

## Apoptosis, Degeneration and Regeneration in Seminiferous Epithelium of Adult Rats after Treatment with EDS

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We aimed to carry out a detailed quantitative analysis of male germ cell apoptosis in seminiferous epithelium in a long period after EDS administration. The apoptosis in adult rat testes was induced by EDS and was assessed by TUNEL method. First signs of seminiferous epithelium regression were manifested by marked increase in number of apoptotic cells on 3<sup>rd</sup> day after EDS treatment. The maximal value of germ cell apoptosis was established on 7<sup>th</sup> day post EDS that coincided with lowest T levels. Quantitative patterns of germ cell death after testosterone deprivation reveal in advance the kinetic of germ cell depletion and regeneration in a long period after EDS.

*Key words:* EDS, apoptosis, spermatogenesis, rat.

### Introduction

Apoptosis is considered as a mechanism by which the testicular germ cells are removed during normal and various pathological conditions [3]. Manipulation of spermatogenesis by deprivation of survival factors provides a basis for detailed study on the regulatory mechanisms of germ cell death. Male germ cell apoptosis in response of androgen withdrawal due to elimination of Leydig cells (LCs) by ethane dimethanesulfonate (EDS) was studied in detail in a short time-window post EDS when LCs were completely missing from the testis [2, 5, 9]. Kinetics of apoptotic events was not examined in a long period after EDS treatment when new LC population developed in the testis. In this respect we aimed to study kinetics of male germ cell apoptosis in seminiferous epithelium after EDS administration and to extend our previous results on kinetics of germ cell death in the testis [1].

## Material and Methods

The apoptosis in adult rat testes was induced by a single, i.p. injection of EDS at a dose of 75 mg/kg body weight. The TUNEL assay for in situ detection of apoptosis [6] and quantitation of apoptotic germ cells [9] were performed on testicular sections on 1, 3, 7 14, 21 and 35 day after EDS.

## Results

First signs of seminiferous epithelium regression were manifested by marked increase in frequency of apoptotic germ cells in comparison with control rat testis (Fig. 1). Germ cell apoptosis was accompanied with depletion of elongating spermatids from the seminiferous tubules first in late and then in early stages of the spermatogenic cycle. The germ cells undergoing apoptosis were mostly pachytene spermatocytes and round spermatids in stages I—VIII. Three day after EDS the germ cell apoptotic index was dramatically increased and 7 days post EDS the parameter was 10-fold higher than control, afterwards decreased but remained still significantly higher compared to control (Fig. 2). The highest values of germ cell apoptosis found by 7 day after EDS corresponded to the lowest plasma level of testosterone (T) ( $< 0.1$  ng/ml in comparison to  $2.14 \pm 0.39$  ng/ml in the control). Elevated germ cell apoptosis decreased after 14 days EDS that coincided with significant rise of T levels on day 14<sup>th</sup> ( $0.51 \pm 0.25$  ng/ml) and 21<sup>th</sup> ( $1.27 \pm 0.3$  ng/ml) even they remained lower than controls.

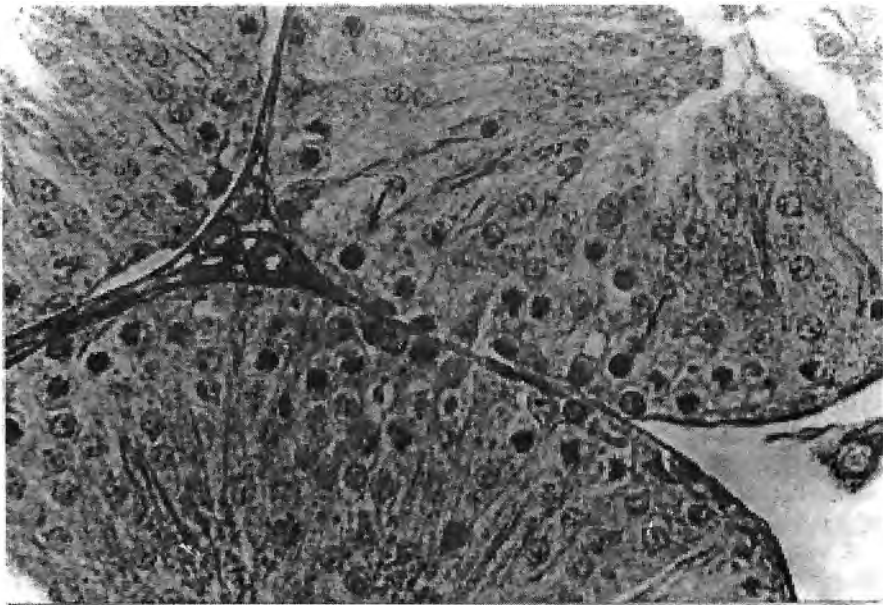


Fig. 1. Testicular cross section of rat at 7<sup>th</sup> day after EDS administration. Apoptotic germ cells — spermatocytes (arrows) are seen in seminiferous tubules in early stages of the cycle ( $\times 400$ )

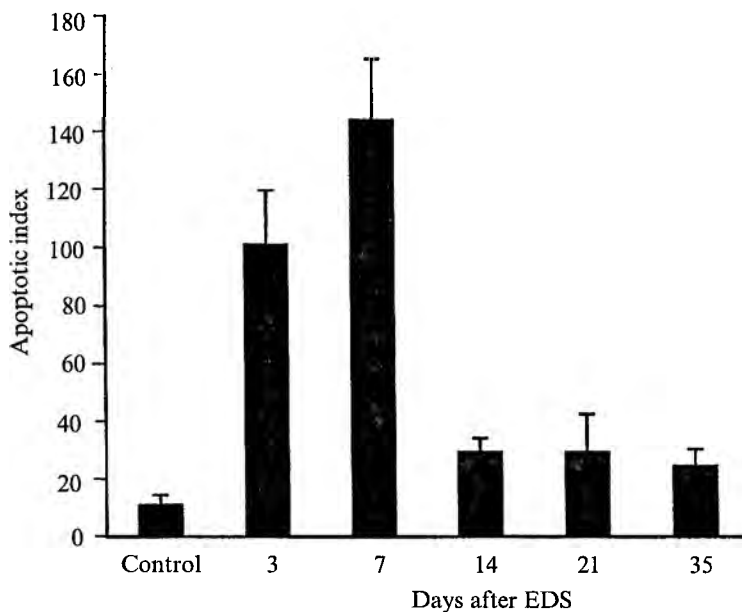


Fig. 2. Temporal changes in apoptotic index following EDS administration. The values of apoptotic index were calculated by multiplying the percentage of tubules containing apoptotic cells by the number of apoptotic germ cells per tubule. Each point represents the mean  $\pm$  SD ( $n=4$ ),  $p<0.001$

## Discussion

Our observation in the present study showed that germ cell death caused by testosterone withdrawal in adult EDS treated rats is mediated by apoptosis. We have demonstrated by detailed quantitative analysis profound time-dependent increase in germ cell apoptosis in seminiferous epithelium after testosterone deprivation by EDS administration. The highest values of germ cell apoptosis we established by day 7th after EDS coincided with the lowest plasma testosterone levels and complete absence of Leydig cells. The induced germ cell death could be interpreted as an “echo” of preceding Leydig cell apoptosis latter documented by Morris et al. [4]. A growing body of evidence has demonstrated that the withdrawal of androgens in adult rats results in the acceleration of germ cell apoptosis at specific stages of the spermatogenic cycle [2, 7]. Our data showed that predominant germ cell types undergoing apoptosis as a result of androgen ablation include pachytene spermatocytes and round spermatids in early (I–VI) and middle stages (VII–VIII) from the spermatogenic cycle. The preferential cell death was reported to occur in androgen dependent stages VII–VIII after testosterone withdrawal [5]. The similar relationship between germ cell apoptosis and T deprivation was reported in adult rats treated with GnRH-antagonist that were also deficient in androgens [8].

Quantitative analysis of time and cell specificity of germ cell apoptosis in the present study develops our previous data [1] that testosterone withdrawal caused stage-dependent loss of haploid germ cells (spermatids) due to differential sensitivity of different germ cell populations. The time-dependent changes in germ cell

apoptosis after EDS administration, we found in the present study, precedes the specific total loss of elongating spermatids and disappearance of pachytene spermatocytes from the seminiferous epithelium. In conclusion our results indicate that quantitative pattern of germ cell death after testosterone deprivation reveal in advance the kinetic of germ cell depletion and regeneration in a long period after EDS. These new findings bring additional support to the concept that germ cell apoptosis is a hormonally regulated process.

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