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GERMINAL CELL LOSS DURING HUMAN SPERMATOGENESIS

ARNOLD B. BARR, DONALD J. MOORE* AND C. ALVIN PAULSEN*

*U.S. Public Health Service Hospital, Staten Island, New York, Department of Anatomy, McGill University, Montreal, Quebec, and *Department of Medicine, University of Washington, Seattle, Washington*

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Summary. Testicular biopsy specimens from fifteen normal adult men were analysed histologically, using Clermont's classification for the germ cell types. After corrections were made for differences in nuclear size and life-span, spermatid/primary spermatocyte ratios ranging from 0·87 to 3·82 (average 2·58) were found. This represents a marked reduction from the theoretical ratio of 4·0 which would be expected. The significance of this attrition rate, or the point at which loss occurs, is not known.

INTRODUCTION

Clermont (1963) clearly demonstrated that human spermatogenesis is not a disorganized process and provided the basis for a rational understanding of the human germinal epithelium. He definitively described the various germ-cell types and showed that the germinal epithelium was organized into recognizable cellular associations or stages, which related to the process of germ-cell maturation. Later, Heller & Clermont (1964) determined the duration of spermatogenesis. Their data also defined the kinetics of individual germ-cell maturation, as well as of the spermatogenic stages.

With this information as a guide-line, the present study was instituted to determine whether or not the observed yield of spermatids from primary spermatocytes during normal human spermatogenesis coincided with the theoretical 4:1 ratio that would be expected.

MATERIALS AND METHODS

Eighteen testicular biopsy specimens were obtained from fifteen normal inmate volunteers. The means of ten or more control sperm counts for each individual ranged from 42 to 239 million spermatozoa/ml of ejaculate. Urinary follicle-stimulating hormone (FSH) excretion, determined by the method of Steelman & Pohley (1953), ranged from 2·9 to 26·3 i.u./24 hr for twelve individuals (in our laboratory the normal range is from 1·8 to 26 i.u./24 hr). In

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three individuals, general gonadotrophin (FSH+LH) levels varied from 1·2 to 3·8 mg-equiv. UPM-1/24 hr, which is within our normal range. (UPM-1 is the general gonadotrophin standard supplied by the Endocrine Study Section, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland.) The biopsies were performed using the technique described by Paulsen (1968), where the specimen is placed into either Cleland's or Zenker's formol fixative, embedded, cut and stained.

Germinal cells were identified and counted according to the classification of Clermont (1963). All cells in from ten to 100 seminiferous tubules per biopsy specimen were counted. In those specimens, where the smaller number of tubules was scored, ten normal-sized circular tubules were randomly selected for quantification from low-power photomicrographs. In other specimens, all circular tubules without artifacts were scored, up to a total of 100 per single cross-section. Seminiferous tubules regarded as exhibiting technical artifacts were discarded from further consideration.

Only the counts for primary spermatocytes and spermatids are presented here. Counts of the various types of spermatogonia are not included because there is not sufficient information to detail their relative life-spans.

TABLE 1

ABERCROMBIE CORRECTION FACTORS FOR VARIOUS GERM-CELL TYPES

Fixative	*Spermatocytes*				*Spermatids*					
	R	*L*	*Z*	*P*	*Sa*	*Sb₁*	*Sb₂*	*Sc*	*Sd₁*	*Sd₂*
Zenker-formol	0·50	0·40	0·39	0·37	0·50	(*	0·55)	0·67	0·71	0·71
Cleland	0·47	0·40	0·39	0·36	0·50	0·53	0·59	0·63	0·70	0·70
Combined groups	0·42			0·36	0·51			0·66		

* Under Zenker-formol fixation, Sb_1 spermatids cannot be easily distinguished from Sb_2 spermatids; therefore these two classifications were combined.

To correct for differences in nuclear size, at least twenty typical representatives of each type of spermatocyte and spermatid from both Cleland- and Zenker-formol-fixed material were measured to determine their average nuclear diameter. The diameters were measured in both axes in most cells; it was then assumed that these nuclei were perfect spheres. In the instance of Sc, Sd_1 and Sd_2 spermatids, measurements were based on their width only. This experimentally determined figure for each cell type was then used to calculate the correction factors for relative nuclear size using the formula of Abercrombie (1946):

$$TC = CC \times \frac{S}{S+D}$$

where TC = true count, CC = crude count, S = section thickness and D = nuclear diameter perpendicular to the plane of section of the tissue. This correction factor, when applied to the various cell classifications or groups, is shown in Table 1.

The life-span for primary spermatocytes has been estimated to be 23·5

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TABLE 2

COMPARISON OF CELL COUNTS BETWEEN ZENKER-FORMOL AND CLELAND-FIXED SEMINIFEROUS TUBULES BY OBSERVER A.B.

Fixative	*Specimen no.*	*Tubules counted*	*Sertoli cells*	*Primary spermatocytes*				*Spermatids*						*All spermatocytes combined*	*All spermatids combined*	*Corrected spermatid/spermatocyte ratio*
				R	*L*	*Z*	*P*	Sz	Sb_1	Sb_2	Sc	Sd_1	Sd_2			
A. Zenker-formol	152	99	1655	0·171	0·101	0·101	1·182	1·924	—*	0·622	0·522	1·030	0·295	1·553	4·303	2·92
	218	87	1543	0·035	0·098	0·296	0·691	0·808	—*	0·433	0·364	0·613	0·196	1·030	2·490	2·43
Average ratio																2·68
B. Cleland	153a	100	2177	0·183	0·173	0·131	0·923	1·71	0·073	0·395	0·306	1·309	0·159	1·410	3·913	2·97
	153b	20	305	0·151	0·295	0·109	0·733	1·35	0·188	0·332	0·353	0·813	0·126	1·206	3·297	2·90
	155b	20	304	0·226	0·112	0·159	1·249	1·69	0·152	0·479	0·406	0·356	0·235	1·746	3·728	2·26
Average ratio																2·83

Cell counts are expressed as 'cells per Sertoli cell nucleolus,' and corrected by Abercrombie's formula for relative nuclear size. Spermatid/spermatocyte ratio is corrected by relative life-span factor of 1·679.

* Under Zenker-formol fixation, Sb_1 spermatids cannot be easily distinguished from Sb_2 spermatids; therefore these two classifications were combined.

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days (Heller & Clermont, 1964) and that for spermatids, 22·2 days; therefore, a further correction factor of 23·5/22·2, or 1·059 has been inserted into the ratio calculations to adjust for this difference.

Tubules were scored by three different observers (D.M., M.C. and A.B.). Observers D.M. and M.C. grouped preleptotene (R), leptotene (L) and zygotene (Z) spermatocytes, while listing pachytene (P) spermatocytes separately; likewise Sa and Sb spermatids, and Sc, Sd_1 and Sd_2 were grouped in their scoring. Account was made of the differing nuclear diameters for each group by determining an 'average' correction factor based on the mean diameter of each cell type.

Observer A.B. expressed his counts as the specific cell type per Sertoli cell nucleolus; D.M. and M.C. expressed their counts as the number of a specific cell type per circular tubule. The counts for each type of spermatocyte, corrected by Abercrombie's formula, were then added together for each specimen; the same was done for the spermatids. The ratio of spermatids to spermatocytes, multiplied by the inverse ratio of their life-spans, was then calculated.

RESULTS

The corrected mean counts for the various cell types scored in each specimen are listed in Tables 2 and 3. Table 2 compares two different fixatives and Table 3

TABLE 3

COUNTS OF PRIMARY SPERMATOCYTES AND SPERMATIDS FROM CLELAND-FIXED MATERIAL

	Specimen no.	*All primary spermatocytes combined*	*All spermatids combined*	*Corrected spermatid/spermatocyte ratio*
A. Counts by Observer A.B. expressed as 'cells per Sertoli cell nucleolus'	84	0·872	3·141	3·82
	86	1·310	3·266	2·66
	135	0·831	2·422	3·10
	151	1·553	4·382	2·99
	153	1·308	3·615	2·92
	155	1·600	3·776	2·50
	158	1·122	2·214	2·09
Average ratio				2·87
B. Counts by Observers D.M. and M.C. expressed as 'cells per tubule'	84	19·21	61·42	3·39
	86	18·02	42·94	2·52
	133	18·06	50·76	2·98
	135	14·13	34·58	2·59
	151	19·29	47·72	2·61
	155	19·63	49·50	2·67
	158	16·08	32·90	2·17
	188	18·31	28·37	1·64
	192	20·58	45·07	2·32
	208	12·98	26·25	2·14
	217	17·78	60·77	3·62
	246	18·32	15·11	0·87
	304	18·26	46·57	2·70
	310	17·99	30·87	1·82
	372	18·91	48·72	2·73
Average ratio				2·45

Cell counts are corrected by Abercrombie's formula for relative nuclear size. The ratio is corrected by the relative life-span factor, 1·059.

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compares two methods for deriving the spermatid/spermatocyte ratio. There was considerable variability in the counts of individual cell types between specimens. Less variability was noted when total spermatocytes and total spermatids were compared, but the range was still considerable. These observations receive further comment later.

A.B. (Tables 2 and 3) used Sertoli cells as his reference point; D.M. and M.C. (Table 3) expressed their results as counts per tubule. Thus, while the total counts cannot be directly compared, the derived ratios are comparable between the two methods.

The most informative data are the ratios given in the last column of each table where, for each specimen, total primary spermatocytes are divided by total spermatids and multiplied by the correction factor for the relative life-spans of these cell types. A.B. (Table 3) found an average spermatid/spermatocyte ratio of 2·87 (range 2·09 to 3·82), while D.M. and M.C. obtained an average ratio of 2·45 (range 0·87 to 3·62) by the 'per tubule' technique.

The ratio of spermatids to primary spermatocytes (Sp/Psp) should theoretically be 4·0, as each primary spermatocyte divides twice before reaching the spermatid stage. However, in all specimens scored, the mean Sp/Psp ratios were less than 4. Indeed, in the majority of observations, eighteen out of twenty-two demonstrated Sp/Psp ratios of less than 3·0.

These data indicate that there is a variable, but significant, loss of potential spermatids during normal human spermatogenesis.

DISCUSSION

The human testis does not easily lend itself to statistical analysis; quantification in other mammals is simple by comparison. This is, in great part, due to the 'mixing' of several stages of the cycle of the seminiferous epithelium in any given tubular cross section (Clermont, 1963). Large standard deviations in the average counts of the various types of spermatogonia, spermatocytes and spermatids have been observed by us and by others (Tjioe, Steinberger & Paulsen, 1967; Steinberger & Tjioe, 1968), even when relatively large numbers of tubules were counted in a single biopsy specimen. The possible reasons for this variability and the statistical problems involved in the quantification of human spermatogenesis have recently been discussed by Steinberger & Tjioe (1968). Examination of our tables shows that the range of the observed data from specimen to specimen is wide. However, the consistent and important finding is that, in each specimen examined, significant numbers of potential spermatids are lost.

A similar quantification of spermatogenesis has been performed in the mouse (Oakberg, 1956), the rat (Clermont, 1962) and the bull (Ortavant, 1963). In these mammals, significant numbers of spermatocytes and spermatids degenerate, mostly during the reduction divisions. The present data show that large numbers of human germinal cells are similarly lost.

Confirmation of these data comes from two other sources. When human seminiferous tubules are prepared by a special 'whole-tubular mount' technique,

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degenerating spermatogonia, spermatocytes and spermatids can frequently be directly observed (Barr, 1967). Secondly, chromosomal abnormalities are seen in spermatocytes during meiotic metaphase and diakinesis in testicular biopsy specimens from males with normal spermatogenesis (Swersie & Paulsen, 1968). This suggests that some of the germinal cell loss occurs in the human during the reduction divisions. Neither the physiological basis nor the biological importance of this large loss of germinal cells in man and in other mammals is known at the present time.

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