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Hormonal Requirements of Implantation in the Rabbit.* (22317)

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The phenomenon of implantation in mammals is accomplished by a definite sequence of events which in part are dependent on the endocrine system. It has been adequately demonstrated(1,2,3) that blastocyst growth in the rabbit is controlled by the secretion of the corpus luteum. Partial progestational proliferation in the ovariectomized rabbit could be produced within 5 days by 0.2 mg progesterone daily(4). The injection into rabbits, ovariectomized 20 hours after mating, of progesterone in dosage up to 1.5 mg a day maintained implantation until the 11th day of pregnancy(5). Crude corpus luteum extracts of similar unitage maintained embryos to full term(6). In ovariectomized rabbits, implantation is maintained for 11 days if at least 0.5 mg of progesterone is given twice daily; 1.0 mg given twice daily after the 11th day of pregnancy maintains the embryo to term(7). In the intact non-ovulated rabbit, implantation of transplanted ova requires an initial high dose of progesterone; subsequent

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treatment prevents the degeneration of the embryo or death of the fetus(8).

Materials and Methods. Sixty-three postpartum and 12 adult virgin rabbits were purchased from Rockland Farms, N. Y. and local dealers and were kept in this laboratory. Two series of experiments were carried out: 1) Eight groups of 3 to 5 animals each were mated twice to fertile bucks and injected intravenously with an ovulating dose of 12 mg of sheep pituitary extract. The does were ovariectomized 24 to 48 hours post coitum and the ovulation points on each ovary were counted. Six groups of does were injected subcutaneously once daily with an oily solution of 0.3, 2 or 25 mg of progesterone 2 or 4 days post coitum. Two other groups were injected subcutaneously once daily with 2 or 25 mg progesterone together with 0.1 mg estradiol. 2) Four groups of 3 does each were mated to a vasectomized buck and injected intravenously with an ovulating dose of sheep pituitary extract. The does were ovariectomized as above; immediately before ovariectomy 24 to 30 fertilized one-day ova were transplanted into the left tubes. The ova were obtained from virgin donors which were superovulated with sheep pituitary extract(9) and mated at the same time as the recipients so that the transplanted ova would be of a corresponding stage with that of the recipient. The experimental does were injected subcutaneously with 2 or 25 mg of progesterone once per day starting either on the day of ovariectomy or the 4th day after mating. In 2 groups 0.01 mg of estradiol was injected once daily. All does were sacrificed on the 10th day after mating; the uteri were examined to determine the number of implantation sites, the diameter of uterine swellings and the weight and length of the fetus. In cases where implantation did not occur, the uteri were flushed with saline and examined under the dissecting microscope. Two control experiments were carried out: (a) Twelve does were mated to fertile bucks, and sacrificed on the 10th day after mating. The number of implantation sites in relation to the number of corpora lutea were counted and the average diameter of uterine swellings, weight and length of the fetus were recorded. (b) Six does were mated to fertile bucks, and were injected subcutaneously once daily with 2 or 25 mg of progesterone. These were sacrificed 15 days after mating and the uteri examined.

Results. In the ovariectomized animals the number of ovulation points on both ovaries ranged from 5 to 18 with an average of 10.9. Implantation occurred in all the animals when the progesterone dose was 2 or 25 mg (Table I). The number of implantations in both uteri ranged from 1 to 3. The number of implantations expressed as a percentage to the total number of ovulation points ranged from 5% to 33% with an over-all average (of 6 experimental groups) of 19.21%. There was no difference in the percentage of implantations between the 2 or the 25 mg groups nor between the groups whose initial injection was on the 2nd or 4th day after mating. Im-

TABLE I. Effect of Progesterone on Implantation and Development in Ovariectomized Rabbits.

Hormones inj. (mg/day)	Start of inj. post coitum (days)	No. of does	Avg No. of ovulations in both ovaries	% of implantations		% of viable embryos		% of ova (or blastocyst) recovered by flushing	
				Range	Mean	Range	Mean	Range	Mean
.3 progesterone	2	3	12.3	0	0			50–73	53
	4	4	7.7	0	0			41–69	52
2 progesterone	2	4	13.3	11–21	19.00	33–50	38		
	4	3	11.3	15–20	17.66	33–66	50		
25 progesterone	2	5	10.4	12–33	23.33	20–80	35		
	4	4	10.2	9–20	15.33	33–66	47		
2 progesterone + .1 estradiol	2	5	7.2	0	0				
25 progesterone + .1 estradiol	2	5	10.1	0	0				

TABLE II. Effect of Progesterone and Estradiol on Implantation of Transplanted Ova in Ovariectomized Rabbits.

Hormones inj., mg/day P*	E*	Doe No.	Left side after sterile mating: No. of ovulation points	No. of transferred eggs	No. of implantations	% of implantations	% of viable embryos
2	—	25	3	27	3	11	33
		26	4	24	4	17	50
		27	5	28	2	7	50
2	.01	28	6	25	3	12	66
		29	5	30	2	7	50
		30	5	30	2	7	50
25	—	31	6	30	2	7	50
		32	7	27	2	7	50
		33	4	27	3	11	100
25	.01	34	5	30	1	3	100
		35	3	30	2	7	50
		36	5	29	2	7	50

* P = Progesterone; E = Estradiol.

plantation failed to occur at a dose of .3 mg progesterone, 2 or 25 mg progesterone in combination with 0.1 mg estradiol. In the group injected with 0.3 mg progesterone daily, 3 to 7 ova (or blastocysts) were recovered from the uterus. In the groups injected with 0.1 mg estradiol with progesterone, no ova (or blastocysts) were recovered from both uteri. A few degenerating ova were recovered from the tubes of 3 animals out of 10.

In the control groups "a" which were mated and sacrificed at 10 days the number of implantations expressed as a percentage of the total number of ovulation points ranged from 71 to 100 with an average of 81 and the percentage of viable embryos ranged from 70 to 100 with an average of 81. Judging by the diameter of the uterine swellings and the developmental stage of the embryos in the ovariectomized animals compared with that in the intact animals, there was no evidence of delayed implantation in the former group.

When the gravid uteri of the ovariectomized animals were dissected 35 to 50% of the embryos were viable. In the control group "b" which were injected with 2 mg of progesterone, the percentage of implantations ranged from 80 to 100 with an average of 93 and the percentage of viable embryos ranged from 67 to 100 with an average of 79. In the control group injected with 25 mg progesterone, the percentage of implantations ranged from 80 to 90 with an average of 82. There were no significant differences between these 2 control groups "b". Also the injection of 2 or 25 mg of progesterone in the intact animals did not exert an effect on the percentage of implantation.

In the second series of experiments where fertilized ova were transplanted in the left horn of ovariectomized animals, the number of implantation sites ranged from 1 to 4 and the percentage of implantations ranged from 3 to 17 (Table II). It was noted that the number of implantations was not corresponding to the number of transferred ova. In fact the number of implantations in the one horn was equivalent to those of 2 horn in the previous experiment.

Discussion. There is general agreement that the presence of the luteal hormone is essential for successful implantation but the mechanism by which it makes implantation possible is not fully known. Results of the present experiment suggest that the hormonal requirement for embryonic development is not identical to that required for implantation. The 2 days following implantation seem to be the most critical during the embryonic life (10). It is possible that substances other than progesterone such as androgens or estrogens are required in certain forms or quantities to ensure normal development. The dose of 2 or 25 mg of exogenous progesterone in the

ovariectomized rabbits is sufficient to establish implantation but not for further development in some of the cases. Courrier and Kehl (11) and Allen and Heckel(12) have determined that while in the early stages about 1 mg of progesterone is sufficient, in later stages up to 5 mg daily is necessary. Meanwhile ovarian hormones are completely unnecessary in experimental extrauterine pregnancy(13,14).

The absence of implantation when 0.3 mg progesterone was injected daily is either due to incomplete progestational conditions of the endometrium or to a delay in the transportation and reception of ova from the tubes to the uterus. It was thought that the injection of a low dose of progesterone may result in delayed implantation. Chang(8) has indicated that the injection of a low dose of progesterone in non-ovulated rabbits for 5 to 6 days, only renders the endometrium similar to that of a rabbit in the 3rd to 4th day of pseudopregnancy. Delayed implantation occurs spontaneously in mice as a result of lactation(15) or may be induced by the administration of ovarian hormones(16). It is possible that implantation was not delayed in the present investigation due to the fact that progesterone is rapidly destroyed (or converted into an inactive form)(17,18), particularly in the ovariectomized rabbit(19). Courrier and Kehl(11) have shown that a definite progesterone:estrogen ratio of 750:1 is required for successful implantation in the ovariectomized rabbit. In the present experiment when the progesterone:estradiol ratio was narrow (250:1 or 20:1), the ova were blocked in the tube and degenerated before reaching the uterus.

It is of great interest to note that the actual number of implantations in the two series of the present experiment did not increase with the increased number of ova in the uterus. This may denote a quantitative basis for the hormonal requirement of implantation. Further experiments are needed to explain the high frequency of embryonic mortality in ovariectomized progesterone-treated rabbits. The role of placental hormones in such animals should not be overlooked.

Summary. The role of ovarian hormones in implantation was studied in rabbits ovariectomized after mating and in ovariectomized pseudopregnant rabbits into which fertilized ova were transplanted. There was no evidence of delayed implantation. One to 3 implantations occurred in ovariectomized rabbits injected with 2 or 25 mg progesterone. The percentage of implantations ranged from 15 to 23 irrespective of dosage or the time at which injection started. Implantation failed to occur when the dose was 0.3 mg progesterone or 0.1 mg estradiol in combination with progesterone. The number of implantations did not increase with the transplantation of ova. This may suggest a quantitative basis for the hormonal requirement at implantation. Hormonal requirements at implantation vary from those needed for subsequent development.

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