REPEATED ACQUISITION OF RESPONSE SEQUENCES: STIMULUS CONTROL AND DRUGS

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Daily Spermatozoal Production, Epididymal Spermatozoal Reserves and Transit Time of Spermatozoa Through the Epididymis of the Rhesus Monkey

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ABSTRACT

One testis and epididymis from each of 8 sexually rested, rhesus monkeys >6 year old was removed during fall (the natural breeding season) to establish the productivity of the testis and the spermatozoal storage capacity of the epididymis. The efficiency of spermatozoal production was quite uniform and averaged 23 ± 1 X 10⁴ sperm per gram of testicular parenchyma per day, although testicular parenchymal weight ranged from 15 to 32 g. Daily spermatozoal production averaged 547 ± 69 X 10⁴ sperm per testis. Thus, the typical rhesus monkey produces about 1.1 X 10⁹ sperm daily during the breeding season. The caput, corpus and cauda epididymis in these sexually rested monkeys contained 0.6 ± 0.1, 2.1 ± 0.3 and 2.9 ± 0.3 X 10⁹ sperm and an additional 1.0 ± 0.1 X 10⁹ sperm were found in the proximal 49–70 mm of ductus deferens. The mean transit times of sperm through the epididymal segments were estimated as 1.1, 3.8 and 5.6 days for the caput, corpus and cauda, respectively. Based on comparisons with data for sexually rested males of seven other species, the transit time of sperm through the caput and corpus epididymidis is quite uniform at 2.0 to 5.5 days, despite a 265-fold difference in epididymal spermatozoal reserves. Thus, the time required for maturation of sperm within the epididymis is less than 5 days in several mammals including the rhesus monkey.

INTRODUCTION

Daily spermatozoal production by the testes, the distribution of spermatozoa within the epididymides, and the time required for passage of spermatozoa through the epididymides have been established for most domestic animals and a few species of laboratory animals. These data are not available for laboratory primates or man, but would be valuable in designing and interpreting experiments or clinical data. The objective of this study was to determine these characteristics for adult rhesus monkeys.

MATERIALS AND METHODS

Eight male rhesus monkeys (Macaca mulatta) >6 year old were available from an experiment which involved surgery on the vertebral column 12–15 months earlier. There was no reason to expect that this surgery altered reproductive function, although this was not proven. Monkeys were individually caged in a room maintained at 20–22°C with ambient light supplemented by artificial illumination to give >12 h of light daily. No mating occurred during 30 days prior to use. Between 9 Sept. and 17 Nov., monkeys were anesthetized with ketamine-HCL (Ketalar, Parke-Davis) and one testis was exposed. The left or right testis was taken alternately. After taking samples of blood from the testicular artery and a testicular vein for other studies, the ductus deferens was clamped below the inguinal ring and the testis-epididymis was extirpated. The free end of the ductus deferens was ligated; then the epididymis with attached ductus deferens was dissected from the testis, freed of fascia, weighed, placed in a plastic bag and frozen at –20°C. The testis was weighed and the tunica albuginea was gently stripped from the parenchyma and weighed. Most of the parenchyma, excluding the mediastinum, was placed into a tared vial, weighed and frozen. Testicular tissue was thawed and homogenized in a Waring blender for 1.0 min (Aman and Lambiase, 1969) in 170–250 ml of an aqueous solution containing 150 mM NaCl, 3.8 mM NaH₂PO₄, 0.05% (v/v) Triton X-100. The homogenates were stored 22–26 h at 5°C before dilution with 0-150 ml of fluid to facilitate counting the spermatid nuclei resistant to homogenization and with a shape characteristic of maturing spermatids (step 13 and 14; Clermont and Leblond, 1955) or testicular spermatozoas (hereafter simply called spermatids). The number of spermatids in the total mass of testicular parenchyma was calculated. The epididymis with attached ductus deferens was thawed. The ductus deferences was severed from the cauda epididymidis and cut into small pieces...
FIG. 1. The right epididymis and ductus deferens of a rhesus monkey showing the demarcation used between adjacent anatomical areas.

which were homogenized in 250 ml of fluid for 2.0 min. The epididymis was divided (Fig. 1) into caput, corpus and cauda, although demarcation between the caput and corpus was arbitrary. Each segment of epididymis was weighed, minced, homogenized for 2.0 min in 250 ml of fluid and further diluted with 0-550 ml of fluid before storage for 22-26 h at 5°C. Each of four technicians used a phase-contrast microscope to count the spermatozoal nuclei in two hemacytometer chambers filled with samples taken from each homogenate. Differences in the number of sperm in successive segments of the epididymis or in their percentage content of the total reserves were evaluated by t-test.

Daily spermatozoal production was estimated (Amann, 1970) by dividing the total number of spermatozoa enumerated in the testis by a time divisor. Based on spermatozoal morphology and the extent of chromatin condensation evident in Epon sections, we concluded that the nuclei resistant to homogenization and enumerated in testicular homogenates were those of step 13 and 14 spermatozoa. Although we assumed that all step 13 and 14 spermatozoa are resistant to homogenization, we do not know of an accurate method to confirm this point. Step 13 and 14 spermatozoa are present in Stages I through VI of the cycle of the seminiferous epithelium (Clermont and Leblond, 1955; 1959). These stages total 46.0 percent of the duration of one cycle which has a duration of 9.5 days (Clermont, personal communication). The product was 4.37 days and this time divisor was used to estimate daily spermatozoal production. Any error in this time divisor probably is a consequence of overestimating the portion of spermiogenesis during which the spermatozoon is resistant to homogenization and has a shape characteristic of a spermatozoon. Thus, the time divisor of 4.37 days should give an accurate or conservative estimate of daily spermatozoal production by the rhesus monkey.

The transit time of sperm through the epididymis was calculated by dividing the number of sperm in each segment by the daily spermatozoal production for the testis attached to the epididymis (Swierstra, 1971; Amann et al., 1974). The relationship epididymal transit time = epididymal spermatozoal reserves / daily spermatozoal production is valid if resorption of sperm in the epididymis is negligible and this latter assumption appears to be valid (Amann et al., 1974; Bedford, 1976).

RESULTS

The number of spermatozoa per gram of testis ranged from 74 to 115 x 10^6 and only two
TABLE 1. Testicular spermatid and epididymal spermatozoal reserves of the rhesus monkey.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SEM (N = 8)</th>
<th>Relative distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body (kg)</td>
<td>9.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Testis parenchyma (g)</td>
<td>23.1 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Testicular spermatids (10^6)a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per gram</td>
<td>102 ± 5</td>
<td></td>
</tr>
<tr>
<td>Total per testis</td>
<td>2390 ± 300</td>
<td></td>
</tr>
<tr>
<td>Spermatozoal reserves (10^9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caput epididymis</td>
<td>576 ± 131</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Corpus epididymis</td>
<td>2083 ± 273</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Cauda epididymis</td>
<td>2866 ± 296</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>Ductus deferensb</td>
<td>992 ± 73</td>
<td>16 ± 1</td>
</tr>
</tbody>
</table>

a Spermatid nuclei resistant to homogenization and with a shape characteristic of a step 13 or 14 spermatid.
b Only part of the ductus deferens (59 ± 2 mm) was available for the counts.

testes contained <10^1 x 10^6 spermatids per gram. The mean number of spermatids per testis was 2,390 x 10^6 (Table 1) which represented a daily spermatozoal production of 547 ± 69 x 10^6. Daily spermatozoal production was not significantly correlated with body weight (r = 0.17), but was correlated with testicular weight (r = 0.96).

Mean weights (± SEM) for the caput, corpus and cauda epididymidis were 1.4 ± 0.1, 1.3 ± 0.1, and 1.0 ± 0.1 g and the length of the corpus epididymidis was 32 ± 1 mm. The epididymis contained 5,526 ± 508 x 10^6 sperm, although values for individual epididymides ranged from 3,760 to 7,590 x 10^6. The 49-70 mm of ductus deferens contained 730 to 1,430 x 10^6 sperm, most of which probably were in the highly convoluted segment adjacent to the cauda epididymidis (Fig. 1). The accountable extragonadal reserves totaled 6,518 ± 545 x 10^6 sperm and we assumed that total extragonadal spermatozoal reserves are ~7 x 10^9 per side for sexually rested rhesus monkeys.

The number of sperm in the cauda was not greater (P>0.05) than in the corpus (Table 1). However, when variation among animals in extragonadal or epididymal spermatozoal reserves was eliminated by expressing the data on a percentage basis, the cauda contained more (P<0.01) sperm than the corpus. In contrast to many species (Table 2), the caput contained only 11 ± 2 percent of the sperm within the epididymis, while the corpus and cauda contained 37 ± 3 and 52 ± 3 percent.

For sexually rested monkeys, transit time of sperm through the epididymis was estimated as 10.6 ± 0.7 days of which 1.1 ± 0.2, 3.8 ± 0.3 and 5.6 ± 0.6 days were required for passage through the caput, corpus and cauda. An additional 2.0 ± 0.8 days were required for passage of sperm through the proximal ductus deferens.

DISCUSSION

Selection of a model animal for experiments in reproductive physiology requires comparative information on basic characteristics of different species. The efficiency of spermatozoal production is high in rhesus monkeys. The value of 23 x 10^6 sperm per gram of testicular parenchyma per day is comparable with that of most species (Table 2), but twice that of dairy bulls. The efficiency of spermatozoal production within the human testis is unknown, but we believe that it would be hazardous to conclude that spermatogenesis in humans is as efficient as that in rhesus monkeys during the breeding season.

The monkeys used in this study were castrated during the breeding season for rhesus monkeys housed under laboratory conditions (Michael et al., 1975; Robinson et al., 1975) and their testicular weights compare favorably with testicular size in free-ranging monkeys during the breeding season (Sade, 1964). The low variability in the number of spermatids per
### TABLE 2. Species differences in daily spermatozoal production, epididymal spermatozoal reserves and transit time of sperm through the epididymis of sexually rested males.

<table>
<thead>
<tr>
<th>Species</th>
<th>Paired testes wt (g)</th>
<th>Daily sperm production by both testes (10⁹)</th>
<th>Per gram parenchyma (10⁸/g)</th>
<th>Extragonadal spermatozoal reserves (10⁹)</th>
<th>Transit time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Caput epid</td>
<td>Corpus epid</td>
<td>Cauda epid</td>
<td>Ductus deferens</td>
</tr>
<tr>
<td>Human</td>
<td>40</td>
<td>19</td>
<td>4.7</td>
<td>38</td>
<td>7.6</td>
</tr>
<tr>
<td>Bull (dairy)</td>
<td>725</td>
<td>9.6</td>
<td>11</td>
<td>50</td>
<td>7.5</td>
</tr>
<tr>
<td>Stallion</td>
<td>340</td>
<td>23</td>
<td>11</td>
<td>126</td>
<td>&gt;165</td>
</tr>
<tr>
<td>Ram</td>
<td>500</td>
<td>36</td>
<td>51</td>
<td>104</td>
<td>&gt;185</td>
</tr>
<tr>
<td>Boar</td>
<td>720</td>
<td>1.2</td>
<td>4.2</td>
<td>5.7</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>49</td>
<td>23</td>
<td>0.26</td>
<td>0.45</td>
<td>&gt;0.70</td>
</tr>
<tr>
<td>Rat (Wistar)</td>
<td>3.8</td>
<td>23</td>
<td>0.15</td>
<td>1.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Hamster</td>
<td>3.0</td>
<td>0.36</td>
<td>0.12</td>
<td>1.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Rabbit</td>
<td>6.4</td>
<td>0.16</td>
<td>25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Based on published data (Almquist and Amann, 1961; Amann et al., 1974; Clermont, 1954; Clermont and Trott, 1969; Hilscher and Hilscher, 1969; Lambiasi and Amann, 1969; Ortavant, 1958; Singh, 1962; Swierstra, 1968, 1971; Swierstra et al., 1974; Rowley et al., 1970), unpublished data for stallions (N = 7) and data in Table 1. The time divisors used to convert testicular spermatozoal reserves to daily spermatozoal production were: bull, 5.32; stallion, 6.00; ram, 4.99; Wistar rat, 6.10; boar, 6.19; monkey, 4.37; hamster, 6.03 and rabbit, 5.35 days. Extragonadal reserves are for paired sides.
gram of testis for rhesus monkeys in this study is similar to findings with adults of other species (Ortavant, 1958; Lambias and Amann, 1969; Amann, 1970). Thus, it is not surprising that daily spermatozoal production is highly correlated with testicular weight \( r = 0.96 \) for monkeys). However, as discussed by Amann (1970), correlations between testicular weight and body weight or between daily spermatozoal production and body weight are too low in adult males to be used for predictive purposes.

During the breeding season the efficiency of spermatozoal production in normal, sexually mature males is very consistent within a species, but seasonal differences in this characteristic occur in rams (Ortavant, 1958) and probably other species (Amann, 1970). A large decrease in testicular size occurs in rhesus monkeys during the nonbreeding season (Sade, 1964). It is quite possible that a decline in the efficiency of spermatozoal production occurs concurrently. For rams (Ortavant, 1958), the efficiency of spermatozoal production is reduced by about 20 percent during the nonbreeding season. A reduction of this magnitude could occur in monkeys housed under laboratory conditions although they produce sperm throughout the year. Age also affects the efficiency of spermatozoal production. At puberty the efficiency of spermatozoal production is very low. In Holstein bulls, this efficiency increases from \( \sim 0 \) to the adult level between 34 and 54 weeks of age (Macmillan and Hafs, 1968; Killian and Amann, 1972); bulls can first ejaculate sperm around 38 weeks of age. The efficiency of spermatozoal production declines with advancing age in rabbits (Ewing et al., 1972).

There are large species differences in extraglandal spermatozoal reserves (Table 2) and in the relative distribution of sperm within the epididymis. Although the anatomical designations of caput, corpus and cauda epididymidis have little relation to the compartmentalization of function within the epididymis (Glover and Nicander, 1971; Crabo and Hunter, 1975), it is a convenient classification. In most species, sperm acquire the ability to fertilize ova while in the distal corpus or proximal cauda epididymidis (Crabo and Hunter, 1975; Orgebin-Crist et al., 1975) so the cauda serves to store mature sperm. The percentage of epididymal sperm found within the caudae ranges from about 53 percent for monkeys and boars to 77–87 percent for rabbits, rams and hamsters. The relatively low value for monkeys could be a consequence of the demarcation we used between the cauda epididymidis and ductus deferens (Fig. 1). Many of the sperm we counted in the ductus deferens probably were in the highly convoluted portion which often is included with the cauda epididymidis (Amquist and Amann, 1961; Lambias and Amann, 1969; Ortavant, 1958; Swierstra, 1971). However, even if \( 1.5 \times 10^8 \) of the \( 2.0 \times 10^8 \) sperm within the proximal ductuli deferentia are assigned to the caudae, the caudae still would contain only 57 percent of the epididymal spermatozoal reserves. Species differences in the storage capacity of the cauda epididymidis also may reflect distensibility of the duct.

Comparisons with more physiological meaning probably can be made by considering epididymal spermatozoal reserves relative to daily spermatozoal production. Differences among species in the ratio of the number of sperm in the caudae epididymisis to daily spermatozoal production may have evolved concurrent with differences in length of the breeding season, duration of estrus and mating behavior of males. Animals with a large reserve of sperm in the caudae epididymisis could successfully breed more females in a shorter time than males of a species where the ratio of spermatozoal reserves to spermatozoal production is narrow.

The ratios for each segment of the epididymis between spermatozoal reserves and daily spermatozoal production also give estimates of the time sperm spend in each segment of the epididymis (Swierstra, 1971; Amann et al., 1974). These estimates (Table 2) are in reasonable agreement with earlier estimates obtained using other techniques (Orgebin-Crist, 1962; Amir and Ortavant, 1968; Swierstra, 1968; Amann, 1972; Hamilton, 1972) and provide the first data for individual portions of the epididymis for most of the species. Transit time of sperm through the caput and corpus epididymidis is surprisingly uniform among species despite the 265-fold difference in epididymal spermatozoal reserves. For each species (Table 2), estimated transit time of sperm through the caput and corpus epididymidis is between 2.0 and 3.5 days. The longest interval is for boars. In boars, sperm apparently acquire their fertilizing capacity in the corpus epididymidis (Crabo and Hunter, 1975; Hunter et al., 1976). The location within the monkey epididymis where sperm acquire their fertility is unknown. However, translocation of the cytoplasmic droplet
occurs before sperm enter the corpus epididymis and 5–15 percent of the sperm from the corpus are progressively motile (Alsum, 1974). Based on these and other data, Alsum (1974) concluded that rhesus monkey sperm in the distal corpus epididymidis have at least limited fertilizing ability. In apparent contrast to other species, human sperm from the caput epididymidis have some fertilizing capacity (Orgebin-Crist et al., 1975) and about 12 percent are progressively motile (Mooney et al., 1972). Thus, the process of sperm maturation requires less than 5 days in all species studied. Depending on the species, however, mature sperm are stored in the cauda epididymidis of the average sexually rested male for 5 to 14 days. The time required for sperm to traverse the cauda epididymidis of a sexually rested male is not dependent simply on the number of sperm stored in the cauda, but rather is a function of storage capacity and daily spermatozoon production (Table 2). There is no evidence that transit time of sperm through the caput and corpus epididymidis is altered by ejaculation frequency, although transit through the cauda epididymidis is shorter in sexually active than in sexually rested males (Orgebin-Crist, 1962; Amir and Ortvant, 1968; Amann et al., 1974). The transit time of sperm through the caput and corpus epididymidis is similar in young and mature Holstein bulls, although there is a two-fold increase with age in time required for sperm passage through the cauda epididymidis (Amann and Almquist, 1976).

ACKNOWLEDGMENTS

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