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Sperm count and sperm motility decrease in old rats

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HIGHLIGHTS

- The sperm motility of old male rats is reduced.
- The amount of expelled sperm is diminished in old male rats.
- In old male rats the proportion of immobile spermatozoa is increased.
- In situ sperm motility is observed at any rat's age.
- Age modifies the copulatory behavior and parameters of the ejaculate in rats.

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ABSTRACT

Sexual behavior declines with age in male rats. The rate and magnitude of this decline may depend on the amount of prior sexual experience and a number of other, unidentified factors. Age-dependent changes in the characteristics of ejaculate quality in rats have not been described earlier, and the relationship between such changes and modifications of sexual behavior is likewise unknown. We have recently developed a technique for the detailed analysis of parameters of ejaculate in rats, and this technique was used for the determination of semen and seminal plug characteristics in rats of different ages. Sexually experienced Wistar rats were tested for sex behavior at the ages of 3, 12 and 24 months. Semen was obtained from the female partner immediately after ejaculation at these tests. Between tests, the males were offered the opportunity to copulate once every 3–4 weeks. The behavioral data showed that the latency to ejaculation was increased only at 24 months. Concerning the characteristics of semen, there was a substantial increase in the proportion of immobile spermatozoa and motility of those moving was much reduced, both at 12 and 24 months of age. There was no relationship between parameters of sexual behavior and those of the ejaculate. Likewise, the size of the seminal plug did not affect the amount of intrauterine spermatozoa. The reduced sperm number together with the increased sperm immobility diminishes the ejaculate quality of old males, which could influence fertility.

1. Introduction

Many sexual functions decline with increasing age. In male rats, several studies have revealed that the intensity of sexual behavior is reduced in old animals [1]. Some studies report increased latency to mount and intromission [2–4] while others fail to detect any difference in these latencies between young and old males [5]. More consistent findings are that the number of mounts without vaginal penetration and the interval between the first intromission and ejaculation increase with age (e.g. [6]). Another rather consistent finding is that the postejaculatory interval, i.e. the time from ejaculation until the following intromission, also increases with age (e.g. [7–9]). In addition to showing a reduced intensity of copulatory behaviors, old male rats also have less intense approach behaviors towards a sexually receptive female than young males [3,10]. There is no reduction in the approach to a non-sexual, social incentive like another male, suggesting that aging produces a specific reduction of sexual motivation while leaving social motivation unchanged [10]. It is not impossible that some of the changes in copulatory behavior and sexual motivation observed in old rats are related to impair motor functions. In fact, motor coordination, general activity and exploration are much reduced in old rats [11,12].

Aging not only diminishes sexual behaviors but also reduces the quality of ejaculation. In men, the ejaculate volume decreases, particularly among men over 50 years, whereas sperm concentration seems to be unaffected by age [13]. Sperm motility is reduced in older men, and studies of sperm morphology reveal that the proportion of sperm with abnormal shape is increased [14]. Whether the reduced quality of semen leads to reduced fertility or not seems to be an open question

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[15–18]. In rats there is an age-dependent reduction of sperm quality (i.e., a decline in the sperm concentration in testis [19]; a reduction in the percentage of motile spermatozoa and an increase in the proportion of spermatozoa with cytoplasmic droplet in cauda epididymis [20]). These changes are similar to those described in men [13]. However, functional parameters like the acrosome reaction and chromatin condensation do not change with age in men [21,22].

It must be observed that the rat data stem from spermatozoa were obtained directly from the testis or epididymis. This means, among other things, that the effects of age on characteristics of the ejaculate remain unknown. Concerning the fertility of old male rats, the limited amount of data available suggests that there is no difference between rats aged 2 months and rats aged about 17 months [23,24], although fetal weight of the offspring of old males was somewhat reduced in the latter study. Thus, male rats and men appear similar in the way that sperm quality is reduced with increasing age, but the consequences for fertility are slight. The male rat's copulatory behavior is a crucial determinant of female fertility [25,26], and it is possible that age-dependent behavioral changes could offset the consequences of reduced sperm quality. Modifications in the reflexes of emission and ejaculation could also alter volume and sperm density in the ejaculate. In the present study, we employed a recently developed technique for studying the characteristics of ejaculated semen in rats [27] with the purpose of describing changes associated with increasing age.

The relationships between parameters of semen quality and characteristics of male sexual behavior were also determined. To these ends, a group of males was subjected to tests for sexual behavior at different ages, and the ejaculated semen was recovered from the female partner's reproductive tract.

2. Material and methods

2.1. Animals

Male and female Wistar rats from the *vivarium* of the Centro Tlaxcala de Biología de la Conducta were used. They were kept under a reversed light/dark cycle (12/12 h, lights on 08:00) in a room with a temperature of 20 ± 2 °C. The relative humidity was around 40%. The animals were provided with commercial rat pellets (Purina Chow, México) and water ad libitum. They were housed in plastic cages (50 × 30 × 20 cm), 3–4 males per cage and 6 females per cage; containing wood chip bedding. The experimental protocol was approved by Tlaxcala University Committee on Laboratory Animals, according to the guidelines of the Mexican Council on Laboratory Animals' Care (NOM-062-Z00-1999).

2.2. Tests for copulatory behavior

Ovariectomized virgin females were brought into estrus by the sequential subcutaneous injection of 10 μg of estradiol benzoate and 2 mg of progesterone (both steroids were purchased from Sigma-Aldrich, St. Louis, MO, USA), 48 h and 4 h before the tests for copulatory behavior, respectively. The doses of estradiol and progesterone employed here are within the standard range employed in similar studies.

A Plexiglas cylinder (50 cm high, 50 cm diameter) with wood shavings on the floor was used for tests of copulatory behavior. All tests were performed during the second third of the dark period. At the beginning of a test, the male was given a five min adaptation period, after which a sexually receptive female was introduced into the cylinder. The following copulatory parameters were registered: mount latency, time in seconds from the introduction of the female until the first mount; intromission latency, the time in seconds from the introduction of the female until the first intromission; the number of mounts with pelvic thrusting; number of intromissions with pelvic thrusting and penile insertion into the vagina; ejaculation latency, time in seconds from the first intromission until ejaculation. The intromission ratio (number of intromissions/number of intromissions + number of mounts) as well as the intromission rate (number of intromissions/ejaculation latency) were calculated. Each test lasted until the end of the first ejaculatory series.

Males with ejaculation latency shorter than 10 min at all six training copulatory tests were selected for the experiment. Experimental tests were performed when the males were 3, 12 and 24 months old. At the beginning of the experiment the male's mean ± SEM weight was 320 ± 10.5 g and at the end of the experiment it was 450 ± 15.8 g. Between tests, the males were offered the opportunity to copulate until ejaculation once every 3–4 weeks.

2.3. Evaluation of seminal fluid obtained from uterine horns

A detailed description of the procedures has been given elsewhere [27,28]. Here we give only brief information of the different steps involved. Immediately after the male's ejaculation the female was transferred from the arena to an empty cage where she was left quiet for 5 min before being anesthetized with sodium pentobarbital, 26 mg/kg intraperitoneally (Pfizer; DF, México). After an abdominal incision, the uterine horns were dissected and tied proximally and distally, removed from the abdominal cavity and immersed in a Petri dish containing physiological saline at 37 °C. Fat and external blood vessels were carefully dissected away, and the uterine horns were then removed from the Petri dish and dried with absorbing paper. Finally, an incision was made at the end of each horn, and the seminal content of both uterine horns was expelled into a 1.5 ml micro-centrifuge tube by applying light pressure. The tube was immediately placed in a thermo-bath at 37 °C. Samples of the ejaculate were used to evaluate the semen parameters as follows: seminal viscosity, using a transfer pipette a drop of semen was collected from the micro-centrifuge tube. The tip of the transfer pipette was positioned on the border of the micro-centrifuge tube, the semen drop was delivered, and the length of the semen filament was measured with a digital caliper (MyCAL Lite, USA) while the pipette was withdrawn. Consequently, viscosity is expressed in millimeters. Sperm count, 10 μl of semen was diluted 1:200 with the help of a Shali pipette. After vigorous shaking, 10 μl of the diluted semen was placed in a Neubauer hemocytometer for counting under a 20× microscope objective. The number of spermatozoa in five squares was counted. The mean was multiplied by 10^6 in order to obtain the sperm count in the uterine sample. Sperm motility, the spermatozoa were classified in three categories according to their motility: progressive, in situ and immobile. The spermatozoa with progressive motility are those with lineal forward movements; in situ motility refers to circular or local movements, and immobile sperm are spermatozoa without movements. Motility was also observed using the 20× microscope objective. One hundred spermatozoa were observed, from left to right, following a line in the middle of the microscope objective. The number obtained in each category was expressed as a percentage. The sperm motility was filmed (using a videocamera TK-C 1380; JVC, USA, connected to a computer HP Pavilion TV PC), in order to confirm the obtained results by a second blind observer. The viability and sperm morphology were determined using a colorant constituted by nigrosin, eosin and sodium citrate, all dissolved in distilled water. Ten μl of semen + 10 μl of the colorant were placed on a microscope slide and mixed with a tooth pick. The stained spermatozoa were considered as dead and those not stained as alive. The normal spermatozoa, with a sickle-shape head and large flagella versus the abnormal ones, with double head and fragmented or zigzag flagella were distinguished. One hundred randomly chosen spermatozoa were evaluated using a 100× microscope objective. Sperm viability and morphology were expressed as percentage.