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The influence of hexestrol diacetate on gametogene function in male rabbit

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Abstract

The research was performed on 24 male sexually mature domestic rabbits, divided in two equal batches. The rabbits from the first batch were administered 0.5 mg/kg of body weight hexestrol diacetate intramuscularly twice a week for four consecutive weeks. Batch 2 was used for control. The testicular tissue samples obtained after orchidectomy were processed in order to obtain histological samples, stained using the Goldner's trichrome method. Examination of histological sections from the control batch showed that a natural aspect of seminal tubule epithelium, without reported injuries, even discrete. In the experimental batch were recorded a number of changes to all categories of the seminal cells. The severity and extent of damages varied greatly from one seminal tube to another and even from one part to another of the same seminal tube. These changes were the appearance of apoptotic cells and apoptotic bodies, vacuolar degenerescence of spermatocytes and spermatides, syncitialisation of spermatides, areas of necrosis accompanied by severe disruption of the seminiferous epithelium. In some areas, the lesions were so severe that the affected area of the cells forms "basal area". If lesions in the "adluminal area" affects only temporarily gametogenetic function the lesions in the "basal area" are irreversible as there are named "reserve cells" which is the starting point of spermatogenesis. Highlighted issue raised on the opportunity use of hexestrol diacetate in therapy or animal production stimulation as it gametogenetic function in males while their risk transfer to humans through consumption of foods of animal origin.

Keywords: gametogenesis, hexestrol diacetate, histopathology, spermatogony.

Introduction

The male hormones and neuro-chemical signals transmitted information at the hypothalamic level, the ante-pituitary, interstitial Leydig cells, Sertoli cells and germinal epithelium semen.

The fertility and fecundity can be, at list theoretic-cally, inhibited by any level of impaired male genitalia [1]. Theoretically, to detect changes in testicular funct-ion in the studies, males take the samples to be assessed at an interval of at least six times during a cycle of the seminal epithelium: 53 days in mice, 52 rats, 64 male rabbits, 81 dogs, 57 in human males and 96 days in a species of primates Rhesus, for a group of germ cells to progress from stage spermatogony A_1 to a differentiated sperm cell [2]. The cells most susceptible to the action of toxic agents are in the process of mitosis or DNA synthesis, collectively known as proliferative spermato-gonial or preleptotene primary spermatogonial [3]. There are exceptions: thus affect RNA synthesis inhibitors categories of germ cells at an advanced stage of differentiation [4]. The most resistant cells to the action of toxic agents are considered to be B_1 sperma-togony, also called "reserve" spermatogonial.

This study aims to objectively evaluate the effects of a synthetic compound called a "estrogen-like" and which is legally a derivative of biochemical hexestrol (hexestrol diacetate) on the cyto-architectonics of the male rabbit gametogene function and dynamics, as species belonging to the group of experimental animals approved legally and scientifically point of view by the existing law of the European Community.

Materials and Methods

The research was conducted on a total of 24 domestic rabbits, male sex, sexually mature aged between 10 and 12 months, with a weight between 2500 and 3000 g, the common breed. Rabbits used in this experiment were divided into two groups of 12 subjects each. Rabbits of the experimental batch received the intramuscular dose hexestrol diacetate 0.5 mg/kg, two times per week for four consecutive weeks, while rabbits in batch 2 were considered the control group. At the end of four weeks, rabbits from these two groups were subjected to bilateral orchidectomy for testicular tissue sampling for histological preparations to carry out necessary investigation to assess the morphological effects of the hexestrol diacetate tested in this study.

Samples thus collected were cross-sectioned into slices of 5 mm, which were introduced Stieve fixing solution for 24 hours. Then the pieces were washed and dehydrated with increasing alcohol concentration (70^0, 95^0, absolute), clarified butyl alcohol (*n*-butanol) and

included in paraffin. They were charged with a thickness of 5 µm sections, and for contrasting sections Goldner trichrome stain. The examination of histological samples was made with an Olympus BX 41 microscope.

Results

Following histological examination of preparations obtained from animals of experimental batch found the presence of lesions in various cell types, but their intensity is different from one seminal tube to another. In the case of seminal tubes, the changes are more subtle, vacuolar degenerescence evidenced by a moderate number of cells (spermatogonial and spermatocytes), intercellular edema greater in some areas, few apoptotic cells present, apoptotic bodies in particular "adluminal area" of seminal tract (Figure 1).

There are some segments of seminiferous tubes, despite of vacuolar degenerescence of cells is given and the number of apoptotic bodies, relatively large, there are numerous sperm cells attach to the seminiferous epithelium, but the vast majority of them have swollen heads and low tinctorial affinity, compared with normal sperm cells, this aspect suggests that those sperm cells are affected by vacuolar degenerescence (Figure 2).

A particular aspect is the appearance of sincitial spermatidis formed on account which, in some cases keep in touch with a somewhat stronger edematiate appearing seminiferous epithelium in their immediate vicinity (Figure 3), and the lesions are more pronounced where there syncytial spermatidis of apoptosis in cellular debris contained in the existing mass in the lumen of the tubule (Figure 4).

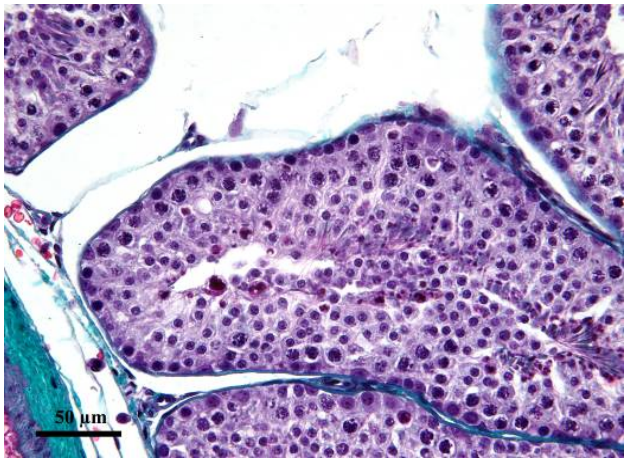

Figure 1 – ***Apoptotic cells and bodies in "adluminal compartment" of seminal tubules (Goldner trichrome stain, ob. 40×).***

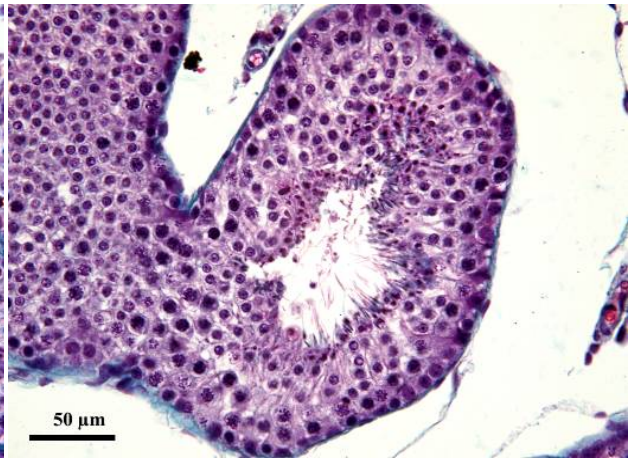

Figure 2 – ***Edema and vacuolar degeneration of sperm cells (Goldner trichrome stain, ob. 40×).***

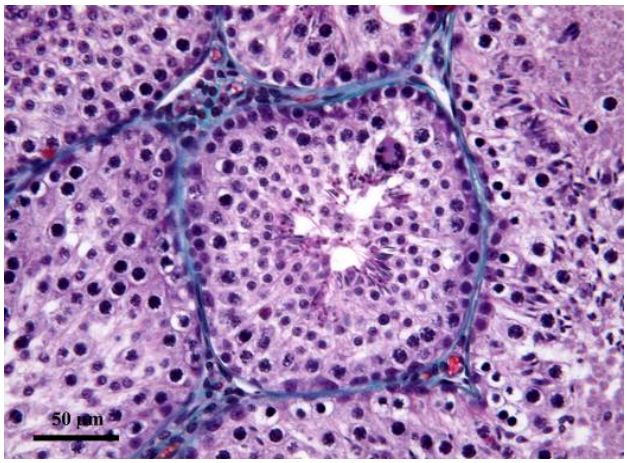

Figure 3 – ***Sincitialisation of spermatides (Goldner trichrome stain, ob. 40×).***

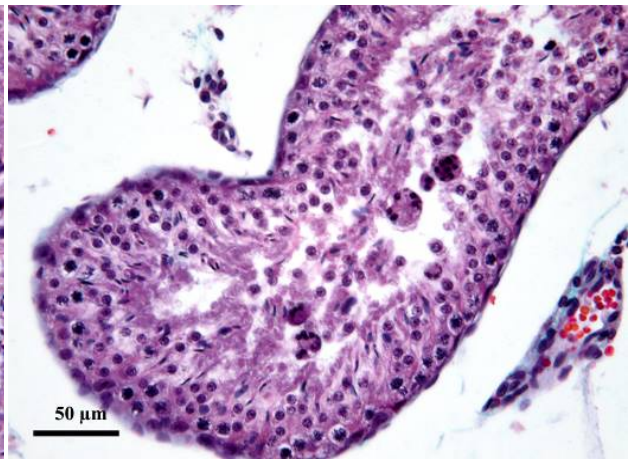

Figure 4 – ***Sincitial cells in apoptosis (Goldner trichrome stain, ob. 40×).***

In some places, the process is more advanced, so seemingly normal-looking cells are very few, edema is ruled by the tubular basement membrane and the lumen is almost obliterated by the proteic debris, resulting from massive disintegration cells, which can distinguish apoptotic cells and sperm from place to place are no longer willing swollen in "adluminal area" seminal tract but appear to be mixed with other cellular debris. Accumulation of debris is so large that the quantity tends to block the lumen tube suggests that degenerative processes were carried out with great brutality and in very short time did not allow the gradual elimination of debris from the lumen (Figure 5).

In some seminal tubules, cell separation is so massive that large areas remain no longer than spermatogonial, located on the basement membrane,

and in some parts and they are destroyed, with debris basal cell membrane in contact with the phenomena of denudation patches of complete (Figure 6). In the case of seminiferous tubes, trials are underway with a special brutality including the vast majority of cells in all stages in spermatogonial a lesser extent, where the degree of damage does not exceed 40% (Figure 7).

By comparison, in most sections examined histological preparations from subjects belonging to control batch, the epithelium is a normal seminal tubules, spermatogenetic cells are formed in large numbers (the epithelium is relatively thick) and where sperm are caught in some sections. They are found in large numbers suggesting that the process of spermatogenesis is complete and functional and runs at a high level (Figure 8). In some seminal tubes are observed some apoptotic bodies in "adluminal area", but they were found in small number.

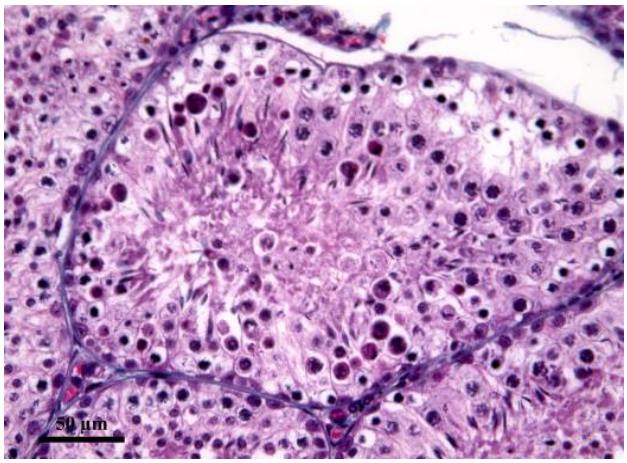

Figure 5 – ***Full obliteration of the seminiferous tubules with cellular debris and protein (Goldner trichrome stain, ob. 40×).***

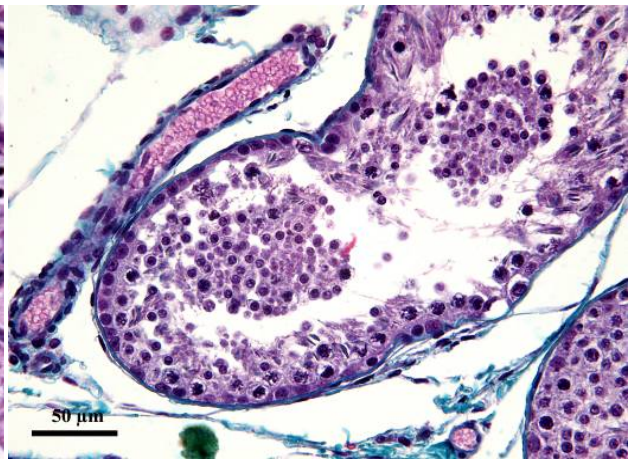

Figure 6 – ***Complete denudation of the seminal cells line (Goldner trichrome stain, ob. 40×).***

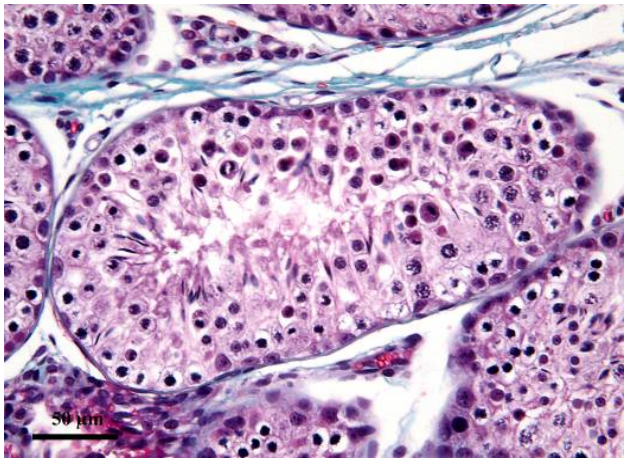

Figure 7 – ***Phenomena of apoptosis and vacuolar degeneration in all stages of seminal line (Goldner trichrome stain, ob. 40×).***

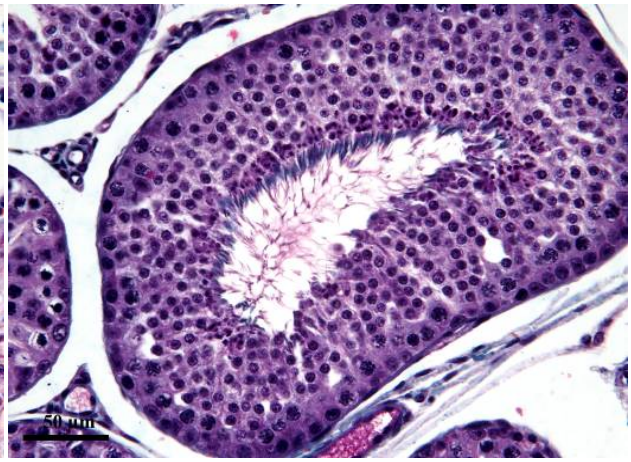

Figure 8 – ***Physiological aspects of the gametogenesis process (Goldner trichrome stain, ob. 40×).***

Discussion

Examination of histological preparations from subjects belonging to the experimental batch, exposed to the hexestrol diacetate affected all categories of seminal line cells, including spermatogonial type A_1 and type B (known as "reserve spermatogonial" or "resistant spermatogonial"), located in the "basal area" of the seminal tubules.

The degree of damages are different in "adluminal area" compared with those found in the "basal area" thus "adluminal area" are pronounced disease (cellular edema, vacuolar degenerescence, the appearance of syncytial of spermatide, apoptotic cells and bodies) with serious disruption or cancellation in certain parts of the process of spermato- and spermiogenesis.

In the "basal area" are affected a moderate number of spermatogonial with significant differences from one tube to another. Given that pathological processes are still underway, we cannot determine the exact number of the cells "basal area" that will be eliminated by apoptosis [5, 6]. This confirms the high degree of toxicity of hexestrol diacetate on seminal cells line and it's ability to overcome both hematological testicular barrier (affecting the cells "basal area") and the barrier consists of Sertoli cells (affecting cells "adluminal area").

In conclusion, the pool of spermatogonial will decrease in proportion to the damage if the action of these substances is extended. Impairment of "reserve" spermatogonial B_1 initial hypothesis of this study confirm that, extended exposure of male rabbits to

hexestrol diacetate action will cause irreversible damage to the seminal epithelium, leading to installation of sterility.

Since the cycle of seminal epithelium in the male rabbit is about 10.6 days and duration of spermatogenetic cycle (time required for a group of germ cells to progress from stage spermatogonial differentiated A_1 to spermatozoa stage) is 4.5 times longer (48 days), most researchers recommended a shutter speed of action of experimental animals to be investigated, equivalent to six time during a cycle of the seminal epithelium (63.6 days in male rabbit). In this case, we obtained data on the toxicity of hexestrol diacetate, which we consider to be relevant as an exposure time of only 30 days [7]. Dose used in this research (0.5 mg/kg hexestrol diacetate) lies within the range of most working protocols used to assess the impact of steroid hormones and synthetic compounds on gametogene function [8]. Choosing the histopathology as method of investigating the effects of hexestrol diacetate is because this method is considered the most sensitive endpoint for detecting testicular toxicity [9]. It is incumbent for the pathologist to have an adequate understanding of the organization and dynamics of spermatogenesis in the species under investigation. Understanding and using consistent terminology for the changes observed and grading the severity of those changes are also important issues in the reporting and interpretation of testicular histopathology [10]. Spermatogenic disruption may reflect a direct effect on the seminiferous epithelium, affecting either the Sertoli cell or any one of the germ cell populations, or it may occur as a secondary response to altered hormone levels, altered vascular supply, or altered fluid balance, either within the testis or within the epididymis [9]. Whether spontaneous or induced, death of germ cells appears to occur predominantly through apoptosis, a process that is closely regulated by the Sertoli cell [9, 10]. This is particularly true for spermatogonia, which may be seen undergoing apoptosis in occasional stage XII tubules. Germ cell depletion is the most common sequel to spermatogenic disturbance and is generally a consequence of germ cell death rather than exfoliation [4]. It may be seen as a generalized or partial depletion of the germ cells or it may only affect a specific cell type [3]. Vacuolation within or between Sertoli cells is a common early sign of Sertoli cell damage. The vacuoles may be solitary and situated among the germ cells at varying depths throughout the epithelium. It is generally not possible to determine by light microscopy whether the vacuoles are intra- or extracellular. In other cases, intracellular micro-vacuolation or swelling may be seen affecting the basal area of the Sertoli cell cytoplasm and causing germ cell displacement and disorganization. Such findings are suggestive of disturbances within the Sertoli cell and may represent alterations in the smooth endoplasmic reticulum or in fluid homeostasis [9]. In the framework of research conducted in 2002 on the influence of synthetic steroid compounds on gametogene function, have been found an increase of Fas–FasL values in the seminal line cells (haploid) and consecutive administration of diethyl-stilbestrol and hexestrol (Fas ligand – FasL) is a trans-membrane protein, with a homotrimeric structure which belongs to the family "tumor necrosis factors" (TNF) and Fas Ligand attachment to it's specific receptor FasR leading to initiation of apoptosis, this phenomenon has a crucial role in the modulation of the immune system to prevent or reduce the development of neoplastic processes [3, 11].

Conclusions

Bi-weekly administration, during 30 days of 0.5 mg/kg hexestrol diacetate leads to damage to all cell types belonging to the seminal line with the emergence of the phenomenon of massive apoptosis, especially in the spermatocytes and pachitene spermatidis, sincitialisation of spermatidis, phenomena of cellular edema and vacuolar degenaration accompanied by massive cell depletion at the level of "adluminal area" of the seminal tubes.

Impaired cells "basal area" and "adluminal area" of seminal tubules in rabbits exposed hexestrol diacetate action confirms the ability of this substance to overcome barriers both hematological and testicular cells that formed under these conditions subjects extended exposure to synthetic steroid action of this compound will cause the complete depopulation of the seminal line leading to the installation of sterility in these males.

We believe that the proposed experimental animal model in this study to assess gametogene toxicity in males of hexestrol diacetate has demonstrated efficiency and specificity both in terms of minimum time exposure of the experimental animals to the action of the agent studied and the advantages of using rabbits in such tests.Highlighted issue raised on the opportunity use of hexestrol diacetate in therapy or animal production stimulation as it gametogenetic function in males while their risk transfer to humans through consumption of foods of animal origin.

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