. It is possible that stimulation arising from the male’s successive intromissions is related to this estrogen surge—without stimulation, no estrogen is secreted and implantation does not occur. In either case, implantation of a fertilized ovum would be prevented by lack of neurogenic stimuli.

In its regular estrus cycle, the female rat has a very short luteal phase. During pregnancy or pseudopregnancy this phase is lengthened, leading to: (1) progestational proliferation of the uterine endometrium which forms an environment suitable for the implantation and nourishment of the fertilized egg, and (2) suppression of the subsequent ovulation which would otherwise occur 4–5 days later.\textsuperscript{19}

A tie between a behavioral event and the lengthened luteal phase seen in pregnancy or pseudopregnancy seems adaptive reproductively. Without such a tie, a second ovulation would occur before the first fertilized ovum had traveled to the uterus, become implanted, and signaled suppression of further ovulation. Although superfetation occurs in other species, it apparently does not in the rat. Also, without such a tie, LTH production would follow LH production and would lead to progesterone formation and endometrial proliferation during every cycle, thus necessitating a 13–14-day estrus cycle rather than the 4–5-day cycle seen when copulatory or equivalent stimulation is not received. It seems to us that the behavioral event which leads to uterine preparation is a function of copulatory stimulation, probably intromission frequency summated over a short period of time. Other experiments are being undertaken to investigate the details of this mechanism.

\textit{Summary.}—Successful pregnancy occurred in 90 per cent of female rats who received 4 or more penile intromissions with the male ejaculate. Only 22 per cent of the females receiving the ejaculate with 3 or fewer intromissions became preg-
nant. These results were independent of differential fertilization. The hypothesis is advanced that, in addition to successful fertilization, a certain amount of neurogenic stimulation is necessary for uterine implantation of the fertilized egg. Possible neuroendocrine mechanisms are discussed.

The hypothesis tested in this study was suggested to us by Dr. Frank A. Beach, and the experiment was supported by USPHS grant MH-04000 to F. A. Beach. The expert technical advice of Dr. Giorgio Bignami was deeply appreciated.

4 Larsson, K., *Conditioning and Sexual Behavior in the Male Albino Rat* (Stockholm: Almqvist & Wiksell, 1956).
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THE MECHANISM OF ACTION OF ALDOLASES, XI. ACTIVATION BY AROMATIC SULFHYDRYL REAGENTS AND β-ELIMINATION OF SELECTED THIOL GROUPS*

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In the course of studies on the active site of rabbit muscle aldolase, we obtained evidence for the presence of four to six sulfhydryl groups which were essential for catalytic activity.1 The presence of substrates such as fructose 1,6-diphosphate and fructose 1-phosphate protected these sulfhydryl groups from the action of a variety of sulfhydryl reagents. One of the reagents employed in these studies was chlorodinitrobenzene, which was found to react selectively with two lysine residues in transaldolase. On the other hand, the only DNP derivative formed with fructose diphosphate aldolase was S-DNP-cysteine.