Induction of ovulation by exogenous gonadotrophin during pseudopregnancy, pregnancy or lactation in rats

K. Taya and S. Sasamoto

*Laboratory of Veterinary Physiology, Faculty of Agriculture,
Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan*

During pseudopregnancy, pregnancy and lactation in the rat there is a characteristic persistent function of the corpus luteum and an interruption of cyclic ovulation. Investigation of the response of ovarian follicles to exogenous gonadotrophins during pseudopregnancy and pregnancy in the rat has led to controversial results (Greenwald, 1966; Rees, Dieten, Bijleveld & Muller, 1968; Brown-Grant, 1969; Ying, Gove, Fang & Greep, 1973; Welschen, Osman, Dullaart, de Greef, Uilenbrock & de Jong, 1975). We therefore examined the effect of HCG on the responsiveness of follicles, their maturation and maintenance at various stages of pseudopregnancy, pregnancy and lactation in the rat.

Methods

Adult female rats of Wistar strain were kept in a room with controlled temperature and lighting (lights on 05.00–19.00 h). Vaginal smears were examined daily. At about 17.00 h on the day of pro-oestrus each female was transferred to the cage of a single intact or vasectomized male and left overnight. The occurrence of mating was checked the next morning by the presence of a vaginal plug or of spermatozoa in the vaginal smear. This day was designated as Day 0 of pseudopregnancy or pregnancy. In the present experiment, the duration of pseudopregnancy was from 11 to 15 days and of pregnancy, 20 days. The day of parturition was designated as Day 0 of lactation. For lactating rats, all litters were adjusted to 8 young on the day of parturition and the young were weaned on Day 21.

The HCG was dissolved in 0.9% (w/v) NaCl and 10 i.u. were injected in 0.2 ml into the tail vein at 17.00 h. On each day of pseudopregnancy, pregnancy or lactation 5 rats were injected unless otherwise stated. The animals were killed on the morning after the HCG treatment and oviducts were examined under a dissecting microscope for fresh ova. The results were analysed statistically by Duncan's multiple range test (Steel & Torrie, 1960).

Results and discussion

The numbers of rats ovulating and numbers of ova per ovulating rat are shown in Table 1. In pseudopregnancy the ovarian follicles were highly responsive to exogenous gonadotrophin: HCG induced ovulation on any day of pseudopregnancy and, apart from Day 1, the numbers of eggs ovulated were high. Follicles were able to respond to HCG throughout pregnancy, but fewer follicles ovulated as the pregnancy progressed until Day 18. A significant increase in the number of ova was noted from Day 18 to 19 ($P < 0.01$) and from Day 19 to Day 20 ($P < 0.01$). This increased response is clearly related to the development of follicles for the post-partum oestrus. A low ovulatory response to HCG during pseudopregnancy and pregnancy in the rat has been reported by van Rees *et al.* (1968), Welschen *et al.* (1975) and Greenwald (1966). However, Brown-Grant (1969) reported that ovulation was induced by HCG in earlier stages of pregnancy and he suggested some evidence for a 3-day cycle of follicular development after Day 6 of pregnancy. Ying *et al.* (1973) reported that rat ovarian follicles were responsive to exogenous gonadotrophins because FSH or LH induced ovulation of similar numbers of ova on any day of pregnancy. Our results agree with those of Ying *et al.* in that HCG can induce ovulation on any day of pregnancy, but the number of eggs ovulated by each rat was low from

Table 1. The effect (mean $\pm$ S.E.M.) of 10 i.u. HCG on the ovulatory response of rats

Days	Pseudopregnancy		Pregnancy		Lactation	
	No. ovulating	No. of ova	No. ovulating	No. of ova	No. ovulating	No. of ova
1	3	3.6 $\pm$ 0.7	5	4.8 $\pm$ 1.1	1	2
2	5	11.2 $\pm$ 0.7	5	10.8 $\pm$ 0.7	0	—
3	5	10.0 $\pm$ 0.7	5	10.4 $\pm$ 0.7	0	—
4	5	10.8 $\pm$ 1.4	5	11.4 $\pm$ 1.3	0	—
5	5	9.8 $\pm$ 1.2	5	10.8 $\pm$ 0.7	0	—
6	5	12.4 $\pm$ 1.0	5	10.4 $\pm$ 0.6	0	—
7	5	10.4 $\pm$ 0.9	5	12.0 $\pm$ 1.1	0	—
8	5	9.4 $\pm$ 1.4	5	10.8 $\pm$ 0.5	2	8, 12
9	5	11.6 $\pm$ 0.7	5	9.4 $\pm$ 1.0	4	6.8 $\pm$ 2.6
10	5	10.8 $\pm$ 1.5	5	8.6 $\pm$ 1.0	2	1, 1
11	5	11.6 $\pm$ 1.0	5	7.2 $\pm$ 0.9	4*	5.5 $\pm$ 3.1
12	—	—	5*	6.8 $\pm$ 0.8	5	8.6 $\pm$ 1.4
13	—	—	5	3.6 $\pm$ 0.9	4	8.5 $\pm$ 2.2
14	—	—	5	1.8 $\pm$ 0.6	5	9.6 $\pm$ 0.7
15	—	—	3*	1.6 $\pm$ 0.03	5	10.8 $\pm$ 0.2
16	—	—	4	2.8 $\pm$ 0.6	5	6.4 $\pm$ 1.2
17	—	—	5	3.0 $\pm$ 0.6	5	10.2 $\pm$ 1.0
18	—	—	5	2.8 $\pm$ 0.6	4	7.8 $\pm$ 1.0
19	—	—	5	7.2 $\pm$ 1.2	5	8.2 $\pm$ 1.1
20	—	—	5	14.2 $\pm$ 1.0	5	11.4 $\pm$ 2.3

* 6 rats/group; all others 5 rats/group.

Day 12 to Day 18. The reason for the differences between our results and those of other workers is not known: strain and hormone differences may be involved. In mice, ovulation can be induced by human pregnancy urine after implantation (Burdick & Whitney, 1941) and Ladman & Runner (1953) observed a low ovulatory response in the middle of pregnancy, and an increased response after Day 17. In lactating rats, no ovulatory response was noted from Day 2 to 7. The ovulatory response was variable after Day 8, suggesting that the stimulus for the initiation of follicular maturation varied because of differences in suckling activity.

We thank Dr K. W. Thompson, Emeritus Professor, University of Wisconsin, for his help in the preparation of the manuscript and the Sankyo-Zoki Co., Tokyo, for the supply of HCG. This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education of Japan (No. 156198 in 1976).

References

- BROWN-GRANT, K. (1969) The induction of ovulation during pregnancy in the rat. *J. Endocr.* **43**, 529–538.
- BURDICK, H.O. & WHITNEY, R. (1941) Ovulation induced in mice by single injections of follutein or untreated human pregnancy urine. *Am. J. Physiol.* **132**, 405–410.
- GREENWALD, G.S. (1966) Ovarian follicular development and pituitary FSH and LH content in the pregnant rat. *Endocrinology* **79**, 572–578.
- LADMAN, A.J. & RUNNER, M.N. (1953) Demonstration of storage and release of gonadotropin by the anterior pituitary of the mouse during gestation. *Endocrinology* **53**, 367–379.
- STEEL, R.G.D. & TORRIE, J.H. (1960) *Principles and Procedures of Statistics*, pp. 107–109. McGraw-Hill, New York.
- VAN REES, G.P., VAN DIETEN, J.A.M.J., BIJLEVELD, E. & MULLER, E.R.A. (1968) Induction of ovulation during pseudopregnancy in the rat. *Neuroendocrinology* **3**, 220–228.
- WELSCHEN, R., OSMAN, P., DULLAART, J., DE GREEF, W.J., UILENBROEK, J.T.H.J. & DE JONG, F.H. (1975) Level of follicle stimulating hormone, luteinizing hormone, oestradiol-17 β and progesterone, and follicular growth in pseudopregnant rat. *J. Endocr.* **64**, 37–47.
- YING, S.Y., GOVE, S., FANG, V.S. & GREPP, R.O. (1973) Ovulation in postpartum rats. *Endocrinology* **92**, 108–116.

Received 7 March 1977