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Original Research Article

Histo-Functional Improvement of Mammalian Testis after Platelets-Rich Plasma Treatment

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ABSTRACT

Background: Among new therapies emerging in the medical field, the use of platelet-rich plasma (PRP) in human reproduction has not yet been explored. Platelet-rich plasma (PRP) has a potential effect on tissue repair through proliferation and differentiation of tissue progenitor cells. The aim of this study was to evaluate the effect of PRP on the testis structure and function in the rabbit model.

Material and methods: A total of 30 male rabbits were recruited in this study. They were allocated into two groups (15 in each group) to receive an injection of PRP (PRP Group), or normal saline (Control Group)

Results: there were statistically significant differences in Means of germinal layer width, Leydig cell number, and Sertoli cell number was significantly higher in the PRP group compared to that in the control group ($P \leq 0.05$). The PRP group had a higher means of sperm concentration and normal morphology compared with the control groups ($P \leq 0.05$).

Conclusion: the platelet-rich plasma is found to have a good potential effect on the testicular tissue that improved the histological and functional aspects and could be considered a promising future treatment for hypogonadism status in many disorders.

Keywords: PRP, reproductive function, testis, Sertoli cells, Leydig cells, the height of the germinal layer

KEY WORDS: Platelet-Rich Plasma (PRP), Tissue Progenitor Cells, Testis.

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INTRODUCTION

The rabbit testis, the male gonad, consists mainly of seminiferous tubules containing the germ cells and Sertoli cells and interstitial tissue containing Leydig cells, demarcated from outside by a thick vascular connective tissue tunica albuginea, sending septa that subdivided the testis into smaller incomplete compartments. The seminiferous tubules are the site of spermatogenesis, while Leydig cells are responsible for secreting male sex hormones, testosterone; to maintain spermatogenesis [1]. Testicular parenchyma consisted of solid straight testicular cords with no lumina. The testicular cords contained two types of cells; pre-spermatogenic cells or gonocytes and Sertoli cells. The pre-spermatogenic cells were large rounded cells with rounded nucleus having two distinct nuclei and pale stained cytoplasm and frequently seen

in mitotic division [2]. These cells were seen in the center of testicular cords and had no contact with the basal lamina. While the Sertoli cells were tall columnar with an oval or spindle-shaped nucleus and pale cytoplasm and their boundaries were less clear. The interstitial tissue contained small singly scattered or grouped spindle, oval, triangular, polyhedral, or elongated Leydig cells, which contained small deeply stained nucleus and pale vacuolated cytoplasm due to the presence of lipid droplet [3].

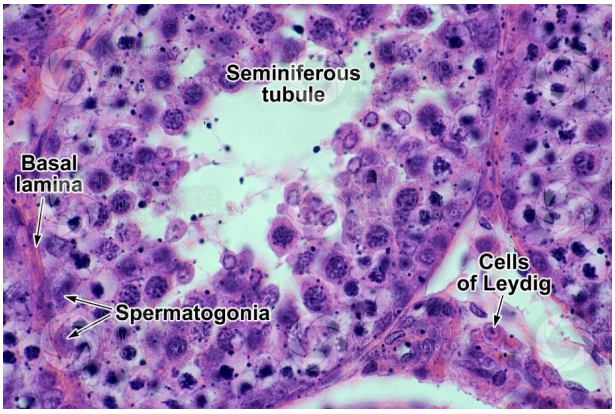

Fig. 1: Rabbit. Testicle. The transverse section shows the details of histological features as labeled above 50X [4].

As a source of growth factors, platelet-rich plasma (PRP) has been widely used in the repair of various tissue injuries. Platelet-rich plasma (PRP) has a potential effect on tissue repair through proliferation and differentiation of tissue progenitor cells. The PRP has beneficial effects on spermatogenesis and reproductive hormone production in testicular torsion. [5,6]. Platelet-rich plasma is defined as an autologous concentration of human platelets that is three to five times greater than the physiologic concentration of thrombocytes in whole blood [7] . Due to its high efficacy and safety, Platelet-rich plasma should be considered as a promising therapy that warrants further investigation on its biological effects on sperm quality, that showed an improvement in vitality and motility, and a significant reduction in vacuolization, DNA fragmentation, and ROS levels [8]. Platelet rich plasma potential therapeutic effect is due to the presence of various factors such as transforming growth factor â (TGF â), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin like growth factor 1 (IGF 1), platelet derived growth factor (PDGF), zinc (Zn), and superoxide dismutase (SOD) [9]. Many of these factors were studied solely on the spermatozoa showing a significant positive effect on its quality and function. The TGF â, FGF, VEGF, Zn, and SOD were found to enhance sperm motility, while IGF 1 and PDGF were associated with normal sperm parameters [8, 10, 11].

It has been found that PRP can improve the structural and functional impairment of the testis in treatment by these agents [12, 13].

Image J analysis was done using the software Image J (Java-based image processing program developed at the National Institutes of Health, USA) version 1.47p whic h has already been installed in a personal computer. Image J software is the keystone in the morphometric study

MATERIALS AND METHODS

A total number of 30 adult male rabbits 24-30 weeks of age and average weight (1.5±0.25) kg, domestic rabbit (Oryctolagus cuniculus); were used in this study. The 30 animals were divided into two groups (15 animals for the control group (Group A) and 15 animals for the experimental group (Group B). Breeding of both groups for two weeks with the same food and under the same environmental factors, then a blood sample was taken from the rabbit of group B (experimental), and PRP was prepared by centrifugation at 3500 rpm for 5-10 minutes then an 80ìl of PRP (single dose) was locally injected in the testis of group B, while group A was injected with normal saline. Take care of the rabbits of both groups for 50-60 days Then after the animals of both groups were sacrificed and the testes were removed. The animals were euthanized by inhalation of chloroform that was soaked in a piece of cotton in an airtight chamber for 3-5 min. Then, the animal was fixed on the dissection stage by its four limbs facing anteriorly. The seminal fluid was collected by using the milking method. collect a drop of semen (0.2 ml) and put it in an Eppendorf tube diluted with normal saline until reaches 1ml (diluted 5X). then put the specimen in a warm environment at degree 15-20 c. we took one

drop from the specimen by micropipette and put it on a slide covered by coverslip and then seminal fluid analysis (SFA) was done to count the sperm concentration (million/ml), check the motility and morphology. These steps must be less than one hour to guaranty we got the exact result of motility.

The testis specimen was prepared for paraffin sections according to [14]. And then kept separately in the containers and these specimens were directly fixed in 10% neutral buffered formalin PH: 7. The fixed tissues then processed for the routine paraffin wax embedding process. Paraffin blocks were sectioned into slices sagittally at 5ìm thickness by using a microtome (Richert - Jung, 2030 MOT Biocut), the microtome work in both manual and automatic way. Ribbons of the section were properly laid down on the surface of hot water (40°C) using a water bath. The ribbons were then collected on clean glass slides, the section will be stained with hematoxylin and eosin for general histological examination.

The prepared histological tissue slide that was stained with hematoxylin & eosin staining was examined for histological evaluation and estimation using a light microscope The observed fields were snapshotted using a Digital Microscope Camera (Model MC500) with 5-megapixel resolution and the pictures were saved in a JPEG format. All the examination procedures took place in the scientific research laboratories at the Department of Human Anatomy, College of Medicine-AL-Nahrain university.

RESULTS

The comparison of testis structure between the control and PRP groups showed a statistically significant differences ($P < 0.05$) in the means of germinal layer width, Leydig cell number, and Sertoli cell number that were significantly higher in the PRP group compared to that in the control group. See (table 1)

The comparison of testis function between the study groups revealed statistically significant differences ($P < 0.05$) in the means of sperm concentration and percentage of normal morphology and motility between the two groups, Control and PRP groups. The PRP group had a higher means of sperm concentration and normal morphology and motility compared with the control groups. (See table 2 below)

Table 1: Histological parameters differences between control and PRP groups.

Testis Structure	Control Mean ± SD	PRP Mean ± SD	P- Value
Germinal Layer (GL)	33.22 ± 6.0	47.7 ± 4.5	**0.001**
Leydig Cell / hpf	3.77 ± 1.5	4.7 ± 0.94	**0.01**
Sertoli Cell /hpf	4.44 ± 1.5	7.9 ± 1.91	**0.001**

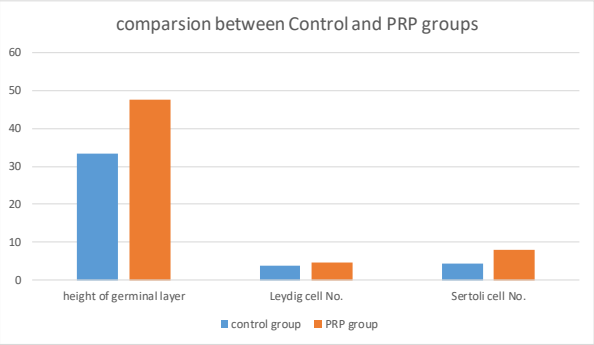

Fig. 1: column charts show the mean differences in histology between two groups, Control, and PRP groups.

Table 2: Differences in parameters of SFA in both control and PRP groups.

Testis Function	Control	PRP	P - Value
Sperm Concentration/Million)	67.5 ± 12.1	240.44 ± 289.3	0.01
Sperm Morphology (%)	72.0 ± 7.5	74.44 ± 7.7	0.01
Sperm Motility (%)	65.33 ± 19.5	74.5 ± 9.6	0.04

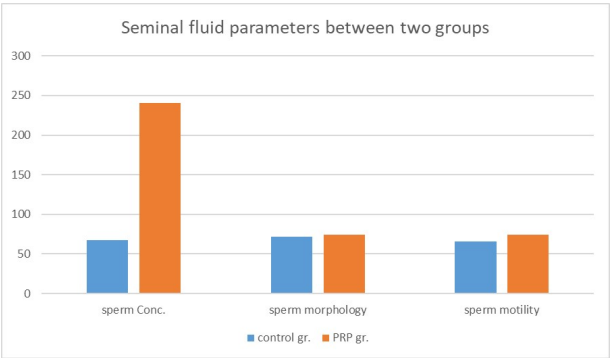

Fig. 2: Column charts show the differences in seminal fluid parameters between Control and PRP groups.

DISCUSSION

Platelet-rich plasma (PRP) has a potential effect on tissue repair through proliferation and differentiation of tissue progenitor cells [12]. This was evident and clear in the data listed in table (1), the height and thickness of the germinal layer that contains the different developmental stages of the male gametes namely, the spermatogonia, spermatocyte

(primary and secondary) and spermatid. The PRP increases the layer width due to the high proliferation rate by the effect of the growth factors provided by the PRP.

Platelet-rich plasma (PRP) has a potential effect on tissue repair through proliferation and differentiation of tissue progenitor cells [12]. This was evident and clear in the data listed in table (1), the height and thickness of the germinal layer that contains the different developmental stages of the male gametes namely, the spermatogonia, spermatocyte (primary and secondary) and spermatid. The PRP increases the layer width due to the high proliferation rate by the effect of the growth factors provided by the PRP.

As seen in table (1), the number of Sertoli cells as well as Leydig were increased significantly due to effect of PRP. Sertoli cells are the somatic cells of the testis that are essential for testis formation and spermatogenesis. Sertoli cells facilitate the progression of germ cells to spermatozoa within the seminiferous tubules [15]. Sertoli cell number has long been thought to be stable in adults with no proliferation of Sertoli cells once adult numbers have been reached. However, new studies indicate that adult Sertoli cells can be made to re-enter mitotic phase under certain experimental conditions. the majority of inhibin alpha-subunit and inhibin/activin beta B-subunit following birth, is immunolocalized in the Sertoli cells of both subunits that increases markedly [16].

The function of activin is to increase FSH secretion at the level of the pituitary. Sertoli cells have nutritive, protective and supportive functions for spermatogenic cells phagocytose regressive sperms and produce an androgen-binding protein and secret constituents of intratubular fluid, which facilitates the lumenation of the seminiferous cords. Furthermore, they form the blood-testis barrier to keep the developing haploid spermatogenic cells protected from the action of immune cells of the blood, hence after PRP therapy; the Sertoli cells may be the corner stones for repair and re-development of testis post traumatic or chemotherapy for instance [3]. The fetal Leydig cells act as a source of activin A in the mouse. In the post-natal human, mouse and rat testes, β_A sub-units have been demonstrated in gonocytes and Sertoli cells, using in situ hybridization and immunohistochemistry [17, 18].

In table (2) the parameters encountered are the sperm concentration, motility and morphology. The PRP is an autologous and endogenous agent which is rich in growth factors and cytokines. It is used, particularly, in regenerative medicine, PRP increased the number of spermatogenic stem cell, count, motility and tail length of the sperm and testosterone level that eventually improves the quality of sperms characteristics, thus; the PRP may be considered as treatment for those patients with low sperm quality [12, 19].

The PRP treatment has been used by many researchers and medical centers to make use of its protective and reparative effects on many body tissues in particular, the reproductive organs and tissue like testis, ovary and even incubation of the semen sample with PRP for time before intrauterine insemination or IVF technique in order to improve the success rate of getting pregnancy. The PRP has a partial protective effect on cryopreservation of human spermatozoa that make it a good promising future treatment for many problems encountered in the field of infertility management [20].

The time needed for the rabbits in the current study before being sacrificed after injection of PRP is approximately ranging between 43-51 days which is the duration of spermatogenesis in rabbit [21].

Conclusively, this study state that PRP increased the number of spermatogenic stem cell, count, motility and the testosterone level significantly [12].

Author Contributions

Thaer M. Farhan has conducted all the steps of the work including design preparation, Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing – original draft; Writing – review & editing

Aiman Al-Maathidy: Data collection, Funding acquisition, investigation, writing- review and editing

Conflicts of Interests: None

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