**Доклади на Българската академия на науките**
**Comptes rendus de l'Académie bulgare des Sciences**

*Tome 66, No 7, 2013*

*MEDECINE*
*Morphologie*

# MORPHOLOGICAL CHANGES IN RAT LEYDIG CELLS REFLECTING THE DECREASED TESTICULAR STEROIDOGENIC CAPACITY DURING AGING

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(*Submitted by Academician P. Vassileva on March 22, 2013*)

**Abstract**

The Leydig cells situated in the interstitial compartment of the mammalian testis are responsible for most of the testosterone produced in males. Previous studies in rats have shown that the ability of Leydig cells to produce testosterone significantly declines with age. The present study was focused on the description of some morphological alterations in rat Leydig cells that could be associated with the decreased testicular steroidogenic capacity during aging. Ultrastructural study of aging rat testes revealed the presence of Leydig cells with intact morphology as well as Leydig cells with different degree of degeneration. The decrease of both smooth endoplasmic reticulum and mitochondria, together with an accumulation of lipid droplets and residual bodies in aging Leydig cells, were observed. Our electron microscopical analysis revealed that the majority of the mitochondria in aged Leydig cells (24-month-old) exhibited significant features of degeneration: increasing in size, swelling, lighten of the matrix, reduction of cristae number, and alterations in the structural integrity of the membranes. During aging progressive increase was established in the number of Leydig cells exhibiting nuclear changes such as chromatin compaction and fragmentation and nuclear shrinkage that could be a sign of elevated apoptotic tendency. The observed morphological alterations in rat Leydig cells reflect the decreased testicular steroidogenic capacity during aging, resulting in decrease of testosterone production.

**Key words:** rat, Leydig cells, electron microscopy, aging

**Introduction.** The Leydig cells (LCs) situated in the interstitial compartment of the mammalian testis are responsible for most of the testosterone (T) produced in males. Previous studies in rats have shown that the ability of LCs to produce T significantly declines with age. The reduced ability of aging LCs to produce T might be caused by extrinsic factors, intrinsic factors or both involving accumulation of reactive oxygen species (ROS) as by product of steroidogenesis [1]. It has been reported that the T levels are reduced with aging but the

numbers of the LCs per testis do not change, suggesting that there must be deficit in the capacity of individual aging LCs to produce T [2, 3]. It is now well established that there is a decline of mammalian testicular spermatogenic and endocrine function with age [4]. The present study was focused on the description of some morphological alterations in rat LCs that could be associated with the decreased testicular steroidogenic capacity during aging.

**Material and methods.** Male Lewis rats at different ages (3, 18 and 24 months, weight 200–330 g) were used in the present study ($n = 6$). Testicular fragments approximately 4–5 mm thick were fixed by immersion in Bouin's fluid for 24 h, embedded in paraffin and prepared for routine histology (staining with haematoxylin-eosin). For electron microscopy testicular fragments were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetraoxide and embedded in Durcopan. Ultrathin sections were observed on EM Opton 109 and microphotographs were made.

All experimental procedures were approved by the Ethical Committee of the Medical University of Plovdiv.

**Results.** Our routine histological analysis demonstrated atrophy of LCs with aging rather than reduction in their number and as a consequence the interstitium of aging testes appeared enlarged (data not shown). At electron microscopical level, typical adult LCs contained abundant cell organelles such as mitochondria with tubular cristae, predominant smooth endoplasmic reticulum, Golgi apparatus and few lipid droplets as cellular inclusions (Fig. 1A, B). Ultrastructural study of LCs in aging rat testis revealed the presence of LCs with intact morphology as well as LCs with different degree of degeneration. The decrease of both smooth endoplasmic reticulum and mitochondria, together with an accumulation of lipid droplets and residual bodies in aging LCs, were observed (Fig. 1C). Our electronmicroscopical analysis revealed that the majority of the mitochondria in aged LCs (24-month-old) exhibited significant features of degeneration: increasing in size, swelling, lighten of the matrix, reduction of cristae number, and alterations in the structural integrity of the membranes. During aging progressive increase was established in the number of LCs exhibiting nuclear changes such as chromatin compaction and fragmentation and nuclear shrinkage that could be a sign of elevated apoptotic tendency (Fig. 1D).

**Discussion.** Our results demonstrated atrophy of LCs during aging rather than reduction in their number. The present data are consistent with previous studies in rat, regarding the effects of aging on LCs structure and functional activity, and suggest that the age-associated diminished T production is related to defects in the steroidogenic pathway [2, 3]. The reduced size of LCs population, observed at light microscopical level, reflects ultrastructural changes in the cytoplasmic organelles and nucleus and these alterations are indicative of decreased steroidogenic capacity of aging LCs. Our ultrastructural observations revealed marked reduction with aging in the major cytoplasmic organells such

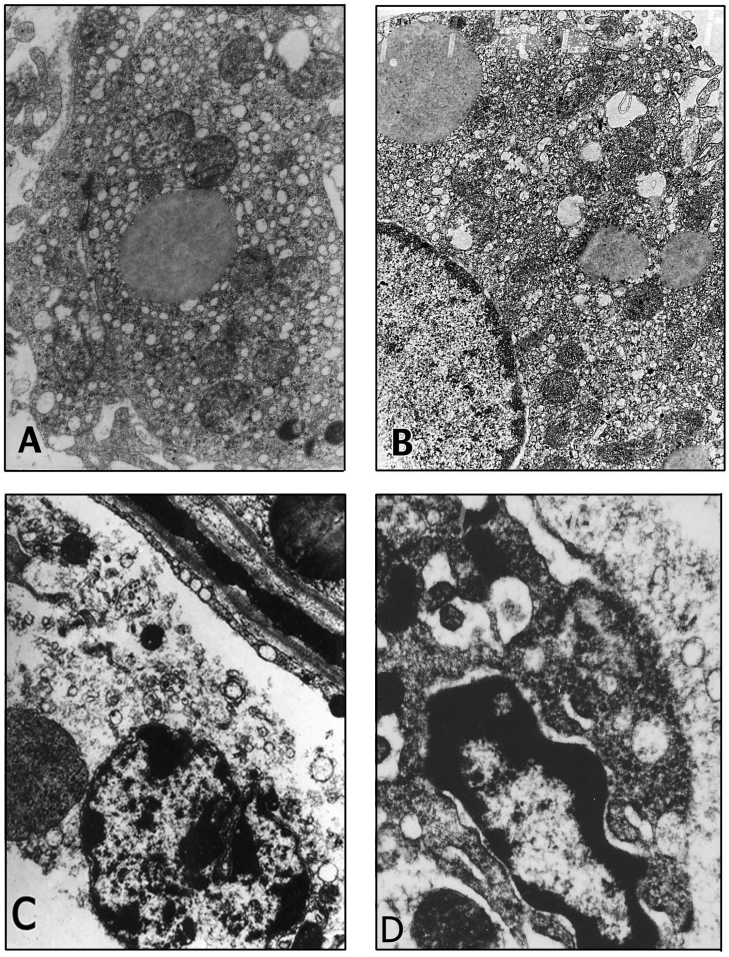


Fig. 1. Electron micrographs: 3-month-old rat controls (A, B) ×12000 – adult type Leydig cell with abundant mitochondria, well-developed smooth emdoplasmic reticulum and few lipid droplets; 18-month-old rat (C) ×4500 – decrease of both smooth endoplasmic reticulum and mitochondria in aging Leydig cell were observed; 24 months of age (D) ×10000 – aged Leydig cell with mitochondria that exhibited significant features of degeneration, apoptotic nuclear changes such as chromatin compaction and fragmentation and diminished cytoplasmic volume

as smooth endoplasmic reticulum, mitochondria and Golgi apparatus. Based on the reported positive correlations between T secretion by LCs and the amount of their smooth endoplasmic reticulum and mitochondria [5, 6], the observed ultrastructural changes in LCs are in accordance with previous results [7, 8] and could be considered as the primary cause for impaired steroidogenesis during aging. According to the free radical theory, the mitochondria might be the biological clock in aging [9]. There is evidence that ROS are produced in LCs both by the mitochondrial electron transport chain as in the other cells and additionally by the steroidogenic pathway [8, 10]. Koeva et al. [11] reported for an alteration

in the balance between pro- and anti-apoptotic Bcl-2-related proteins in favour of pro-apoptotic protein Bax in aged LCs suggesting the consequential triggering of a mitochondrial-dependent pathway of apoptosis. Moreover, previous data revealed an age-related down-regulation of a number of genes that scavenge and/or repair ROS-induced damage in aged LCs [12]. In our study, we found a significant age-related mitochondrial degeneration in LCs and these findings can be interpreted according to the concept of a mitochondrial-dependent programming death observed during aging [13]. In accordance with the above-mentioned data, the progressive alterations in LCs nuclei found in the present study could be a possible sign of their increased apoptotic tendency during aging.

In conclusion, the observed morphological alterations in rat LCs reflect the reduced testicular steroidogenic capacity during aging, resulting in decrease of T production.

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