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Platelet-rich plasma induces morphofunctional restoration of mice testes following doxorubomycine hydrochloride exposure

Zaporozhan Valeriy[a], Kholodkova Olena[b*], Kuleshova Olena[b]

[a] *Department of Obstetrics And Gynecology, Odessa National Medical University, Odessa, Ukraine*
[b] *Department Of Human Anatomy, Odessa National Medical University, Odessa, Ukraine*

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*** Correspondence to:**
Kholodkova Olena
Department Of Human Anatomy,
Odessa National Medical University,
Odessa, Ukraine
e-mail: kholodkova_l@rambler.ru

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ABSTRACT

According to the World Health Organization (WHO) about 8% of couples during the reproductive period of life are faced with the problem of infertility. Thus, the efforts of doctors and researchers are aimed at finding of effective treatment methods for spermatogenesis disorders. Platelet-rich plasma (PRP) has therapeutic effect due to the presence of growth factors. This work aimed to investigate the function of PRP on mice males' gonads in experimental toxic affection. After toxic affection of the gonads by doxorubomycine hydrochloride (DH) mice got three injections of PRP. PRP was isolated from whole blood with the processing unit SmartPrep (Harvest Corp.). The introduction of PRP under the skin of scrotum renews the testis microstructure and the state of spermatogenic epitelium in mice, normalizes the amount and functioning of the Sertoly cells. This effect, probably, is conditioned by the activity of the growth factors, which freed from platelets' α-granules after the PRP injection. Thus, the application of the platelet rich plasma is the effective method for restoration of the gonads of mice males in the condition of toxic affection.

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1. Introduction

According to the World Health Organization (WHO), about 8% of couples during the reproductive period of life are faced with the problem of infertility (Grudzinskas and Yovich, 1995). In technologically advanced countries such as USA, Germany, Denmark etc. about 15% of couples suffers from infertility (Bashamboo et al., 2010). Data obtained over the last 20 years show about 30% of fertilization problems cases are caused only by male factor, according to some sources -about 50% of cases (Maffini et al., 2006; Olesen et al., 2007; Bashamboo et al., 2010). So, the efforts of doctors and researchers are aimed at finding of effective treatment methods for spermatogenesis disorders. Using the platelet-rich plasma (PRP) is one of the promising technologies. PRP is a plasma with platelets' concentration more than 1000000/mcl since it was proved that only this concentration has stimulating effect (Marx, 2001). PRP has therapeutic effect due to the presence of growth factors (Coppinger et al., 2004; Qureshi et al, 2009; Weyrich et al., 2009). In addition, interest to the PRP is high because it is 100% biocompatible, safe, wouldn't cause patient's infection, as it is made with patients' own blood.

The aim of the present study, an investigation of the impact of PRP on male mice' gonads in experimental toxic affection of testicles were performed.

2. Material and methods

Animals

Sixty adult male ICR mice, weighting 29-23 g were utilized in this study. Mice were fed a standard mice chow and tap water ad libitum. Temperature 22±2C° and humidity 50-55% were performed. Animals received human care in accordance with the European Communities Council Directive of November 24 1986 (86/609/EEC) http://ec.europa.eu/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf.

Table 1. Morphometry of seminiferous epithelium (conventional units, M± m)

Index of specific areas	Control	1st group 4 weeks	1st group 6 weeks	2nd group 4 weeks	2nd group 6 weeks
Spermatogonia	4.67±0.7	2.95±0.42*	2.85±0.64*	7.0±0.81*	6.95±0.44*
Sertoli cells	1.71±0.3	1.14±0.12*	1.35±0.43	1.69±0.14	1.72±0.5
Leydig cells	0.71±0.2	0.8±0.36	0.7±0.17	0.7±0.26	0.65±0.02
Diameter of seminiferous tubules	1.72±2.8	21.09±3.17*	19.05±2.06*	9.95±2.1*	9.69±1.75*
Space between cells	7.86±1.1	8.43±1.66	9.8±1.92	7.35±1.72	6.52±1.13
Nuclei of cells	21.43±2.1	16.85±1.24*	17.6±1.05*	19.95±2.28	20.82±2.3
Cytoplasm	41.85±3.6	32.66±3.5*	38.6±4.46	43.3±3.27	44.0±2.01
Interstitium	6.57±0.9	8.85±1.04*	7.9±0.28*	6.25±1.22	5.48±0.9

* - significant difference relative to control ($p<0.05$)

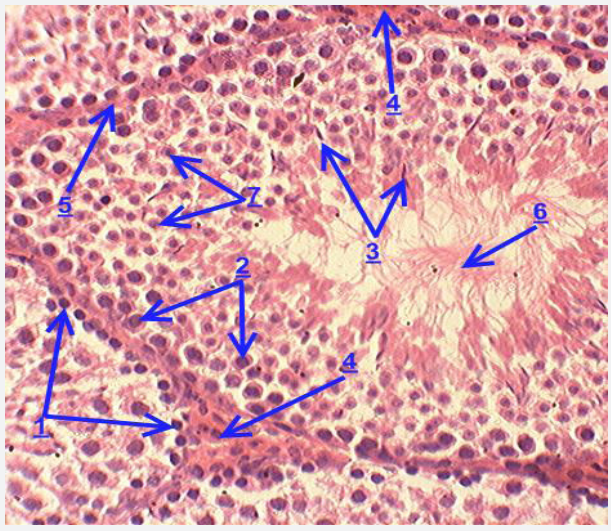

Fig. 1. Light microscopy of testicular tissue in control group (H&E, magnification x 400): 1-spermatogonia, 2-spermatocytes of the 1st order, 3-sperm, 4-interstitium, 5-basement membrane, 6-seminiferous tubules, 7-spermatocytes of the 2nd order.

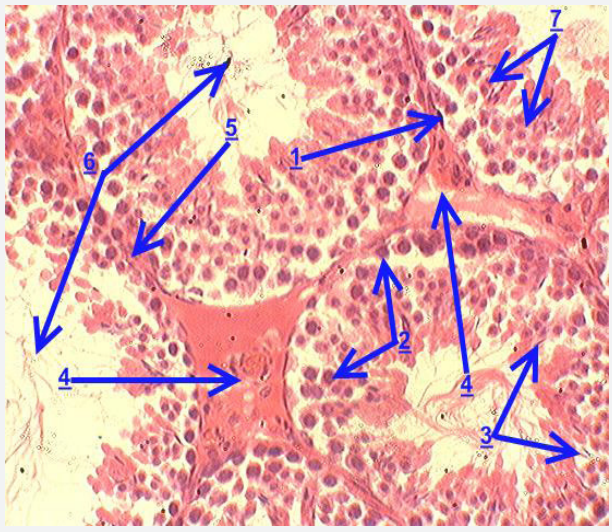

Fig. 2. Light microscopy of testicular tissue in 1st group on the 4th week (H&E, magnification x 400): 1-spermatogonia, 2-spermatocytes of the 1st order, 3-sperm, 4-interstitium, 5-basement membrane, 6-seminiferous tubules, 7-spermatocytes of the 2nd order.

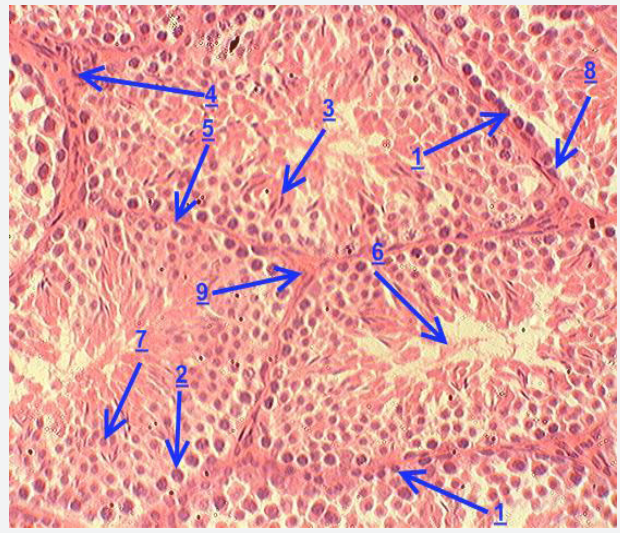

Fig. 3A. Light microscopy of testicular tissue in 2nd group on the 4th week (H&E, magnification x 400): 1-spermatogonia, 2-spermatocytes of the 1st order, 3-sperm, 4-interstitium, 5-basement membrane, 6-seminiferous tubules, 7-spermatocytes of the 2nd order, 8-Sertoli cells, 9-Leydig cells.

Experimental design

Mices were randomly allotted into two experimental groups: 1st with toxic affection, 2nd group was injected with PRP after the toxic affection; each group contains 20 mices. A separate group of mices served as intact control.

Experimental procedures

Toxic affection of the gonads was induced by two intraperitoneal injection of doxorubomycine hydrochloride (DH) (single dose-2 mg/kg) with an interval of a week. Two weeks after DH treatment, development of toxic affection was confirmed by histopathologic evaluation.

PRP was given under the scrotum three times with 2 weeks intervals (volume-0.1 ml per injection). PRP was isolated from whole blood with the processing unit SmartPrep (Harvest Corp.). Control mice were injected with the same volume of isotonic NaCl as the animals of the 2nd group.

The first day of the experiment was considered the last introduction of DH or PRP in the respective groups. At the end of experiment, all animals were anesthetized by inhalation of the ether vapor. The anesthetized animals were sacrificed after 4 and 6 weeks and testis tissues were removed for the histopathological investigation.

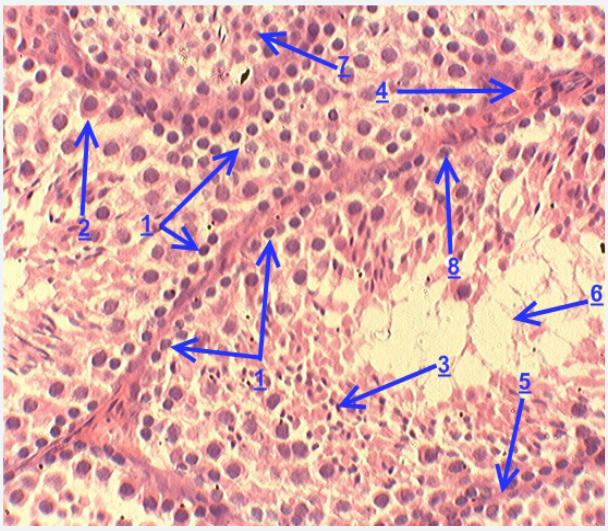

Fig. 3B. Light microscopy of testicular tissue in 2nd group on the 4th week (H&E, magnification x 400): 1-spermatogonia, 2-spermatocytes of the 1st order, 3-sperm, 4-interstitium, 5-basement membrane, 6-seminiferous tubules, 7-spermatocytes of the 2nd order.

Histopathologic evaluation

The testis specimens were embedded in the paraffin blocks after they had been fixed in 10% neutral formalin solution. Sections of 5 μm were obtained, deparaffinized and stained with hematoxylin and eosin (H&E). The testis tissue and spermatogenesis was examined and evaluated in random order with standard light microscopy also photometric system "Videotest-Master" in conjunction with the light-optical microscope LEICA-DMLS. Morphometric measurements were performed in the software package "Videotest-Morphology" with internal measuring standard.

Statistical analysis

Further processing of the data was performed using statistical analysis software package "Statistics-6" for Windows 11.0.

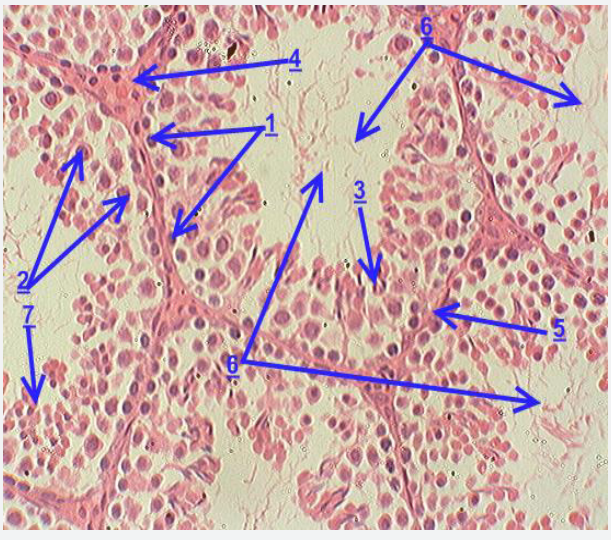

Fig. 4. Light microscopy of testicular tissue in 1st group on the 6th week (H&E, magnification x 400): 1-spermatogonia, 2-spermatocytes of the 1st order, 3-sperm, 4-interstitium, 5-basement membrane, 6-seminiferous tubules, 7-spermatocytes of the 2nd order.

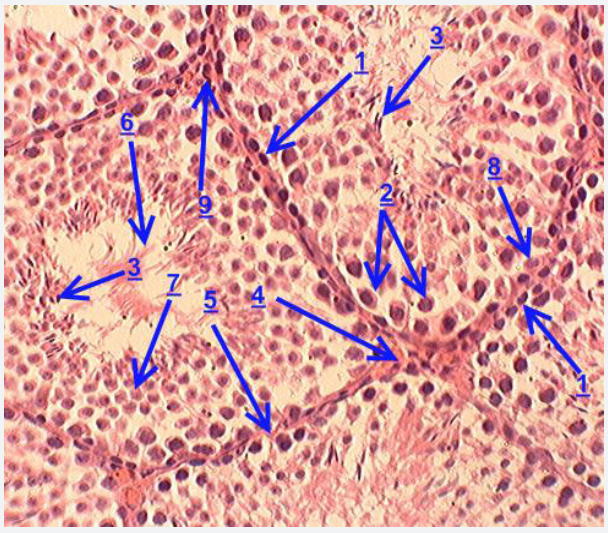

Fig. 5A. Light microscopy of testicular tissue in 2nd group on the 6th week (H&E, magnification x 400): 1-spermatogonia, 2-spermatocytes of the 1st order, 3-sperm, 4-interstitium, 5-basement membrane, 6-seminiferous tubules, 7-spermatocytes of the 2nd order, 8-Sertoli cells, 9-Leydig cells.

The differences were considered significant when probability was less than 0.05.

3. Results

Histopathological changes on the 4th week of the experiment

Control testis showed the presence of normal testicular architecture and regular seminiferous tubular morphology with normal spermatogenesis (Fig.1). In testis of the 1st group it was found the amount of spermatogonia was reduced dramatically, indicating the inhibition of spermatogenesis (Table 1, Fig. 2). In testis after PRP correction, this index was even higher than in control. Number of Sertoli cells in 1st group decreased more than 30% compared to the control group, in the 2nd group the number of cells didn't differ from the control. Diameter of the seminiferous tubules in the 1st group testis was significantly expanded, while in the 2nd group, this figure was more than two times less than in the 1st group but significantly higher when compared to the control group. The reduction of the nuclei specific area and cytoplasm in the 1st group testis indicates the depletion of cellular structure and inhibition of spermatogenesis. On the 4th week in the 2nd group testis these figures were similar to the intact ones.

In the 2nd group testis depression of the intensity of the inflammatory response which lead to decrease of basement membrane's thickness and interstitial area with reduce of it's hyperchromity were observed (Fig. 3A, B). The significant increase in all spermatogenic cell series was mentioned. Both straight and convoluted tubules were still slightly expanded, however, with tend to their recovery.

Histopathological changes on the 6th week

Significant extension of seminiferous tubules when compared to the control still remained (Fig. 4). Number of spermatogonia were still significantly reduced, resulting almost full absence of spermatocytes of the 1st, 2nd order and the newly formed sperm cells. The volume of interstitium was increased, the basement membrane was thickened and hyperchromed that

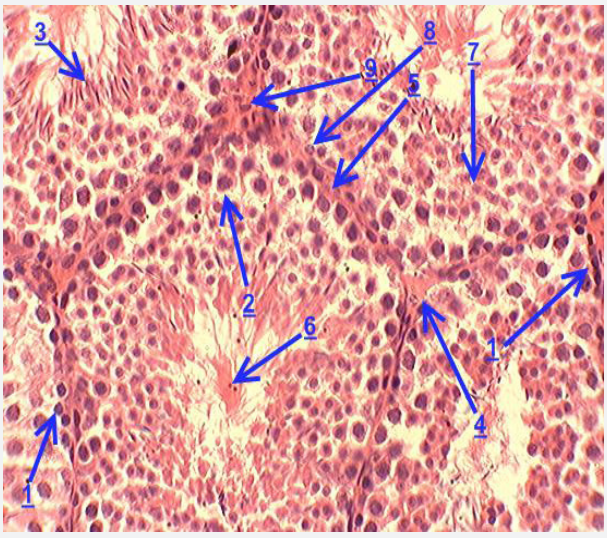

Fig. 5B. Light microscopy of testicular tissue in 2nd group on the 6th week (H&E, magnification x 400): 1-spermatogonia, 2-spermatocytes of the 1st order, 3-sperm, 4-interstitium, 5-basement membrane, 6-seminiferous tubules, 7-spermatocytes of the 2nd order, 8-Sertoli cells, 9-Leydig cells.

indicates the processes of swelling and inflammation in the testicles. Also there was a detachment of spermatogenic epithelium. Ducts of seminiferus tubules were significantly expanded and filled with protein secretion of Sertoli cells (protein detritus); newly formed sperm were almost absent.

In testis of the 2nd group a large number of newly formed spermatocytes of the 1st and 2nd order were observed (Fig. 5 A, B). Interstitium and basement membrane were still somewhere expanded, but their structure were better organized than in the 1st group testis at the same period, inflammation and edema were not observed at all. The specific area of seminiferous tubular duct was almost identical to intact animals' one, and there was a large number of sperm in the duct lumen. All these signs indicate the active regeneration in the testis under the influence of PRP.

4. Discussion

Doxorubomicine hydrochloride administration caused the development of testis tissue affection as the result of toxic influence of DH which is used for cancer chemotherapy (Kraus-Berthier, 2005; Zaporozhan et al., 2005). DH quickly penetrates the cell, interacts with DNA, prevents the nucleic acids synthesis and inhibits mitotic activity (Sparano, 1999). Cancer treatment cause a variety of toxic effects detected in testis (Chatterjee and Kattardis., 2002; Gambacorti-Passerini et al., 2003). In our research main manifestation of this process was approximately 40% decrease of the number of spermatogonia what indicates the spermatogenesis depression (Grudzinskas and Yovich, 1995; Nalesnik et al., 2004). As a result, the number of newly formed spermatocytes and sperm reduced dramatically. Also development of inflammatory reaction both in the interstitium and basement membrane was mentioned as confirmed by the increased in the specific area of interstitium, the presence of protein detritus in the seminiferous tubules, detachment of spermatogenic epithelium, and basement membrane thickening. Affection of the tubule was heterogeneous in nature and depended on the stage of spermatogenesis, which was at the time of DH administrarion. The obtained data are similar to those of toxic damage of testicles of various origin (Kahan, 1989; Lampe et al., 1997; Tung and Cunningham, 2007; Nakayama et al., 2008).

The results of pathomorphological and morphometric investigations showed restoration of experimental animals' testicular microstructure after exposure of PRP. The spermatogenesis resumed, and Sertoli cell number and function normalized. The width of ducts of seminiferous tubules reached indexes of the control testis, in many ducts a large number of newly formed sperm revealed. It indicates the renewal of the morphofunctional state of gonads after the use of PRP in case of experimental toxic injury. Obviously, this effect is due to the activity of growth factors that are released from α-granules of platelets after PRP injection: Epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), etc. (Bir et al., 2009; Battinelli et al., 2011; Sanchez-Gonzalez et al., 2012). The basis for this conclusion is a list of the properties of these biologically active substances: the stimulation of cell growth and differentiation, enhancement of angiogenesis and collagen synthesis, antiapoptotic effect, accelerating cell migration and others. Given the fact that, e.g., cancer chemotherapy leads to hypogonadism and reduced fertility, and such patients are forced to take samples of seminiferous epithelium in sperm bank for cryopreservation and further treatment (Anger et al., 2003; Scrader et al., 2003; Seshadri et al., 2014), the use of PRP will help to avoid additional financial costs and anxiety. Thus, one can assume the use of platelet-rich plasma is an effective method of restoring the state of the gonads after toxic damage.

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