## No Effect of Voluntary Exercise on Ovarian Follicle in Rats

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Abstract: The present study was to clarify the relationship between voluntary exercise and follicular growth or ovulation. Rats kept for 4 weeks in a rotating drum with free access to the wheel, food and water ran 2–12 km per day. The number of ova shed after superovulation treatments and the number of non-atretic follicles were not influenced by voluntary exercise. These experiments demonstrate that spontaneous voluntary exercise does not affect either the number of ova shed or the number of non-atretic follicles in superovulating rats or control rats.

Key words: follicle, ovulation, rat, voluntary exercise

Laboratory rodents engage in voluntary exercise when they are given access to running wheels, and the amount of voluntary exercise varies with the reproductive condition of the animals [6, 9–11, 13, 15]. Laboratory rodents increase their running when they are required to do so in order to obtain food. In rats, forced exercise inhibits sexual maturation [7, 8], and forced swimming or wheel running disrupts the four-to-five-day estrous cycle of adult rats [5], but it is not clear that this correlates with exercise and follicular growth or ovulation. The present study was to clarify the relationship between voluntary exercise and follicular growth or ovulation.

One hundred and forty female Wistar-Imamichi strain rats, 30 days old were obtained from the Institute for Animal Reproduction, and studied at 9 weeks old. During the measurement phase, each animal lived continuously in a Natsume rotating drum with free access to the wheel, food and water. The circumference

of wheel was 1 m. The rotating drums were located in a room maintained at 24 ± 2°C and under a lighting regimen of 14L: 10D (lights on 07:00 to 21:00 h). Vaginal smears were judged according to the criteria of Zarrow et al. [17]. Body weight, 24 hr food intake and 24 hr physical activity (number of wheel revolutions) were recorded at the start of the light period. Ovaries were removed from 13-week-old control rats at 09:00 h on the day of metestrus. Ovaries were fixed in Lavdovski's solution, dehydrated and embedded in paraffin wax, serially sectioned at 15  $\mu$ m and stained with haematoxylin and eosin. Follicles were classified as non-atretic or atretic according to the criteria of Braw and Tsafriri [3], and the diameter of the follicle was also recorded as 250-549  $\mu$ m or greater than 550  $\mu$ m. Rats were treated with pregnant mare's serum gonadotropin (PMSG) (Serotropin, Teizo Co. Ltd. Tokyo Japan) at 09:00 h on the day of metestrus, and their ovaries were removed 54 hr later. The number of non-

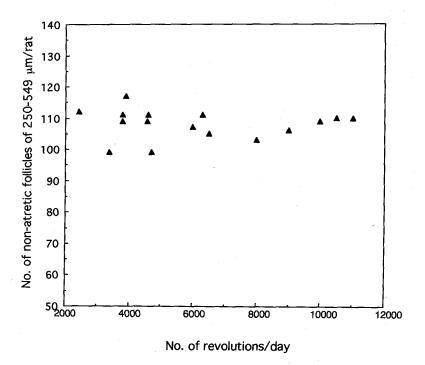


Fig. 1. Relationship between spontaneous voluntary exercise and the number of non-atretic follicles 250-549  $\mu$ m in diameter in control rats.

atretic follicles over 550  $\mu$ m in diameter was counted as mentioned above. Control animals, 13-week-old, were killed at 05:00 h on the day of estrus. At autopsy the ova were removed from the fallopian tubes under a dissecting microscope and counted. Other rats were treated with PMSG at 09:00 h on the day of metestrus, and human chorionic gonadotropin (hCG) (Gonatropin, Teizo Co. Ltd.) was given intramuscularly 54 hr after PMSG administration. They were killed 20 hr after hCG administration. At autopsy the ova were removed and counted.

The results are presented in Figs. 1, 2 and 3. For every rat the number of wheel revolutions was generally constant. Food intake of the exercised 140 rats was  $16.0 \pm 4.1$  g/day (Mean  $\pm$  SD) and that of the control 50 rats was  $12.3 \pm 3.4$  g/day. At the start of the experiments, at 9 weeks of age, the body weight was  $226.1 \pm 2.7$  g (Mean  $\pm$  SD). The body weight at autopsy was  $263.9 \pm 7.1$  g (Mean  $\pm$  SD) for the experiment rats, and  $282.6 \pm 6.6$  g (Mean  $\pm$  SD) for the control. The relationship between voluntary exercise and the number of non-atretic ovarian follicles in the control rats is shown in Fig. 1. The voluntary exercise affected neither the number of non-atretic follicles  $250-549~\mu m$ 

diameter nor the number of ova shed in the control or superovulating rats.

The present study was undertaken to examine the relationships between voluntary exercise and the number of ova shed and the number of non-atretic follicles in adult rats. In rats forced exercise inhibits sexual maturation [7, 8], and forced swimming or wheel running disrupts the sexual four-to-five day estrous cycle of adults [5]. One of the first investigators [2] to comment on the link between food and fertility may have viewed the relationship in terms of energy supply and demand. Relationships between nutrition and reproduction have been documented in mammalian species ranging from rodents to human beings: reproduction is suppressed when calories are unavailable.

Women athletes and ballet dancers also have irregular menstrual cycles during intensive training, and this effect is readily reversed when the training regimen is relaxed [1, 12, 16]. In female Rhesus monkeys, exercise-induced amenorrhea is reversed when the females are allowed to compensate for their increased energy expenditure by increasing their caloric intake [4]. The idea that exercise affects reproduction via its effect on the metabolic energy supply is supported by data show-

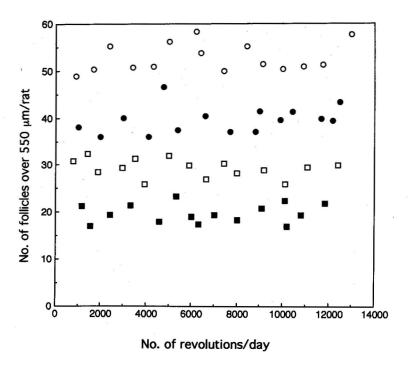


Fig. 2. Relationship between spontaneous voluntary exercise and the number of non-atretic follicles over 550 µm in control rats (■) and rats treated with 20 IU of PMSG (□), 30 IU of PMSG (●) and 40 IU PMSG (○).

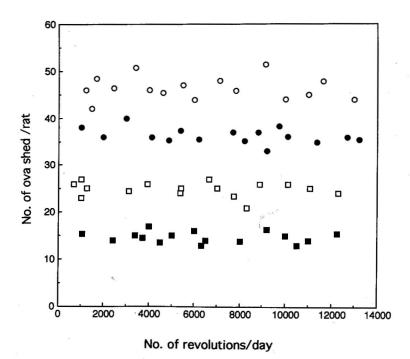


Fig. 3. Relationship between spontaneous voluntary exercise and the number of ova shed in control rats (■) and superovulating treated rats (□; 20 IU of PMSG injected at metestrus and 20 IU of hCG injected 54 hr after PMSG administration, (●) 30 IU of PMSG and 30 IU of hCG, (○) 40 IU of PMSG and 40 IU of hCG.

ing an interaction between exercise and food availability [14]. Rats were therefore fed *ad libitum* in these experiments.

No change in the number of ova shed or in the number of non-atretic follicles was observed in the voluntary exercising rats (2000–12000 revolutions per day). Under these experimental conditions (food and water ad libitum), voluntary wheel running exercise had no effect on the number of ova shed or on the number of ovarian follicles. These experiments demonstrate that spontaneous voluntary exercise does not influence either the number of ova shed in superovulating rats or control rats, or the number of non-atretic follicles.

## References

- 1. Abraham, S.F., Beumont, P.J.V., Fraser, I.S., and Llewllyn-Jones. 1982. Br. J. Obstet. Gynaec. 89: 507-510.
- 2. Allen, D.M. 1961. J. Agr. Sci. 56: 69-79.
- Braw, R.H. and Tsafriri, A. 1980. J. Reprod. Fert. 59: 267-272.

- Cameron, J.L., Nosbisch, C., Helmrich, D.L. and Parfitt, D.B. 1990. Endocrine Society Meetings: 1042.
- Carberg, K.A. 1985. Proc. Soc. Exp. Biol. Med. 179: 21– 24.
- Gerall, A.A., Napoli, A.M., and Cooper, U.C. 1973. Physiol. Behav. 10: 225–229.
- 7. Manning, J.M. and Bronson, F.H. 1991. Am. J. Physiol. 258: R1395-R1401.
- 8. Manning, J.M. and Bronson, F.H. 1991. Am. J. Physiol. 260: R717-R723.
- 9. Richards, M.P.M. 1966. Anim. Behav. 14: 450-458.
- 10. Richter, C.P. 1943. The Harvey Lectures. 38: 63-103.
- 11. Robyn, C. 1982. Semin. Perinatol. 6: 254-264.
- 12. Ronkainen, H., Pakarinen, A., and Kauppila, A. 1984. Gynecol. Obstet. Invest. 18: 183-189.
- 13. Slonaker, J.R. 1924. Am. J. Physiol. 68: 294-315.
- 14. Wade, G.N. and Schneider, J.L. 1992. Neuroscience and Biobehavioral Reviews 16: 235-272.
- 15. Wang, G.H. 1923. Com. Psychol. Monogr. ii, No. 6.
- 16. Warren, M.P. 1980. J. Endocrinol. Metab. 51: 1150-1157.
- Zarrow, M.X., Yochim, J.M., and McCarthy, J.L. 1964.
  pp. 24–27. *In*: Experimental endocrinology, A sourcebook of Basic Techniques. New York, Academic Press.