Changes in the lipid inclusion/Sertoli cell cytoplasm area ratio during the cycle of the human seminiferous epithelium

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Summary. The area occupied by Sertoli cell lipid inclusions—electron-lucent lipid vacuoles (LLV) and electron-dense lipid droplets (DLD)—at each stage of the cycle of the seminiferous epithelium was measured on electron micrographs in young adults and elderly men, and expressed as the ratio “area occupied by lipid inclusions/area occupied by the Sertoli cell cytoplasm”. For LLV this ratio increased from stage I to stage III, and decreased from stage IV to stage VI in young adults. These results suggest that the development of LLV is synchronized with the spermatogenic process: the residual bodies released in stages I and II are phagocytized by Sertoli cells and transformed into LLV; the amounts of LLV decrease in the subsequent stages of the cycle and increase again when new residual bodies appear. In elderly men the ratio LLV/Sertoli cell cytoplasm was 1.9–2.9 times higher than in young adults at each stage of the cycle. This increase may be related to the increased germ-cell degeneration observed in ageing testes, DLD were less abundant than LLV and the DLD/Sertoli cell cytoplasm ratio did not undergo cyclic changes in young adults or elderly men.

Introduction

Sertoli cells have been reported to exhibit cyclic changes in follicle-stimulating hormone binding and action (Parvinen *et al.*, 1980), as well as in the secretion of androgen-binding protein (Ritzén *et al.*, 1982), and plasminogen activator (Fritz *et al.*, 1981). In addition, a number of light- and electron-microscopic studies have shown variations in the form and content of Sertoli cell organelles and inclusions during the cycle of the seminiferous epithelium in a variety of mammalian species (Fawcett, 1975). Recently, the cyclic accumulation and depletion of Sertoli cell lipid inclusions during the rat spermatogenic cycle, suggested by various reports (Lacy, 1960; Niemi & Kormano, 1965; Posalaki *et al.*, 1968; Kerr & de Kretser, 1975), have been corroborated by morphometric studies (Kerr *et al.*, 1984).

Human Sertoli cells display numerous droplets and lipid vacuoles, the latter belonging to the group of phagolysosomes (Holstein & Roosen-Runge, 1981; Schulze, 1984). Although no evidence has been presented for a correlation between the lipid content of Sertoli cells and the cycle of the seminiferous epithelium in man, according to the quantity and composition of lipid inclusions, four functional stages of the human Sertoli cell have been distinguished (Schulze, 1974).

The aim of the present report was to describe the variations observed in the surface area occupied by lipids in the human Sertoli cell during the cycle of the seminiferous epithelium and the possible relationship between these changes and the spermatogenic process.

Materials and Methods

Testicular biopsies were obtained about 2 h after death from both testes of 30 adult men who died in traffic accidents (19 men) or from other accidents (6 men) and had not suffered from testicular, endocrine or related diseases. The men were classified into two groups according to their age: 15 young adults (from 24 to 42 years of age), and 15 elderly men (from 61 to 89 years of age).

Small fragments (1 mm³) of each testicular specimen were fixed in 3% cacodylate-buffered glutaraldehyde, post-fixed in 1% veronal-buffered osmium tetroxide, and embedded in araldite. Semi-thin sections were stained with toluidine blue. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined with a Philips-300 electron microscope.

For the quantitative study, 20 electron microscopic fields (4200 μm^2) from each of the 6 stages of the cycle were selected at random in each testis, photographed at $\times 2000$, and examined at a final magnification of $\times 10\,000$. On each electron micrograph the areas occupied by the two types (electron lucent and electron dense) of lipid inclusions and by the Sertoli cell cytoplasm were measured with a semi-automatic image analyser (Kontron, Zeiss, Oberkochen, FRG). The results were expressed as the ratio "area occupied by lipid inclusions/area occupied by the Sertoli cell cytoplasm". For each stage of the cycle the average values per testis were calculated. From these values the means and standard errors of the mean were obtained. The significance of differences between means was evaluated by analysis of variance.

In the young adults and elderly men, only seminiferous tubules showing qualitatively normal (complete) spermatogenesis were photographed (Figs 1–6). In elderly men, the tubules selected were similar to those of young adults except for more abundant lipid inclusions, a lower number of spermatids, and a slightly reduced tubular diameter; many of the tubules not selected showed different degrees of maturation arrest of spermatogenesis at the level of spermatids, spermatocytes or spermatogonia, a thickened tunica propria, a reduced tubular diameter, and larger amounts of lipid inclusions than the tubules with complete spermatogenesis. The reason for excluding measurements of the tubules with maturation arrest was the absence of a recognizable cycle of the seminiferous epithelium.

Results

Two types of lipid inclusions could be distinguished in the cytoplasm of most Sertoli cells (Figs 1–6): (1) small electron-dense lipid droplets (DLD), and (2) large electron-lucent lipid vacuoles (LLV) (Fig. 3). The latter were the most abundant (Figs 1 & 2, 4 & 5) and, in some Sertoli cells principally in elderly men they tended to coalesce, forming large accumulations (Figs 5 & 6). Residual bodies and secondary lysosomes suggesting intermediate stages between residual bodies and LLV were seen (Fig. 3), mainly in stages VI, I and II of the cycle.

The changes throughout the cycle in the LLV/Sertoli cell cytoplasm ratio are shown in Table 1. Differences between samples from the same testis were lower than between testes. In young adults this ratio increased significantly from stage I to stage III, and then declined significantly from stage IV to stage VI. In elderly men the LLV/Sertoli cell cytoplasm ratio was higher than in young adults in all stages of the cycle; this ratio increased significantly from stage II to stage III, thereafter declining significantly from stage IV to stage VI, and remained constant throughout stages VI–II.

Fig. 1. Seminiferous tubule from a young adult man (27 years of age) showing complete spermatogenesis and a moderate amount of lipid inclusions (li). Toluidine blue, $\times 450$.

Fig. 2. Stage III of the cycle in a 26-year-old man. Pachytene spermatocytes (P) and Sa spermatids (Sa) can be seen. The Sertoli cells (Se) show numerous lipid inclusions (Li). $\times 3000$.

Fig. 3. Part of a Sertoli cell in another seminiferous tubule (stage II) from the same man as in Fig. 2, showing two types of lipid inclusions: small electron-dense lipid droplets (DLD) and larger electron-lucent lipid vacuoles (LLV). A secondary lysosome (asterisk) suggesting an intermediate stage between a residual body and an electron-lucent lipid vacuole is seen. $\times 14\,000$.

Table 1. Ratio of area occupied by lipid inclusions/area occupied by the Sertoli cell cytoplasm ($\times 10^4$) during the 6 stages of the cycle of the human seminiferous epithelium in young adults and elderly men

Lipid inclusions	Stages of the cycle					
	I	II	III	IV	V	VI
Electron-lucent lipid vacuoles (LLV)						
Young adults	236 $\pm$ 11 ^a	365 $\pm$ 15 ^b	612 $\pm$ 25 ^c	543 $\pm$ 21 ^c	395 $\pm$ 19 ^b	318 $\pm$ 15 ^{ab}
Elderly men	684 $\pm$ 37 ^{cd}	672 $\pm$ 38 ^{cd}	1227 $\pm$ 72 ^{ef}	1062 $\pm$ 59 ^e	849 $\pm$ 47 ^{de}	661 $\pm$ 36 ^{cd}
Electron-dense lipid droplets (DDL)						
Young adults	43 $\pm$ 3 ^g	50 $\pm$ 4 ^g	40 $\pm$ 3 ^g	45 $\pm$ 4 ^g	41 $\pm$ 3 ^g	47 $\pm$ 4 ^g
Elderly men	85 $\pm$ 7 ^h	71 $\pm$ 6 ^h	76 $\pm$ 6 ^h	80 $\pm$ 8 ^h	85 $\pm$ 8 ^h	75 $\pm$ 7 ^h

Values are expressed as means $\pm$ s.e.m.

Values with different superscript letters are significantly different ($P < 0.05$).

The DLD/Sertoli cell cytoplasm ratio throughout the 6 stages of the cycle in young adults is shown in Table 1. Although the values in elderly men differed significantly ($P < 0.05$) from those in young adults in each stage of the cycle, the DLD/Sertoli cell cytoplasm ratio did not undergo any significant changes during the cycle in young or elderly men.

Discussion

The two types of Sertoli cell lipids presumably have a different function. The most abundant lipids (LLV) are contained in electron-lucent vacuoles which are defined by a membrane; they have been considered as telolysosomes derived from both phagocytosed germ cells and residual bodies released from spermatids during spermiogenesis (Schulze, 1984). This notion arises from the observation that the number of lipid vacuoles increases considerably following iatrogenic damage to the germinal epithelium causing germ cell degeneration (Schulze, 1984), whereas lipid vacuoles are inconspicuous in seminiferous tubules with germ cell hypoplasia, such as in Del Castillo's syndrome (Chemes *et al.*, 1979; de Kretser *et al.*, 1981), Klinefelter's syndrome (Nistal *et al.*, 1982), cryptorchidism (Schulze *et al.*, 1976), and hypogonadotrophic hypogonadism (de Kretser, 1968). The small electron-dense lipids (DDL) are not surrounded by a membrane and correspond to the usual lipid droplets found in most cell types; if the Sertoli cell synthesizes steroid hormones, as has been postulated (Lacy, 1962; Fawcett, 1975), these lipids might represent steroid precursors.

The results of this study show that the LLV/Sertoli cell cytoplasm ratio undergoes cyclic changes synchronized with the cycle of the seminiferous epithelium, reaching a peak at stage III. This agrees with the origin attributed to these vacuoles (telolysosomes) and suggests that the

Fig. 4. Seminiferous tubule from an elderly man (77 years of age) showing complete spermatogenesis and numerous lipid inclusions (li). Toluidine blue, $\times 450$.

Fig. 5. Stage IV of the cycle in a 74-year-old man. One binucleate A spermatogonium (A), and one zygotene spermatocyte (Z) can be seen, together with Sertoli cells (Se) filled by electron-lucent lipid vacuoles (LLV). $\times 3000$.

Fig. 6. Sertoli cell from the same testis as in Fig. 5, showing coalescence of electron-lucent lipid vacuoles (LLV). DLD: electron-dense lipid droplets. $\times 21\,000$.

residual bodies, formed during the elongation and complete differentiation of spermatids at stages VI to I of the cycle (Clermont, 1963; Holstein & Roosen-Runge, 1981) and phagocytosed by Sertoli cells (Fawcett, 1975; Russell, 1980; Holstein & Roosen-Runge, 1981), are transformed into LLV during stages I through III. These lipids decrease in the subsequent stages of the cycle and increase again when new residual bodies appear. These cyclic changes in lipid content are similar to those reported in the rat seminiferous tubules which show a maximum lipid content during stages IX–XIV of their cycle (Kerr *et al.*, 1984), following the release of spermatid residual bodies and the increase in secondary lysosomes in the Sertoli cells (Morales *et al.*, 1986).

The increase in LLV beginning at stage I in humans also agrees with the loss of late prophase–anaphase II spermatocytes reported in the human testis, even in young adults (Johnson *et al.*, 1983). This loss occurs at stage VI when pachytene spermatocytes give rise to round spermatids; about 43% of these spermatocytes undergo degeneration (Johnson *et al.*, 1983) and are probably phagocytosed by Sertoli cells and transformed into LLV. Abundant residual bodies have been observed at this stage.

The LLV/Sertoli cell cytoplasm ratio in ageing testes undergoes cyclic changes similar to those observed in young adults, although this ratio was about 1.9–2.1 times that measured in young adult testes at each stage of the cycle except for stage I in which this ratio was 2.9 times higher than in stage I in young adults. An augmented Sertoli-cell lipid content in ageing human seminiferous tubules has been reported previously (Lynch & Scott, 1950; Paniagua *et al.*, 1985) and may be attributed to the elevated number of degenerating germ cells observed in ageing testes (Paniagua *et al.*, 1987). The disproportionate increase in lipids observed at stage I in elderly men might be due to an increased loss of spermatocytes at stage VI of the cycle (unpublished observations).

In contrast with LLV no cyclic changes in the DLD/Sertoli cell cytoplasm ratio were observed. If these lipid are used for steroid biosynthesis (Lacy, 1962), such synthesis seems to be unrelated to the stage of the cycle. DLD are also increased in elderly men. This has two possible explanations: (1) DLD are derived from lipids contained in the LLV, and therefore an increase in LLV would be associated with an increase in DLD; and (2) steroid biosynthesis is decreased in ageing Sertoli cells, leading to an accumulation of unused precursors.

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