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Estimation of daily sperm production in rats and monkeys

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Abstract. Daily sperm production in rats and monkeys were estimated by extended histometric method. Individual seminiferous tubules were separated, fixed, processed and embedded. Semi-thin sections were stained with toluidine blue and the different cell types quantitated with Chalkley's eye piece. The daily sperm production estimated was compared with that obtained by the homogenization method. The results indicate that daily sperm production obtained by both the methods were comparable. The present histometric method has an added advantage of requiring very small amount of biopsy material to give precise daily sperm production estimations.

Keywords. Testicular homogenization; histometry of testis; daily sperm production.

Introduction

The assessment of testicular function is an essential aspect of androgenic evaluation. A proper evaluation of spermatogenesis depends on a correlation between distinct morphological cell types and the kinetics of their division. This requires pulse labelling of germ cells in the premitotic and premeiotic stages with [^{3}H]-thymidine and subjecting testicular tissue to autoradiography. This is a tedious process. Nevertheless, the studies carried out on a limited number of species have clearly shown that the rate of spermatogenesis varies between species (Johnson *et al*., 1980a; Neaves, 1975; Amann and Lambiase, 1969; Amann *et al*., 1976; Berdson, 1977). Two alternative methods have been developed in recent years by Amann and co-workers. The simpler of the two methods is based on the enumeration of maturation phase spermatids present in testicular homogenates and to express the data in terms of the number of sperms produced per day by a gram of testicular parenchyma (Amann and Howards, 1980). The second method is based on histometric analysis of testicular biopsies where the different cell types are visually estimated in histological sections and data expressed as number of sperms produced by a unit g of testicular parenchyma per day (Amann 1970).

In the study reported here we have extended the histometric method of enumerating the different cell types in histological sections of individual tubules in contrast to that described by Amann (1970) where histological section of the entire testis parenchyma was used. The data obtained by histology was correlated with data obtained by the testicular homogenate method as described by Amann (1970). These studies have shown that there is a good correlation between the two methods.

Materials

Testis from 4 month old rats (Holtzman strain, n = 6) and adult bonnet monkeys (n = 6) were taken for the present study. The weight of the whole testes as well as weights of

Abbreviation used: DSP, Daily sperm production.

tunica albuginea and parenchyma were taken. In case of monkeys a small portion of the testicular tissue was taken for homogenization (Amann and Lambiase, 1969; Amann *et al*., 1976) and the remaining part for histometric analysis. In rats the entire left testis was taken for homogenization and the right for histometric analysis (Amann, 1970).

Estimation of daily sperm production by homogenization method

The testicular parenchyma was cut into small pieces placed in 0·25 mol/1 sucrose solution buffered to pH 7·5 with 0·02 mol/1 Tris (hydroxy methylamino methane) HCl and homogenized in a fluid containing 150 mM NaCl, 0·05% (v/v) Triton X-100 and 3·8 mM NaN_3 (Amann and Lambiase, 1969; Amann *et al.,* 1976). Round spermatids were counted using a haemocytometer and such evaluations were made in duplicate by two separate observers. The daily sperm production (DSP) was estimated by dividing the number of round spermatids by the product of weight of parenchyma and time divisor assigned to that particular species (Amann, 1970). The time divisor for rats was taken as 9·2 days (Johnson *et al*., 1980a) and for the bonnet monkeys it was taken as 4·37 days (Amann *et al*., 1976). Parenchyma volume was calculated by dividing parenchyma weight by assumed specific gravity of l·05g/ml (Johnson *et al.,* 1980a). The estimation was carried out using the formula given in table 1.

Table 1. Data used in estimation of DSP by homogenization method.

	Rat (n = 6)	Bonnet monkey (n = 6)
Wt. of testis (g)	1·640	25·976
Wt. of tunica albuginea (g)	0·108	0·712
Wt. of parenchyma (g)	1·533	24·732
Total No. of spermatids ($\times 10^6$)	270·8	487·5
Time divisor	9·2	4·37

$$\text{DSP} = \frac{\text{No. of round spermatids}}{\text{Wt. of parenchyma} \times \text{time divisor}}$$

Estimation of DSP by histometry

For histometric analysis the individual seminiferous tubules were separated under a transilluminated stereomicroscope. Seminiferous tubules measuring 1–2 mm in length were dissected out and fixed in modified Karnovsky's fluid (David *et al*., 1973). The tubules were further processed and embedded in Araldite and serially sectioned at 0·5 μ thickness. The sections were stained with toluidine blue and observed under bright field optics at × 400 or × 1000 magnification. A differential count of the cell types in sections in stage VII and XII at least 1000 different points in the seminiferous tubule were made using a Chalkley's eye piece in the microscope (Chalkley, 1943). The tubule diameter and the diameter of the spermatid nuclei were determined by taking the mean of 125 measurements per specimen with an occular micrometer. The DSP was calculated by the formula given in table 2. The results obtained by both the methods were compared.

Table 2. Data used in estimation of DSP by extended histometric method.

	Rat (n = 6)	Monkey (n = 6)
Wt. of parenchyma (g)	1·494	25·671
Parenchyma Vol. (mm^3)	1·3687	26·95
Diameter of round spermatid nuclei (um)	7·3089	3·762
Volume of round sp. nuclei (mm^3)	2·3192	2·7593
Time divisor	9·2	4·37

$$\text{DSP} = \frac{\text{Round spermatid (\%)} \times \text{volume of parenchyma}}{\text{Parenchyma weight} \times \text{volume of single round nuclei} \times \text{time divisor}}$$

Results and discussion

The results are summarized in tables 1 and 2. Sections of individual seminiferous tubules stained with toluidine blue from rat and monkey testis are shown in figure 1. The results show that some of the testicular parameters, such as testicular weight, weight of parenchyma etc. obtained in our study were comparable to those published by others (Johnson *et al.,* 1980a; Neaves, 1975).

Evaluation of spermatogenesis by enumeration of homogenization resistant spermatids revealed that for rats DSP/g parenchyma obtained was not considerably different from previous estimates reported using the same method (Johnson *et al*., 1980a; Amann *et al*., 1976; Robb *et al*., 1978). The value of $19{\cdot}7 \times 10^6$ sperms/g of testicular parenchyma per day is comparable to values reported by Amann *et al.* (1976). Furthermore, results of our studies indicate that DSP rate as determined by homogenization methods yielded slightly lower value as compared to histometric method (table 3).

Table 3. DSP in millions per g parenchyma by homogenization and histometric method.

	Method	
Species	Homogenization	Histometry
Rat	19·70	22·59
Monkey	4·34	4·752

The homogenization method is easy and practical. This method apart from failing to throw specific light on the different cell types and their correlation, requires large amount of tissue. The histometric method reported in the present study though time consuming, has the distinct advantage of requiring a very small bit of the tissue and it should therefore serve as the method of choice for studying spermatogenesis in small biopsy specimens. The sperm production is uniform in all regions of the testis and DSP/g parenchyma evaluated from small biopsy specimen did not yield inferior result as compared to the over all DSP/g value obtained for whole testis (Johnson *et al*., 1980b). The histometric method can be used to precisely estimate DSP in small bits of tissues weighing as low as 9 mg.

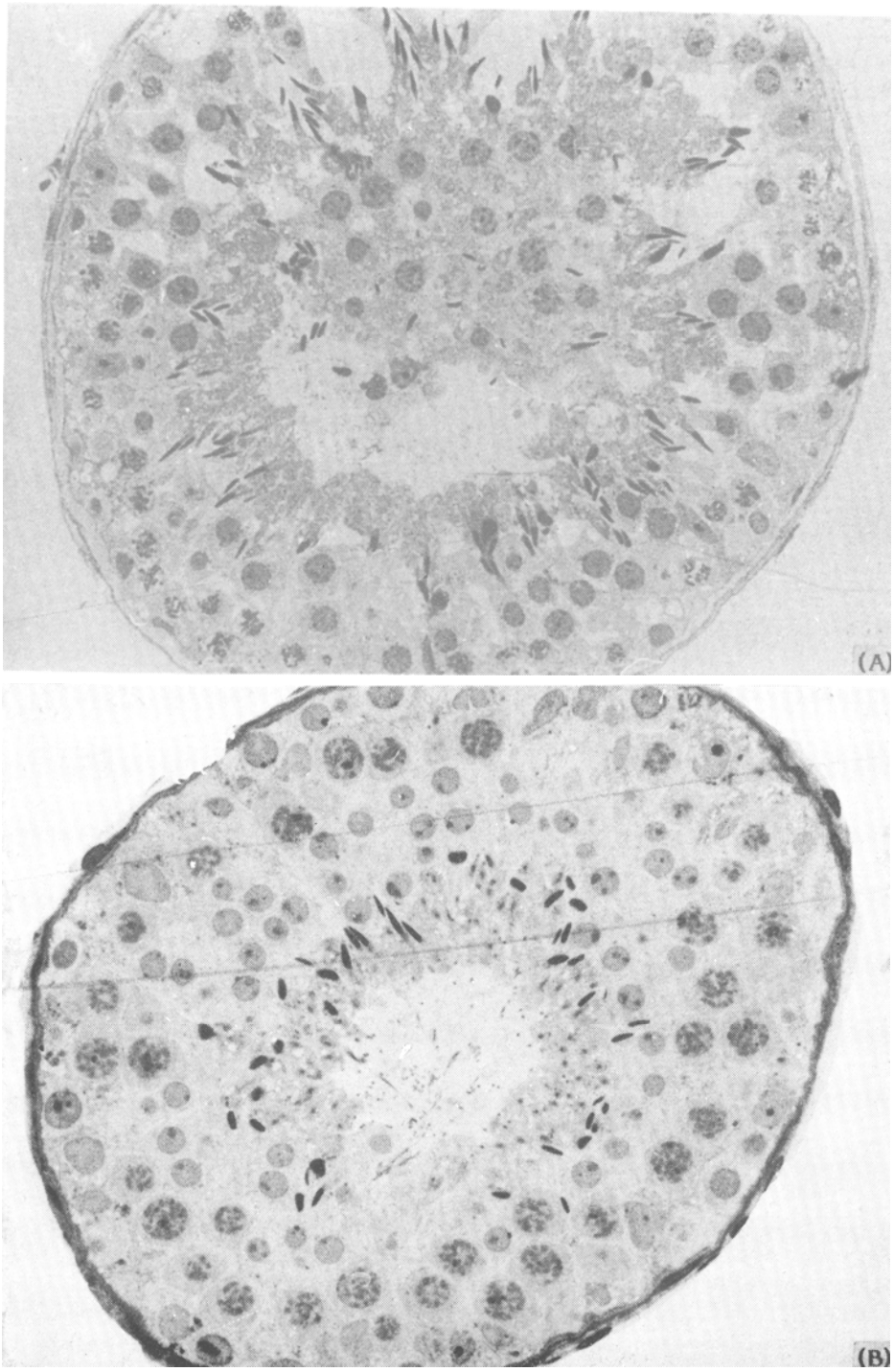

Figure 1. A. Semi-thin sections (0·5 μm) of rat seminiferous tubule in stage VII stained with toluidine blue showing nuclei of round spermatid and maturation phase spermatids (×400). **B.** Semi-thin sections (0·5 μm) of bonnet monkey seminiferous tubule in stage XII stained with toluidine blue showing nuclei of round spermatid and maturing spermatids (×400).

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