Induction of ovulation, mating, and conception in androgen-treated immature rats

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Summary. Attempts were made to induce pregnancy in androgen-treated immature rats. Treatment with PMSG alone, which causes ovulation in normal immature rats, failed to cause ovulation in androgenized rats. However, treatment with PMSG plus LHRH was effective in causing ovulation. After ovulation, some of the normal and androgenized rats mated. Normal mated rats became pregnant but androgenized mated rats did not. However, when a pituitary gland was transplanted from a normal rat into the kidney capsule of an androgenized rat to maintain functional corpora lutea, implantation occurred in some of the mated animals. The positive decidual reaction in the uteri of such androgenized rats was similar to that observed in normal rats. These results suggest that the uterine sensitivity to blastocyst implantation of androgenized immature rats may be normal.

Introduction

The administration of androgen to neonatal female rats results in anovulatory sterility accompanied by persistent vaginal cornification in adulthood. This syndrome may arise from disorders of the hypothalamus and subsequent changes in the cyclic release of luteinizing hormone (LH) from the pituitary gland (Barraclough, 1966; Wagner *et al.*, 1966). Attempts are often made to induce pregnancy in such androgenized rats, but they often end in failure (Kramen, 1974; Hahn & McGuire, 1978). Matton & Maurasse (1985) have reported that endometrial scratching in immature rats elicits a better response in androgenized rats than in controls after induction of ovulation or steroid replacement therapy, and that the reduction in the response is age-dependent. It is not known whether uterine sensitivity to blastocyst implantation is normal in androgenized immature rats. Immature rats just before puberty can be induced to have a normal pregnancy when the first ovulation is followed by a single injection of PMSG (Sawada & Ichikawa, 1978). In the present study, I examined the ovarian response to exogenous hormones (PMSG alone and PMSG plus LHRH) in immature rats treated with androgen, and investigated whether the uteri of such rats could support implantation such as is observed in normal immature rats.

Materials and Methods

Animals. Female Sprague–Dawley rats were used. They were kept at $24 \pm 1^\circ\text{C}$ and on a 14-h photoperiod (lights on from 05:00 to 19:00 h), and were given water and standard commercial pellet food. On the 5th day after birth, female young were given subcutaneous injections of 1 mg testosterone propionate (Sigma Chemical Co., St Louis, MO, U.S.A.) in 0.02 ml sesame oil (Matton & Maurasse, 1985). This treatment produces 100% sterility. Uninjected litter-mates served as controls. The young were allowed to grow with the mother. They were weaned at 21 days of age and used in this study at 30 days of age.

Ovulation. On Day 30 at 08:00 h, the rats were injected s.c. with 4 i.u. PMSG (Primantron, Schering AG, Berlin, West Germany) in 0.2 ml saline (9 g NaCl/l). This treatment induces ovulation in 100% of normal immature rats by the morning of Day 33 (Ichikawa *et al.*, 1982). When LHRH was also given, it was injected s.c. 56 h after the PMSG, and tubal ova were recovered and counted under a dissecting microscope.

Mating and implantation. To determine whether the animals were capable of mating and implantation, they were caged with males on the evening of 32 days of age. The presence of spermatozoa in the vaginal smear the following morning was defined as Day 1 of pregnancy. On Day 1, a pituitary gland was transplanted from a normal rat into the kidney capsule of some androgenized rats under ether anaesthesia to maintain functional corpora lutea (Sawada *et al.*, 1968, Hahn & McGuire, 1978). All animals were killed on Day 10 and the numbers of animals with implantations were recorded.

Uterine decidualization. After ovulation was induced in the immature rats, attempts were made to induce uterine decidualization. To induce functional corpora lutea, the above-mentioned method of transplantation of a pituitary into the renal capsule was used. Transplantation was done when the rats were 33 days old. On Day 5 after the pituitary transplant, the endometrium of the right uterine horn was traumatized with a bent needle by the technique of De Feo (1963). The contralateral horn served as the control. On Day 10, the stimulated and intact horns were excised, cleaned of fat and adhering tissue, and weighed separately. When the stimulated horn weighed at least 3 times as much as the control, the increase was considered significant (De Feo, 1963).

Statistics. Fisher's exact probability test was used to compare the percentage of differences between groups. Significant differences in weights between groups were evaluated by Student's *t* test. Statistical tests of the data were taken to be significant at the 5% level.

Table 1. Effect of administration of PMSG and LHRH on ovulation of androgen-treated immature rats (PMSG injected at 30 days of age; LHRH 56 h later)

Group	Dose of		No. of rats ovulating (%)	Mean $\pm$ s.e.m. no. of eggs recovered
	PMSG (i.u.)	LHRH (ng)		
1*	4	—	6/6 (100)	7.8 $\pm$ 0.6
2	4	—	0/6 (0)	0
3	4	400	6/6 (100)	7.5 $\pm$ 0.7
4	4	800	6/6 (100)	7.7 $\pm$ 0.6

*Normal immature rats.

Table 2. Effect of administration of PMSG and LHRH on mating and conception of androgen-treated immature rats (PMSG injected at 30 days of age; LHRH 56 h later)

Group	Dose of		No. of rats mating (%)	No. of rats with implantations on Day 10 after mating (%)	Mean $\pm$ s.e.m. no. of implantations/rat*
	PMSG (i.u.)	LHRH (ng)			
5†	4	—	8/12 (67)	8/8 (100)	7.0 $\pm$ 0.5
6	4	400	5/12 (42)	0/5 (0)	0
7	4	800	5/11 (46)	0/5 (0)	0
8‡	4	800	6/12 (50)	3/6 (50)	7.0 $\pm$ 3.6

*Excludes rats without visible implantation sites.

†Normal immature rats.

‡Pituitary gland was transplanted from a normal rat under the kidney capsule of the androgenized rat on Day 1 after mating.

Results

Induction of ovulation, mating, and implantation

When normal and androgenized rats were injected with 4 i.u. PMSG on the morning of Day 30, vaginal opening took place in both groups of animals on the evening of Day 32. Eggs were observed in the ampulla of the oviducts of the normal rats on the morning of Day 33 (Table 1, Group 1), but

Table 3. Effect of administration of PMSG and LHRH on decidual response in the uterus of androgen-treated immature rats (PMSG injected at 30 days of age; LHRH 56 h later)

Group	Dose of		Positive response (%)	Uterine weight (mg)†	
	PMSG (i.u.)	LHRH (ng)		Traumatized horn	Control horn
9*	4	—	6/8 (75)	376 ± 47 (152, 249)	94 ± 16
10	4	800	5/7 (71)	438 ± 72 (136, 152)	103 ± 6

*Normal pseudopregnant rats: pseudopregnancy was induced by transplantation of a pituitary under the renal capsule.

†Values are mean ± s.e.m. with the weights of traumatized uterine horns without deciduomata given in parentheses.

not in the androgenized rats (Table 1, Group 2). Treatment with PMSG plus LHRH induced ovulation in all of the androgenized rats (Table 1, Groups 3 and 4).

Mating occurred in about half of the rats that ovulated (Table 2). When the mated animals were autopsied on Day 10, all of the normal rats were pregnant (Table 2, Group 5), but none of the androgenized rats (Table 2, Groups 6 and 7). Some of the androgenized rats that received a pituitary transplant after mating became pregnant (Table 2, Group 8).

Induction of deciduomata

The response of androgenized rats and normal rats to traumatization of the endometrium was almost the same (Table 3).

Discussion

Treatment with PMSG alone causes ovulation in normal but not in androgen-treated immature rats (Brown-Grant *et al.*, 1964). In this study, treatment with PMSG alone gave results that suggested the absence of an endogenous LH surge in androgenized immature rats. At 30 days of age (before the beginning of persistent vaginal oestrus), the pituitary LH content of androgen-treated rats is significantly lower than that in littermate controls (Barracough, 1966). However, ovulation can be readily induced by an endogenous LH surge that follows injections of LHRH and PMSG in androgenized immature rats.

The percentage of androgen-sterilized rats that mated was not significantly different from that for normal controls (Brown-Grant, 1975), or markedly lower than that of normal controls (Kramen & Johnson, 1971; Hahn & McGuire, 1978). The mating response found in this study in androgenized immature rats was not significantly different from that for normal controls.

In androgen-sterilized rats, vaginal cervical stimulation by mating fails to maintain corpora lutea after induction of ovulation (Zeilmaker, 1964). Normal immature rats that were mated became pregnant, but mated androgenized immature rats did not. Therefore, to maintain functional corpora lutea, transplantation of a normal pituitary gland into the renal capsule was needed to allow pregnancy and uterine decidualization in androgenized rats. This treatment permitted pregnancy and a positive decidual reaction to occur in some of the androgenized rats. In androgenized rats with transplants the uterine sensitivity to decidual reaction was about the same as that of normal immature rats. These results suggest that the uterine sensitivity to blastocyst implantation of androgen-treated rats may be normal.

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