Nidation in Progesterone-Treated, Estrogen-Deficient Hamsters, *Mesocricetus auratus* (Waterhouse).* (25723)

M. R. N. Prasad,† Margaret Ward Orsini and Roland K. Meyer
Depts. of Anatomy and Zoology, University of Wisconsin

Gestation in the mammal is divisible into 2 portions, the period of free and attached ovum. In a few animals such as bear, roe deer, Mustilids, seals, armadillo the period of free ovum may be extremely long, extending into months. In most mammals the free period is short and very definitely defined, but in mouse and rat, gestation, concurrent with lactation, may be prolonged, due to retardation in implantation associated with a large number of suckling young. The exact mechanism controlling extent of delay and induction of nidation in both types of delay cited above is still not clear. For a brief review of the literature the reader is referred to(1). Recently experimental studies have been made of factors inducing and controlling delay in non-lactating rats. The publications indicate that time of ovariectomy after fertilization, and kind, quantity, and time of administration of exogenous hormone are important factors. The adrenal has also been implicated. These variables may account for what appear to be inconsistent, contradictory results(2-10). Time of ovariectomy during period of natural delay of implantation in the armadillo also affects implantation and subsequent viability of embryos(11). Loeb(12) demonstrated that time of removal of corpora lutea before implantation in the guinea pig determines whether nidation will occur. Bloch(13) reported that progesterone induces implantation at normal time in non-lactating mice castrated after mating. In prepubertal mice, induced to ovulate and mate by administration of exogenous gonadotropins, implantation does not occur, blastocysts delay and have been recovered as late as 22 days; implantation can be induced by exogenous progesterone (Smithberg and Runner)(14). This paper describes results of experiments designed to determine effect of ovariectomy, adrenalectomy, hypophysectomy and progesterone and estrone on implantation in the golden hamster.

Methods and results. The golden hamster, unlike the rat, has a very regular estrous cycle (Ward(15)), and has no postpartum ovulation or estrus (Deanesly(16)). Moreover, the hamster has a very short progestational, or free-living stage in its gestation; implantation begins on day 5, at approximately 4 days and 8 hours developmental age (Ward (17)). All hamsters used were virgins of breeding age in which several cycles had been followed. Females in estrus were placed with males of proven fertility in the evening and left overnight. Copulations were observed in most and confirmed in all by presence of sperm the following morning, *i.e.*, day 1 of pregnancy. A group of 5 intact pregnant hamsters were given 2-4 mg of progesterone daily from days 2 or 3 of pregnancy to determine whether exogenous progesterone would prevent implantation. These animals were laparotomized on day 8 and the number of sites recorded. One animal was killed at this time, another killed at time of normal parturition (15 days and 12 hours—as determined from approximate time of ovulation, 2 a.m. of morning when sperm were found, (Ward(15,17)), and the remaining 3 were treated until day 20 and killed. Observations at laparotomy indicate no effect on time of implantation. These results agree with those obtained by Samelwitz, Dziuk and Nalbandov (20), and Cochrane and Meyer(9), who administered 4-16 mg of progesterone daily for variable periods after mating and obtained no significant effect on implantation and development. The remaining females were subjected to surgery at known period of pregnancy prior to implantation. Beginning at time of surgery they were maintained with, or without (control groups), daily injections of progesterone, and laparotomized when conceptual swellings would be easily visible if implantation had occurred. When implantations were found, the

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† Present address: Dept. of Zoology, Delhi, India.

TABLE I. Effect of Various Treatments on Implantation in Hamster.

No. of animals	Day of surgery	Daily treatment before laparotomy	Day of laparotomy	No. of animals without sites at laparotomy	No. of animals with sites at laparotomy	Daily treatment after laparotomy	Day of autopsy	No. animals without sites at autopsy
Group A—Ovariectomized, progesterone given from day of operation to autopsy								
1	1	2 mg prog.	7	1	0	2 mg prog. + 1 γ estrone	11	1
3	1	4	7	1*	2‡	4 mg prog. + 1 γ estrone	11	1
1	2	2	7	0	1‡	0		0
11	2	4	7–9	1*	10§	4 mg prog. + 1 γ estrone	13–14	1
1	3	2	7	0	1‡	0	7	0
9	3	4	7–10	0	9‖	0	7 or 17	0
1	4	2	7	0	1‡	0	7	0
1	4	4	7	0	1‡	0	7	0
Group B—Ovariectonized and adrenalectomized								
5	2	4 mg prog.	6	1*	4‡	4 mg prog. + 1 γ estrone	11	1
Group C—Ovariectomized, no progesterone given								
4	2	0	9	4	0	1 γ estrone	11	4
1	3	0	7	1‡	0			1
2	4	0	6 & 8	2‡	0			2
Group D—Pituitary autograph to kidney								
4	2	0	10–11	4†	0	1 γ estrone	16–17	4
2	3	0	10–11	2†	0	*Idem*	17	2
1	2	0	Both horns flushed day 4, 56 hr after operation. No blastocysts recovered.					
Group E—Hypophysectomized only								
8	2	0	7–10	6	2‡	1 γ estrone	13–17	6
Group F—Hypophysectomized and given progesterone								
4	2	4 mg	9		4‡			

* 1 horn flushed at time of laparotomy.
† 1 animal of group had one horn flushed at time of laparotomy.
‡ Killed.
§ 9 pregnant animals carried to day 13-14, 1 non-pregnant animal carried to day 13, no implantation occurred.
‖ 4 carried to day 17, 5 killed at time of 1st laparotomy.

animal was usually killed. Number and conditions of implantations were recorded and the tracts fixed in AFA (3 parts 95% alcohol, 1 part commercial formalin, 1 part glacial acetic, and 5 parts water), dehydrated in alcohol and cleared through benzol to benzyl benzoate. After clearing they were re-examined for completeness of ovariectomy and correspondence of development to stage when killed. Variation in experimental design is shown in Table I, which also summarizes procedures and results. Animals in Table I are divided into the following groups: Group A: Bilateral ovariectomy plus 2 or 4 mg of progesterone daily. Group B: Bilateral ovariectomy and adrenalectomy plus 4 mg of progesterone daily. Group C: Bilateral ovariectomy; no progesterone. Group D: Hypophysectomy and pituitary autograft to kidney capsule; no progesterone. Group E: Hypophysectomy; no progesterone. Group F: Hypophysectomy plus 4 mg progesterone daily. Ovaries and adrenals were removed by usual methods. Unilateral hysterectomies and laparotomies were made by ventral approach. Hypophysectomy was done by parapharyngeal approach under ether and semisterile conditions. When pituitary gland was to be transplanted, it was sucked into a sterile cannula and placed in 0.9% saline at room temperature. Autografts of pituitary were made by placing the gland under left kidney capsule following method of Everett(18). The sella turcica of hypophysectomized animals was examined with magnifying lens at autopsy to determine whether removal was complete.

Daily weights were recorded for all animals. Synthetic U.S.P. progesterone was used throughout. Two or 4 mg of progesterone, or a combination of progesterone plus 1 γ of estrone, was injected daily subcutaneously in .25 cc of corn oil. Hamsters were maintained on Rockland Rat or Purina Laboratory Chow. Corn sugar was added to drinking water 3-4 days after hypophysectomy. Adrenalectomized hamsters were provided with 0.9% saline until autopsy. In 13 instances where implantation sites were present after bilateral ovariectomy, animals were maintained after laparotomy on the same hormonal treatment as that given before laparotomy; 9 to late gestation; 4 to prolonged gestation. At autopsy, number and condition of implantation sites and conceptuses were recorded. Where no implantation sites were found at laparotomy, the same prelaparotomy regime plus 1 γ of estrone daily was given for additional 4-6 days. One uterine horn from 5 of these animals was flushed at time of laparotomy, *i.e.,* 2 of the 3 from Group A, 1 from Group B, and 1 from each of the first 2 subgroups of Group D. Both horns from one animal in Group D were flushed on day 4, approximately one day prior to time of expected implantation (Ward(17)), to determine how long the blastocysts would remain viable. A similar attempt was made to determine condition of the blastocysts in the control Group C, where one animal castrated on day 4 was laparotomized and the uterus flushed on day 6, approximately one day after implantation. Since small amounts of estrone added to progesterone cause implantation in the non-lactating rat, the finding that 89% of hamsters ovariectomized and given progesterone alone implanted led us to eliminate all possible exogenous estrogen (from food and progesterone solvent), Orsini and Meyer(19). For this 18 hamsters were placed on a diet of water and glucose alone from day 2 to day 7. Sixteen were ovariectomized and given 4 mg of progesterone daily, 6 received it in corn oil, 5 suspended in saline, 5 dissolved in Trioctanoin, a synthetic oil obtained from Eastman Kodak. The remaining 2 were intact, serving as controls on the effect of the diet alone. All showed implantation sites comparable to the normal stage of development when laparotomized on day 7.

Discussion. In Group A of Table I, 3 or 11% of animals ovariectomized and given progesterone failed to implant. It is possible that the oviduct may have been traumatized during the operation. Eleven of total ovariectomized females had unilateral pregnancies suspected to be attributable to this cause, as the number of conceptuses in the single horn was not sufficient to suggest unilateral ovulations.

In Group E 6 of 8 animals hypophysectomized failed to implant. No pituitary fragments were found in 8 animals. It is possible, however, that fragments too small to be detected accounted for implantation in the remaining two.

Progesterone in doses used caused no detectable delay in nidation in hamsters made estrogen deficient by hypophysectomy, ovariectomy, or ovariectomy and adrenalectomy, (Table I, Groups A, B, and F). These findings support the concept that estrogen is not required for implantation in the hamster, whereas in the rat progesterone alone prevents nidation and estrogen induces it(4,5,9,10). In the hamster estrogen produced at ovulation and immediately thereafter may be sufficient for progesterone alone to produce implantation subsequently. The work of Loeb(12), who found that removal of corpora from the guinea pig during first 2 days was not followed by implantation, but that removal of corpora at 75 hours was followed by implantation and apparently normal development, and Bloch (13), who was able to secure implantation in castrate non-lactating mouse by progesterone alone, supports this possibility. Both suggest that the ovary may sensitize the uterus in this early stage. In prepubertal mice in which mating and ovulation were induced by gonadotrophins evidence was found for both estrogen and progesterone secretion during the first 2 days of gestation, Smithberg and Runner (14). Subsequently in their untreated animals, the corpora degenerate, the blastocysts survive but do not implant. However, if these animals are given progesterone daily, implantation occurs.

Group D, with hypophysectomy and autograft to the kidney in the hamster is unlike that in the rat(18,21,10). Whether failure to

implant in the hamster under these conditions is due to a lack of luteotropin or luteinizing hormone has not been determined.

Cleavage stages, morulae and early blastocysts were flushed from the uteri of normal hamsters prior to implantation, yet in no case were ova or blastocysts recovered from the few experimental animals flushed before or after expected time of normal implantation. No evidence was found of abortive implantations in cleared, non-pregnant tracts. Moreover, implantations found in pregnant tracts (progesterone treated) appeared comparable to stages in the normal hamster. This suggests that without progesterone ova or blastocysts degenerate; at what stage is not known.

In experiment designed to eliminate possible estrogen contamination the entire group of animals lost weight when maintained on glucose, but all showed implantations demonstrating that exogenous estrogen is not necessary for implantation in the hamster. One possible source for contamination is in the progesterone itself. This, however, does not seem probable.

In no instance where implantation had not occurred at time of laparotomy, regardless of treatment, was it subsequently induced by estrone. This suggests that blastocysts were not alive at time estrone was begun.

Summary. 1. In the hamster, when ovarian hormones are lacking, due to ovariectomy, or hypophysectomy, implantation does not occur and blastocysts are not recovered. 2. Implantation does not occur in hypophysectomized hamsters with pituitary autografts. 3. Doses of progesterone, 2-4 mg daily, do not affect time of implantation in the intact hamster. 4. Implantation occurs with no apparent delay in hamsters made estrogen-deficient by ovariectomy, ovariectomy and adrenalectomy, or hypophysectomy and given daily injections of 2-4 mg progesterone. In experiments designed to eliminate estrogen in injection media and diet, implantation also occurred when progesterone was given to ovariectomized hamsters. This suggests that progesterone alone is required for maintenance of blastocyst and nidation in the hamster.

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