

Reproductive ability of pubertal male and female rats

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Abstract

Ten Fisher rats 50 to 55 days of age made up the pubertal group, and ten rats 90 to 95 days of age served as the controls. The testicular and epididymal weights and volumes of the pubertal males were lower than those of the controls ($P < 0.001$). There was also a difference in relative epididymal weight ($P < 0.001$). The sperm of pubertal males was morphologically abnormal in 58.2% of cases, as opposed to only 3.8% in the controls ($P < 0.001$). The mean number of spermatozoa in the control group was $11.9 \times 10^6/\text{ml}$ and their viability was 99.6%, while these values could not be determined for pubertal rats. Serum testosterone was higher in the pubertal animals than in the controls (2.52 ± 1.46 vs 0.92 ± 0.34 nM, $P < 0.01$). The ovaries of control females were heavier than those of pubertal females ($P < 0.001$) but there was no difference in their relative weights. Serum estradiol was similar in both groups (75.5 ± 12.8 vs 81.8 ± 14.7 nM, $P > 0.05$). At the beginning of gestation, the pubertal dams weighed less than the controls ($P < 0.001$) but following uterectomy the body weights were equal. Pubertal dams delivered fewer pups than the controls (8.1 ± 2.5 vs 10.4 ± 1.3 , $P < 0.05$). There was no difference in the body weights of their offspring or in the weights of their placentas. The results suggest that, in contrast to their female counterparts, pubertal male rats are not fully mature and have not reached complete reproductive capacity at 50-55 days of age.

Key words

- Puberty
- Reproduction
- Gonads
- Sex hormones
- Pregnancy

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Introduction

We have previously reported that there was a lower incidence of pregnancies after mating pubertal females and males (50 to 55 days old) compared to control adult rats (90 to 95 days old). The gestation index in the pubertal females was significantly lower compared to the controls (1).

The beginning of puberty in female Sprague Dawley rats is the day of vaginal opening, which is immediately followed by the first ovulation and sexual receptivity around the 33rd day (2). Between 41 and 54 days of age, irregular estrous cycles occur in females. Normal estrous cycles were seen after 54 days of age. Around the 33rd day there is an abrupt increase in testicular weight

in male rats. Other secondary sexual characteristics mature between 41 and 54 days and males reach adult body weight after 54 days of age (2). Female sexual receptivity begins before the complete perforation of the vagina. Robb et al. (3) considered male Wistar rats to be pubertal at 50 days of age, when spermatozoa were first found in the tail of the epididymis. Sperm production/g of testis increased until 75 days of age and testicular weight increased until 100 days of age.

Zanato et al. (4) found that the first significant increase of plasma testosterone in male Wistar rats occurred from 40 to 50 days of age and a progressive increase was observed thereafter to a maximum at 76 days. Plasma testosterone then became gradually lower, reaching adult levels at 97 days of age. The release of luteinizing hormone-releasing hormone in female Wistar rats appears to be associated with the opening of the vagina. However, the timing of vaginal opening may be influenced by the diurnal rhythm of the endocrine changes occurring during the pubertal period (5,6). The cholinergic system, through the muscarinic receptors, substantially contributes to the accurate timing of female puberty and ovarian growth in the rat (7). It seems that pubertal females have the same reproductive ability as adults (5).

In the present study we investigated the reproductive ability of pubertal male and female rats (50-55 days old) in comparison with adult (90-95 days old) pairs.

Material and Methods

Animals

Fisher rats were raised under controlled housing conditions (temperature, $22 \pm 1^\circ\text{C}$; light schedule: 14-h light and 10-h dark). Laboratory food and tap water were supplied *ad libitum*.

The animals were bred and maintained according to the Guide for the Care and Use of Laboratory Animals (Institute of Labora-

tory Animal Resources, Commission on Life Sciences, National Research Council, 1996). The stipulations for intramural animal care and the human use of laboratory animals in experimental work were followed. The experiments were approved by the Ethics Committee of the School of Medicine, University of Split, at the time of grant approval.

Pubertal rats (50-55 days old) and their offspring represented the pubertal group, and adult rats (90-95 days old) and their offspring served as the controls.

Experiment 1

Ten males of the pubertal group weighing 171 ± 18 g (mean \pm SD) and ten males of the control group weighing 287 ± 22 g were used in this study. At the appropriate age, 2 ml of blood was taken from the jugular vein to determine serum testosterone levels. Blood samples were centrifuged at 4°C and serum testosterone levels were measured using a commercial radioimmunoassay kit (Testosterone Directe Kit, Immunotech, Marseille, France). Subsequently, the animals were killed, the testes and epididymis were removed and weighed and their volume was determined in a precisely graduated laboratory dish. One epididymis was used for sperm counts and the determination of epididymal sperm viability and morphology. Epididymal sperm count was assessed by adding a 5- μl sample of sperm from the cauda epididymis to 0.5 ml of a solution of 5 g Na bicarbonate + 1 ml 40% formalin. The sample was dispersed and an aliquot was placed in a Burkert-Turk chamber. The number of sperms in the four outlying squares was determined. Epididymal sperm counts were reported as the number of spermatozoa per ml, using a modification of the Zanevald and Polakoski method (8). With this technique, because of dilution, it is possible to measure sperm counts above 1×10^4 spermatozoa per ml.

Epididymal sperm morphology was assessed by a modification of the method of

McClain et al. (9). Another sperm sample from the cauda epididymis (5 μ l) was placed on a slide and smeared. Two hundred sperm per animal were evaluated for morphology. The types and numbers of abnormal forms, characterized primarily by separation of the sperm head from the tail, were recorded and the percent of abnormal sperm was calculated for each animal. Finally, a third sperm smear was covered with a drop of 2% eosin B and allowed to dry. Viability was assessed using a light microscope. A total of 200 sperm were evaluated per animal and the percent of viable (unstained) and dead (stained) sperm was calculated.

Ten pubertal and ten control females were used in this experiment. At the appropriate age, 2 ml of blood was taken from the jugular vein and centrifuged at 4°C and serum 17 β -estradiol was measured with a commercial radioimmunoassay kit (Estr-ctk-4 Kit, DiaSorin s.r.l., Saluggia, Italy). Subsequently, the animals were killed and the ovaries removed, weighed and fixed in formalin for histological analysis. Morphology was studied on 5- μ m thick paraffin sections stained with hematoxylin-eosin.

Experiment 2

Ten pregnant pubertal females and ten control females were weighed on the first and last days of pregnancy. On the 21st day

of pregnancy, the rats were anesthetized and laparotomized. The uterine horns were opened and the numbers of fetuses, resorbing conceptuses and stillbirths were determined. Subsequently, the fetuses were removed and the females were weighed without them. The number and sex of the fetuses, their body weights and the weights of the placentas were determined.

Statistical analysis

Data were analyzed statistically by the Mann-Whitney test (body weight; the absolute and relative weights of the testes, epididymis and ovaries; the volume of the testes and epididymis; serum testosterone and 17 β -estradiol levels; sperm morphology, and the number of fetuses). Two-way Kruskal-Wallis ANOVA without replication was used to determine the differences in body weights of the offspring and in the weights of the placentas. The level of significance was set at 0.05.

Results

Experiment 1

The testes and epididymis were significantly lighter and of less volume from pubertal male rats compared to controls (Table 1). A significant difference was demonstrable

Table 1. Comparison of the reproductive organs of pubertal (50-55 days old) and adult control (90-95 days old) Fisher rats.

Organ	Weight (mg/pair)		Relative weight (mg/100 g body weight)		Volume (ml)	
	Pubertal	Adult	Pubertal	Adult	Pubertal	Adult
Testis	165.5 \pm 29.1*	277.2 \pm 20.9	96.6 \pm 11.0	99.7 \pm 9.5	1.94 \pm 0.28*	3.01 \pm 0.11
Epididymis	14.38 \pm 5.01*	80.74 \pm 4.91	8.27 \pm 2.12*	28.01 \pm 1.93	0.39 \pm 0.04*	0.85 \pm 0.50
Ovary	6.54 \pm 1.25*	10.09 \pm 1.35	5.58 \pm 0.78	5.62 \pm 0.50	ND	ND

Data are reported as means \pm SD for 10 rats per group. ND, not determined.

*P<0.001 compared to adult control (Mann-Whitney test).

in the relative weights of the epididymis (Table 1) but there was no difference in the relative weights of the testes (Table 1). In the pubertal group, 58.2% of the sperm were abnormal, while in the control group deviations were found in only 3.8% of the sperm ($P < 0.001$). The mean number of sperm in the control males was $11.9 \times 10^6/\text{ml}$ and their viability was 99.6%. These parameters could not be measured in the pubertal males, suggesting that the number of sperm was below $1 \times 10^4/\text{ml}$ in the pubertal group. Serum testosterone concentration in the pubertal group was higher than in the control group (pubertal = 2.52 ± 1.46 vs control = 0.92 ± 0.34 nM; $P < 0.01$).

The ovaries of the control females were heavier than those of the pubertal females (Table 1) but there was no difference in the relative weights (per 100 g body weight) of the ovaries (Table 1). There was also no difference in serum 17β -estradiol concentration between the pubertal and the control groups of females (pubertal = 75.5 ± 12.8 vs control = 81.8 ± 14.7 nM; $P > 0.05$). Histological analysis of the ovaries showed that all the forms of the follicles and a similar amount of corpora lutea (5-6 per section) were present in both groups.

Experiment 2

Pubertal females weighed less than control females ($N = 10$, pubertal = 117 ± 13 vs control = 176 ± 8 g; $P < 0.001$) at the beginning of pregnancy. They were also different on the 20th day of pregnancy (pubertal = 229 ± 12 vs control = 252 ± 22 g; $P < 0.01$) but there was no difference after hysterectomy on the 21st day (pubertal = 181 ± 18 vs control = 188 ± 18 g; $P > 0.05$).

The pubertal dams had fewer pups than the adult control dams (pubertal = 8.1 ± 2.5 vs control = 10.4 ± 1.3 ; $P < 0.05$). Two-way Kruskal-Wallis ANOVA showed that there was no difference in fetal body weight (pubertal = 4.40 ± 0.30 vs control = 4.47 ± 0.29 g;

$F = 3.08$, $P > 0.05$), or in the weight of the placentas (pubertal = 0.4 ± 0.07 vs control = 0.39 ± 0.05 g; $F = 1.73$, $P > 0.05$). There was only a sex difference in body weight, with the males of both groups being heavier than the females (males: 4.56 ± 0.28 vs females: 4.34 ± 0.27 g; $F = 24.80$, $P < 0.001$). Grouping by sexual interaction was not observed in fetal body weights ($F = 3.04$, $P > 0.05$) or in the weights of the placentas ($F = 0.759$, $P > 0.05$). No evidence of dead fetuses or resorbed conceptuses was found in either group at maternal autopsy on the 21st day of pregnancy.

Discussion

The present study demonstrated that male rats were not fully mature at 50 to 55 days of age as shown by the reproductive organ weights, serum testosterone levels and results of sperm analysis. This finding suggests a possible reason for less successful achievement of pregnancy at the age of 50-55 days than in adult rats (90-95 days), which we had noted in our previous study (1). The gestation index for the females of the pubertal group was significantly lower (3.8%) than that of the controls (36.5%) (1). Moreover, our preliminary data showed a higher gestation index when pubertal females were mated to adult males.

The difference in growth rate between male and female rats begins during the peripubertal period. Between 33 and 40 days of age, rat growth hormone (rGH) pulse amplitudes are increased more than ten-fold in both sexes. The duration of rGH pulses is significantly longer at this time in males versus females, a pattern that continues throughout adulthood. Between 41 and 54 days of age, rGH pulse amplitudes are similarly increased by two-fold in both sexes (2).

However, other endocrine factors such as testosterone, 17β -estradiol and corticosterone may be comparatively more important than rGH for the emergence of the sex

difference in growth rates during the peripubertal interval. Testosterone concentration steadily increases over 15-fold from 28 days of age throughout adulthood. Over this period, testosterone concentrations in females remain near the detection limit for radioimmunoassay. By contrast, plasma 17β -estradiol concentrations are higher in female rats compared to male rats. It is commonly considered that puberty lasts until 54 days of age. Animals over 54 days have been generally treated as adult, sexually mature animals (2). Some studies on the maturation of male Wistar rats prolonged their investigation to 100 days of animal life when, according to their results, serum testosterone became established at the levels of a full-grown animal (4).

In the present study we investigated the reproductive ability of male and female pubertal rats (50-55 days old). The results showed that in pubertal female rats (50-55 days old), ovarian relative weight, histological analysis of ovaries, serum 17β -estradiol levels and ability to carry live fetuses to term do not differ from adult females (90-95 days old). We may conclude that pubertal females have the same reproductive ability as adults. Our results are consistent with those of Hashizume and Ohashi (5), who reported that the timing of sexual receptivity, ovulation and release of gonadotropins during puberty is similar to that of proestrus in adult rats. They also noted that almost all female rats mated to full-grown males become pregnant during the first estrous cycle.

The primary functional consequence of copulation is the initiation of pregnancy. In rats, pregnancy initiation is dependent not only on the deposition of sperm in the female's reproductive tract but also on the delivery of appropriate levels of copulatory stimulation. In rats, the estrous cycle is brief and the initiation of a functional luteal phase depends on the stimulation received from copulation. In young virgin laboratory rats of some strains, a single ejaculatory series is

sufficient for the initiation of pregnancy in nearly all cases (10). In female rats, it has been estimated that the initial (vaginal opening) and final (establishment of positive feedback and ovulation) stages of sexual maturation are in most cases separated by time and regulated by different testosterone metabolites. A considerable decrease in testosterone and its biotransformation to 5α -reduced androgens are necessary for the induction of the initial puberty stage. The duration of the interval between the beginning and the end of pubertogenesis depends on the manner of testosterone metabolism. The transformation of testosterone to 5α -reduced androgens stimulates vaginal opening but delays the onset of the first ovulation. The activation of testosterone aromatization promotes a reduction in the time interval or even the temporal coincidence of the initial and final puberty stages (11).

Aging in female laboratory animals is associated with a decline in the capacity to mate and the ability to carry live fetuses to term. This decline in reproductive function may be related to dysfunction of the ovary and/or uterus. The maintenance of mammalian pregnancy is dependent on progesterone (P4), which is secreted by the corpus luteum during rat pregnancy. It has been concluded that luteal function in aged laboratory animals is normal during pregnancy, since the concentrations of P4 in the peripheral circulation have been shown to be relatively similar in young and aged rats (6,12,13). This statement is in agreement with our finding in the present study of the same number of corpora lutea in the ovaries of pubertal and adult females.

In a study by Hashizume and Ohashi (5), the question of the continuation of pregnancy and fetal development in pubertal rats was not considered. We monitored pregnant females during pregnancy and obtained some interesting findings. Pubertal dams started pregnancy weighing less than the controls but they were able to catch up by the end of

gestation and were of similar weight following hysterectomy and fetectomy on the 21st day of gestation. They carried a smaller number of fetuses but there was no difference in pup body weight or placental weight between the two groups. These results differ from those reported by Hashizume et al. (14), who found the offspring of control mothers to be heavier at delivery than the offspring of pubertal mothers. The same investigators also found that pubertal dams mated immediately or shortly after vaginal opening to fertile adult males are apparently reproductively equal to normal full-grown females, which is in agreement with the results reported here.

The present results indicate that the pubertal males were probably responsible for the delivery of fewer pups by pubertal dams. The relative weight of the epididymis of pubertal males was significantly smaller than that of adult animals. The pubertal males had 58.2% morphologically abnormal sperm, characterized primarily by separation of the sperm head from the tail, while the control males had only 3.8%. Because of the very low sperm counts (below $1 \times 10^4/\text{ml}$) in the pubertal males, it was not possible to measure the epididymal sperm count and sperm viability. Data in the literature about sperm production and maturation at critical periods of life are very scanty. Robb et al. (3) found that testicular weight increased rapidly up to 75 days of age and growth continued until 100 days of age. Sperm production/g testis reached the adult level of 24×10^6 spermatozoa/day by 75 days of age and daily sperm production/rat also stabilized at this age. At

45 days of age, only a few spermatozoa were found in the epididymis. Prior to 75 days of age, the head + body contained more spermatozoa than did the tail of the epididymis. At 75 days of age, the epididymal tail weighed only 65% of the weight at 125 days compared with a value of 83% for the head + body (3,15). The period from 51 to 66 days of life was called young adult period by Tentler et al. (16). In the present study, the serum testosterone level was significantly higher in pubertal males than in control males. It is known that serum testosterone level is low before puberty, starts rising at puberty (from the 28th day of life), reaches the highest level at approximately the end of puberty (54th day of life), and then decreases slowly as the animal ages (2).

According to the present findings, female rats of 50-55 days of age appear to be sexually mature, ready to become pregnant and to carry their pregnancies to term. However, in spite of presenting an amount of corpora lutea similar to that presented by adult animals, the possibility of occurrence of pre-implantation loss contributing to the lower number of live fetuses cannot be excluded. Pubertal males, on the contrary, were not fully mature and may have been responsible, at least in part, for the delivery of fewer pups by pubertal dams.

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