Determination of the elongate spermatid–Sertoli cell ratio in various mammals

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Summary. Criteria were devised for determining the elongate spermatid–Sertoli cell ratio in various mammalian species at the electron microscope level. When data from particular species were pooled, the values were: rabbit, 12:17:1; hamster, 10:75:1; gerbil, 10:64:1; rat, 10:32:1; guinea-pig, 10:10:1; vole, 9:75:1; and monkey, 5:94:1. The elongate spermatid–Sertoli cell ratio is a measure of the workload of the Sertoli cell and is a prime factor determining their efficiency. The higher the ratio, the higher the sperm output is likely to be per given weight of seminiferous tubule parenchyma for a particular species.

Introduction

The number of spermatozoa provided in the ejaculate is determined by a number of factors but the major influence is the number of spermatozoa produced in the testis. In mammals that breed continuously testicular sperm production appears to be related to the size of the testis, especially the seminiferous tubule compartment. Here the kinetics of spermatogenesis dictate how many germ cells (spermatagonia) become committed to the spermatogenic process and also the time it takes these germ cells to go through various cell divisions and transformations to become a spermatozoon. The index of sperm production, or the daily sperm production, is expressed as the number of spermatozoa produced per day by the two testes of an individual, whereas the index of efficiency of sperm production is the number of spermatozoa produced per unit weight or volume of testicular tissue (Amann, 1970).

The efficiency of sperm production may also relate to the efficiency of the somatic cells or Sertoli cells, which co-exist with the germ cells of the seminiferous tubule, to support a specific number of germ cells. For non-seasonally breeding animals, the Sertoli cell number appears to become fixed early in development because no further mitosis of these cells occurs (Clermont & Perey, 1957; Nagy, 1972; Bergh, 1981). This allows use of Sertoli cells as a standard for quantitation of nearby germ cell types (Clermont & Perey, 1957; Oakberg, 1959). Most germ cell types are related to the lateral surfaces of the Sertoli cell (Weber et al., 1983) and a ratio of round germ cells to Sertoli cells may be determined by morphometric analysis (Roosen-Runge, 1955; Wing & Christensen, 1982). Clusters of elongate germ cells, on the other hand, are embedded in a single Sertoli cell (Weber et al., 1983) in deep cylindrical recesses (Wong & Russell, 1983). It is the ratio of elongate spermatids to Sertoli cells that may be a prime factor determining daily sperm production since the number of Sertoli cells is fixed in the adult. The ratio of elongate cells (spermatids) to Sertoli cells is not easily analysed by morphometric means because both cell types are irregularly shaped and, at the light microscope level, the cell boundaries are indistinct (Amann, 1981). Rolshoven (1941) and Wing & Christensen (1982) have used light microscope serial sectioning to
estimate this ratio for the rat. In this report we extend previous findings by using electron microscopy to visualize Sertoli cells and elongate germ cell boundaries. The resulting quantitative determinations for different mammals can be used in a relative sense to determine whether a greater germ cell–Sertoli cell ratio in a particular species leads to a higher sperm production per unit weight of seminiferous tubule parenchyma.

**Materials and Methods**

**Animals**

Sprague–Dawley rats (*Rattus norvegicus* weighing 275–325 g), gerbils (*Meriones unguiculatus*, weighing 70–76 g), vole (*Microtus ochrogaster*, weighing 50 g), rabbits (*Oryctolagus cuniculus*, weighing 4.4–6.6 kg), mice (*Mus musculus*, weighing 25–30 g), hamsters (*Mesocricetus auratus*, weighing 120–130 g), monkey (*Macaca fascicularis*, weighing 5 kg), and opossums (*Didelphis virginiana*, weighing about 9 kg) were used. All the animals were adult and, except the opossums, were housed in the SIU Vivarium and fed and watered *ad libitum*. Opossums were trapped locally in their natural habitat in Goreville, Illinois, during the breeding season and were killed soon after. The number of animals of each species is indicated in Table 1.

**Tissue preparation**

All animals except the monkey were killed by an intraperitoneal injection of pentobarbital sodium. The monkey was given phencyclidin hydrochloride, intramuscularly. For rats, perfusion fixation was accomplished by a retrograde abdominal aorta method (Vitale, Fawcett & Dym, 1973); for mice, vole, gerbils and hamsters, perfusion was through the heart; and for guinea-pigs, rabbits, monkey and opossums, the perfusion-fixation was through a needle inserted into the testicular artery, as it lay deep to the testicular capsule. In all of the aforementioned, the testicular vessels were first cleared with saline (9 g NaCl/l) and subsequently perfused with a 5% solution of glutaraldehyde, buffered with 0.15 M-sodium cacodylate (pH 7.4) for 30–45 min. Testes were diced into small (1 μm) blocks with a razor blade and these blocks were fixed with the same fixative for an additional 45 min. Subsequently, they were washed three times in buffer (10 min each wash), and post-fixed in an osmium : ferrocyanide solution (Russell & Burguet, 1977) for 1 h. Tissues were dehydrated in ascending concentrations of ethanol, infiltrated with propylene oxide and embedded in Araldite.

Thick sections (1 μm, toluidine blue-stained) were first examined under the light microscope and the blocks were trimmed according to the specific stage desired as well as the orientation of particular seminiferous tubules with respect to the block face (for rationale of orientation see below). Tissue sections showing silver–gold and gold interference colours were examined on a Philips 201 electron microscope. Staging criteria for each species studied were as follows: rats, Leblond & Clermont, (1952); mice, Oakberg (1956); guinea-pig, Clermont (1960); vole, Schuler & Gier (1976); hamsters, Clermont (1954); rabbits, Swierstra & Foote (1963); monkey, Clermont & Leblond (1959); and opossums, Orsi & Ferreira (1978). Gerbils were not staged, but elongate spermatid–Sertoli cell ratios were obtained from tubules in which elongate spermatid nuclei were condensed and these spermatids were deeply embedded in the Sertoli cells.

**Results**

To obtain the elongate spermatid–Sertoli cell ratio for the species described above, specific knowledge of the general shape, relationships and especially the configuration of the Sertoli cell at particular phases of spermatogenesis were required. Recent studies from this laboratory (Wong & Russell, 1983; Weber *et al.*, 1983; Russell & Karpas, 1983) have provided this information through
construction of a model of Sertoli cells in the rat and monkey. These studies depicted the Sertoli cells to be irregularly columnar and two basic, stage-dependent configurations of the Sertoli cell were demonstrated. The configuration designated Type A (Wong & Russell, 1983) was the predominant configuration and was characterized by deep recesses at the apical surface of the cell—the area which contained the elongate spermatids. In the Type B configuration, the Sertoli cell recesses were shallow and contained only the spermatid heads. This configuration was seen less frequently and observed only near the time of sperm release (Russell & Karpas, 1983). Since the relationship of the Sertoli cells to elongate spermatids was most extensive when Sertoli cells assumed the Type A configuration, this configuration proved to be the most valuable for the determination of the elongate spermatid–Sertoli cell ratio. Specific stages displaying the Type A configuration Sertoli cell were utilized for quantitation (see Table 1), for these showed the elongate spermatids deep within Sertoli recesses.

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<th>Table 1. Elongate spermatid–Sertoli cell ratios in various species</th>
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Plate 1, Fig. 1 shows an isolated cluster of rat spermatids as they are seen embedded in a single Type A Sertoli cell. The area in the figure between the two lines marks the region in which appropriate sections could be taken for a determination of the elongate spermatid–Sertoli cell ratio. Sections which were too low (nearer the basal surface of the Sertoli cell) did not always include all of the spermatid heads, because these heads were positioned at various distances from the basal lamina (Weber et al., 1983). Sections which were too high (near the apical surface of the Sertoli cell) often showed the elongate spermatid flagella related to both the cell of interest and a neighbouring Sertoli cell (Weber et al., 1983). Optimal sections were those that were oblique with respect to the long axis of the seminiferous tubule (Pl. 1, Fig. 2). As a result of these observations in the rat, criteria were devised for other species and were used to select appropriate sections and/or areas of the tubule. Sections near the rostral end (tip) of any one elongate spermatid of an embedded cluster were rejected (see Pl. 1, Fig. 1), since they may have missed some spermatids. Sections which
showed the flagellum of an elongate spermatid ambiguously positioned between two Sertoli cells (see Pl. 1, Fig. 1) were too high and it was difficult to determine which Sertoli cell contained the recess to the flagellum in question. For the rat, mouse and hamster the area between the lines comparable to that shown in Pl. 1, Fig. 1 was relatively extensive and clumps of spermatids embedded in a single Sertoli cell were frequently found in toluidine blue-stained sections (Pl. 1, Fig. 2) and in thin sections (Pl. 2, Figs 3 & 4).

Over 200 micrographs were originally taken, and from these 132 fitted the criteria described above. The data, as obtained in spermatid counts from these 132 electron micrographs, are provided in Table 1. Data were not obtained from the mouse or opossum, since the elongate spermatids in these animals were often very near the tubular lumen or were shared along their flagella by two or more Sertoli cells. Although recognized as desirable, equivalent numbers of counts for each species or even from animals of the same species, were not obtained. This was due to relative differences in the extent of acceptable sectioned areas (Pl. 1, Fig. 1) or the variation in the quality of section or unacceptability of section planes. The mean ratio of elongate spermatids to Sertoli cells differed little in animals of the same species (Table 1).

Discussion

The elaborate relationship of elongate spermatids, as they are embedded in a single Sertoli cell, permits us to determine the number of elongate spermatids within individual Sertoli cells. This type of study has not previously been undertaken at the electron microscope level. Using serial sections taken oblique to the long axis of the rat seminiferous tubule, Rolshoven (1981) determined, in an extensive quantitative study (1000 counts), that there was a bimodal distribution in the numbers of elongate spermatids per Sertoli cell with a mean (our estimate from their graph) of about 9-5–10 (range 6–12). Trans-illuminated tubules were used by Roosne-Runge (1955) to visualize the number of late spermatids per rat Sertoli cell. He found an average of 8-85 (range 6–12) elongate spermatids per Sertoli cell. Wing & Christensen (1982) used a light microscope serial sectioning technique similar to that of Rolshoven (1941) and found an average of 8.35 (range 5–11) elongate germ cells per rat Sertoli cell.

We believe the electron microscopic studies of the type undertaken herein are more credible, since the boundaries of the Sertoli and germ cells in question are visualized and not in doubt. The

PLATE 1

**Fig. 1.** Drawing, modified from Wong & Russell (1983), showing an isolated cluster of rat spermatids as they were positioned within a reconstructed Sertoli cell. The area between the lines is approximately that through which sections may pass to be judged acceptable for use in the present study. The upper boundary of the area (thin line) marks the area where some spermatids lose their exclusive relationship with one Sertoli cell and are related also to an adjoining Sertoli cell. The thick line indicates the area in which all spermatids heads are within the plane of section.

**Fig. 2.** Light micrograph of a toluidine blue-stained thick section showing an apparently appropriate area for use in electron microscopy. The section, taken oblique to the long axis of the seminiferous tubule, shows clusters of elongate spermatids, each cluster (outlined) apparently associated with a single Sertoli cell. × 1800.

PLATE 2

**Figs 3–4.** Electron micrographs showing clusters of elongate spermatids embedded in apical recesses of single Sertoli cells in the rat (Fig. 3) and monkey (Fig. 4). Each spermatid is numbered. The number of elongate spermatids in the Sertoli cell of the rat (14) exceeds the high end of the range reported by other authors (see 'Discussion'). Fig. 3, × 5600; Fig. 4, × 7200.
methodologies used by all investigators to obtain quantitative data assume that each Sertoli cell is intimately related to one or more elongate spermatids. This assumption is not necessarily true since some Sertoli cells may ‘rest’ for a spermatogenic cycle and take on elongate spermatids during the next cycle, although there is no evidence to substantiate this assumption. Our mean elongate germ cell–Sertoli cell ratio for the rat was 10-32:1 and is considerably higher than values reported by Roosen-Runge (1955) and Wing & Christensen (1982), and similar to that obtained by Rolshoven (1941). The determinations made in this study are a minimum since some spermatids may have been missed due to the plane of section. We believe that the specific strain of animals, their adult age and weight and nutritional status may have some influence on these ratios.

This study has extended previous studies by using a number of species for comparative purposes. Fawcett (1979) has stated that the number of spermatids per Sertoli cell is variable and very low in animals such as the ground squirrel, for which there may be 2–4 spermatids per Sertoli cell. We note that in several species the mean ratio of elongate spermatids to Sertoli cells appears very similar (9:75, vole; 10:10 guinea-pig; 10:64, gerbil; 10:32, rat; 10:75 hamster), yet in the monkey the mean number is markedly lower (5:94) and in the rabbit it is substantially higher (12:17). The means indicate an apparent flexibility amongst species in the numbers of elongate spermatids embedded in Sertoli cell recesses.

There is an impressive list of functions attributed to the Sertoli cell (Fawcett, 1975; Dym, Madhwa Raj & Chemes, 1977), and there is every indication that each Sertoli cell interacts with elongate spermatids in a way which facilitates or is essential for the process of spermatogenesis (Russell, 1980, 1983). Exceeding the upper limit of the elongate spermatid–Sertoli cell ratio may be detrimental to all of the germ cells within that particular Sertoli cell since the Sertoli cell could not effectively ‘handle’ all of the germinal elements and some or all would degenerate. Metabolites reaching the elongate spermatids would also be diluted because of the greater number of spermatids. The physical capacity of Sertoli cells to accommodate and move germ cells or develop necessary cell specializations might be exceeded. Degenerations of elongate spermatids in normal animals are rare, but are known to occur (Russell & Clermont, 1977). There are germ cell degenerations in the normal rat just before elongation (Stage VII) which would indicate that the number of spermatids about to attain an exclusive (or nearly exclusive) relationship with a single Sertoli cell is regulated at this point in spermatogenesis.

It would appear that the ‘efficiency’ of the Sertoli cell, for a particular species, could be measured, in part, by the mean number of spermatids within each Sertoli cell. Efficiency would be related to the daily sperm production or the sperm production per unit weight or volume of the testis or seminiferous tubule tissue. Several factors must be considered before any calculation of sperm production based on elongate germ cell–Sertoli cell ratios could be undertaken. For example, the size and volume of the monkey Sertoli cell (Russell & Karpas, 1983) is substantially less (4110 versus 6012 $\mu$m$^3$, respectively), than that of the rat (Wong & Russell, 1983). Therefore the ability to accommodate more germinal cells may be dependent on the size of the Sertoli cell and also to a certain degree on the size and/or shape of the elongate spermatids. Certainly, other factors such as the percentage area which is occupied by seminiferous tubules versus intertubular space or the duration of the cycle of the seminiferous epithelium would be a factor in these determinations. For example, the sperm production in man is similar to that of the rat, although there is a 10-fold difference in testicular mass (Johnson, Petty & Neaves, 1980). This difference was partly due to the higher proportion of area occupied by the intertubular and boundary tissue in men, but when comparisons of testicular (tubular) parenchyma in rats and men were made, the rat was about 7 times more efficient (Johnson et al., 1980). Micrographs in Figure 4 of Johnson et al. (1980) elegantly depict the relative abundance of spermatids and relative sparcity of Sertoli cells in the rat as compared with man. The elongate spermatid–Sertoli cell ratio is probably therefore the reason for the dramatic difference in sperm production in rat and man.

The number of the elongate spermatids in the testis is the product of the number of elongate spermatids per Sertoli cell and the number of Sertoli cells per testis. The sperm production rate is
the number of spermatozoa in the testis divided by the length of the spermatogenic cycle (Amann, 1970) which in the Sprague-Dawley rat is 12.9 days (Clermont & Harvey, 1965). Wing & Christensen (1982) have determined that there are 21.9 x 10^6 ± 1.70 (s.e.m.) x 10^6 Sertoli cells in an adult rat. Accordingly, we determine that approximately 235.6 x 10^6 elongate spermatids are present in one testis (471.2 x 10^6 spermatozoa per pair of testes). The daily sperm production rate per testis is calculated as 18.29 x 0.13 x 10^6 (36.58 x 2.6 x 10^6 for 2 testes). Using, in our calculations, the mean testis weight given by Wing & Christensen (1.40 g/testis), we would predict that 13.07 ± 0.9 x 10^6 spermatozoa/g testis would be produced. These data (and those from other laboratories) do not account for the small number of spermatozoa that are resorbed in the normal rat during spermiation (Russell & Clermont, 1977).

The results of the present study, specifically referring to the rat, indicate a slightly higher sperm production rate than that calculated by Wing & Christensen (13.42 x 10^6/testis; 9.61/g testis), yet a lower production rate than that cited by Johnson et al. (1980) for homogenization techniques (35.4 x 10^6 and 20.0 x 10^6, respectively), or for histometric techniques (26.7 x 10^6 and 15.03 respectively). Table 2 of Amann (1981) compares previously published data for spermatozoa/testis and sperm production/g parenchyma for several species. The most efficient producer shown is the rabbit. The monkey (rhesus) is less efficient, and the hamster and rat are intermediate on the scale. These data parallel our elongate spermatid-Sertoli cell ratio (although a time divisor was not utilized) data in a comparative sense, and fit our hypothesis that the elongate spermatid-Sertoli cell ratio is a basic determinant of spermatogenic efficiency.

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References


Oakberg, E.F. (1959) Initial depletion and subsequent recovery of spermatogonia of the mouse after 20 R of gamma rays and 100, 300 and 600 R of X-rays. Radiation Res. 11, 700-719.


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