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Injection of Platelet Rich Plasma Intra-Ovarian to Treat Inactive Ovaries in Rabbits

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ABSTRACT

In this investigation, we examined the efficiency of PRP as a therapy for inactive ovary (IO) in rabbits. IO in rabbits was induced by administration of D-galactose (200mg/kg) (dissolved in saline, 0.2mL/rabbits/day) for ten consecutive days. Rabbits divide into three groups. After preparing two concentrations of PRP solution, the PRP was injected intraovarian through surgical intervention. Then two weeks later, FSH and estrogen were measured, histopathological examination for ovaries were made according to the routine procedure, and finally, the mating trial was made to evaluate the fertility status. Ten days after D-galactose administration IO had significantly higher levels of FSH (2.30±1.32) and (2.80±0.80) and lower levels of E2 (5.55±0.78) and (5.36±0.91), in comparison to the control group ($P<0.05$) (1.99±0.80 and 7.16±1.195) respectively. The results after injection of PRP in two different concentrations indicated that the highest value significant ($P<0.05$) was in E2 levels (7.87±0.9) and (8.08±2.21), and the lowest significant ($P<0.05$) was in FSH levels (1.60±0.57) and (1.49±0.38) comparison with others induction D-galactose. Histopathological examination showed an increase in follicles in PRP groups compared to galactose group. It was concluded that PRP had a protective effect against galactose toxicity and improved follicular growth.

Key words: Inactive Ovary, D-Galactose, Platelet-Rich Plasma (PRP).

INTRODUCTION

The ovaries are inactive, they are quiescent and show no evidence of cyclicity or ovarian structures associated with cycles (Zulu et al. 2002). According to some theories, the inability of regular ovarian activity can be caused by insufficient gonadotropin release or synthesis to cause follicular development and maturation or by the ovaries' failure to react to gonadotropins. Inactive ovaries have been treated using various hormonal, nutritional, and management therapy (Majeed and Sadik 1997; Majeed and Taha 2000). A naturally occurring monosaccharide, galactose, combines with glucose (another monosaccharide) to produce the disaccharide lactose (Sunehag et al 2003). In addition, galactosemia is a rare genetic metabolic condition, which is itself defined by an abundance of galactose in the body (Leslie 2003). Galactose 1-phosphate accumulates to dangerous levels in a variety of tissues when the enzymes required for further galactose metabolism, such as galactose-1-phosphate uridyltransferase, are substantially reduced or absent in people with galactosemia (Ahmadian et al. 2020). Usually, this toxic overdose leads to ovarian failure. PRP, or platelet-rich plasma, is one striking illustration. It comprises a high-density platelet concentrate and is frequently used to treat various issues, including surgical or diabetic wounds, osteoarthritis, skin damage, and soft tissue damage (Marx et al. 1998; Nikolidakis and Jansen 2008; Lacci and Dardik 2010; Lubkowska et al. 2012; Kavadar et al. 2015). Megakaryocytes in the bone marrow produce platelets, which are nucleated blood cells with a lifespan of 7 to 10 days in humans and a little less time in mice (Van der Meijden and Heemskerk 2018). Due to the granules in them, they are simple to isolate and actively participate in tissue repair and wound healing (Messora et al. 2011; Amable et al. 2011). More than 800 distinct proteins, mostly local mesenchymal stem cells (MSCs), are present in the -granules, which exert a paracrine influence on adjacent cells and speed up tissue regeneration (Amable et al. 2011). PRP could be considered a potential alternative treatment method for several issues related to female infertility, including POI

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(Ahmadian et al. 2020). Based on the regenerative capabilities of PRP that have already been extensively studied in human (Anitua et al. 2004; Bendinelli et al. 2010) and veterinary (Lange-Consiglio et al. 2014; Lange-Consiglio et al. 2015) medicine, this work suggests a therapeutic approach for treating ovarian failure. As a result, the objectives of this study include determining the hormone levels of FSH and estrogen in serum from rabbits fed a D-galactose diet before and after PRP therapy.

MATERIALS AND METHODS

Animals

Twenty-five female rabbits, averaging 1.5-2kg in weight, older than 6 months, fed a bread-hay mixture, and not pregnant, were utilized in this investigation. Each rabbit was housed separately for one week. and kept in a typical setting with a temperature of 22.2°C, unfettered access to food, and water. Al-Basrah University, College of Veterinary Medicine approved all experimental protocols.

The Creation of a Rabbit IO Model

Rabbits were split into two main groups—the D-galactose group and the control—after a week of acclimatization. The D-galactose group (n=16) received 0.2mL/rabbits/day of D-galactose for 10 days (Wang et al. 2019). The control group's (9 total) nine rabbits got an equivalent volume of ordinary saline. Two rabbits from each group were randomly sacrificed after one day from the last injection, and serum and ovarian tissue samples were taken to verify the emergence of IO.

PRP Procedure

For blood testing, five healthy female rabbits were chosen. To achieve this, blood was drawn with a syringe directly from the heart of sedated rabbits. Centrifugation was performed on the blood and anticoagulant mixture at 22°C for 10min at 2100rpm. The second centrifugation was carried out at 4000rpm for 6min at 22°C followed the transfer of supernatant into the new tubes. The supernatant was then discarded while PRP was kept. After enrichment, both the low and high concentrated platelet densities increased significantly. The ovaries received a recent injection of PRP.

PRP Injection Inside the Ovary

Rabbits were separated into three groups, each with seven animals, following the induction of IO:

I: OI Rabbits assigned for low concentration PRP (PRP-L) intraovarian injection (n=7)
II: PRP Rabbits with high concentration PRP (PRP-H) intraovarian injection (n=7)
III: OI Rabbits the control group with no treatment (n=7)

Surgical Procedure

Following a general anesthetic, the surgical procedure was performed intramuscularly with a Ketamine-Xylazine (15mg-5mg/kg bwt) mixture. The dorsum close to the upper flank position was shaved and cleaned with solutions of 10% iodopovidone and 70% ethanol the day following the final D-galactose dose. To reach the left and right ovaries, a tiny incision was then created on either side. These does were injected with a 0.5mL PRP solution made from 3mL aspiration from the heart. According to their groups' PRP-a and PRP-b, ovarian injections were administered. The wounds were ultimately stitched up and cleaned with an iodopovidone solution. For five days, 50 mg/kg of Neomycin was administered to rabbits to prevent bacterial infections.

Sampling and Histopathologic Examinations

After PRP injection, ovarian samples were collected for two weeks for histological analysis. Per group, three rabbits were chosen at random for this purpose. Overdoses of ketamine and xylazine were used to humanely euthanize rabbits, and the ovary was excised and fixed in 10% formalin. The specimens were immersed in paraffin for histological analysis, and three consecutive 5µm thick slices were cut. Then, hematoxylin and eosin (H&E) staining solution was applied to the sections. Ovarian sections were evaluated for any pathogenic reaction and structural alterations. The number of primary, secondary, and antral follicles was counted and compared to that of the control rabbits.

Using ELISA to Measure Serum Levels of FSH and Estrogen

To assess any connections and feedback between serum levels of FSH and Estrogen in IO circumstances before and after PRP treatment, systemic FSH levels were determined using a suitable ELISA kit. Blood samples were taken for this purpose two weeks following PRP injection.

Mating Trial

To determine the fertility status of the rabbits, three remaining rabbits from each group were confined with male rabbit that had been proven fertile for five days. Following parturition, each mother rabbit was kept in a separate cage, and the number of healthy kits each delivery was recorded.

Statistical Analysis

All acquired results were presented as means standard deviations and assessed using IBM SPSS statistics. Data at each interval following PRP injection to determine the important differences between groups. The significance level was set to $P<0.05$.

Table 1: Concentration of PRP –L on serum D-galactose

Treatment	Control group	Induction D-galactose group	PRP-L group
FSH (mIU/mL)	1.07±0.17c	2.30±1.32a	1.60±0.57b
Estrogen (pg/mL)	7.55±1.73a	5.55±0.78b	7.87±0.93a

Values (Mean±SD) bearing different letters in a row differ significantly ($P<0.05$).

Table 2: Concentration of PRP –H on serum D-galactose

Treatment	Control group	Induction D-galactose group	PRP-H group
FSH (mIU/mL)	1.99±0.80b	2.80±0.80a	1.49±0.38b
Estrogen (pg/mL)	7.16±1.195b	5.36±0.91c	8.08±2.21a

Values (Mean±SD) bearing different letters in a row differ significantly ($P<0.05$).

RESULTS

Effect of PRP –L on Serum D-Galactose

Ten days after administration of D-galactose, the IO had significantly higher levels of FSH (2.30±1.32) and lower levels of E2 (5.55±0.78) than the control group. In addition, 0.5mL of PRP-L injected intra-ovarian into the galactose rabbit groups significantly changes the hormone levels. The results proved that a statistically significant difference (P<0.05) was found between the levels of E2 (7.87±0.9) and FSH (1.60±0.57) between the D-galactose induction group and the other groups (Table 1).

Effect of PRP –H on Serum D-Galactose

Ten days after administration of D-galactose, the IO had significantly higher levels of FSH (2.80±0.80) and considerably lower levels of E2 (5.36±0.91) than the control group (P <0.05) (1.99±0.80 and 7.16±1.195). In addition, 0.5mL of PRP-H injected intra-ovarian into the galactose rabbits significantly changes the hormone levels. E2 levels had the highest significantly value (P<0.05) (8.08±2.21), and FSH levels had the lowest significant value (P<0.05) (1.49±0.38) in comparison to the other induction D-galactose group (5.36±0.91 and 2.80±0.80) (P<0.05) respectively Table 2.

Intra-Ovarian Injection of PRP Improved Fertility Status of IO Rabbits

After two weeks from surgery, three does from each group subjected to mating trials. The results proved that the average number of kits in PRP –L group was 3 kits/birth while in PRP –B was 4 in which it closer to control group where it 6 kits/birth. It was significantly higher (P<0.05) than IO group (1 kit). Following administration of D-galactose for induction of IO, histopathological examinations showed that there was a separation of granulosa cells from oocytes and atresia had affected all stages of follicular development (Fig. 2a). There was an absence of normal follicle development in rabbits treated with D-galactose. (Average follicle number: Control=12.42±1.43; D-galactose=0±0). Consequently, the number of atretic follicles in the control group was 3.60±0.82, while in D-galactose was 58.76±6.64. In the D-galactose group, follicles in different stages of development displayed detachment, degeneration of granulosa cells, shrinkage, and atresia Fig. 2 compared to the control group Fig.1a. In Fig. 2, there was an increase in the atretic follicles after injection of D-galactose.

After PRP Injection, the Ovarian Function was Observed to be Enhanced.

According to the findings of histopathological studies, rabbits treated with PRP-L and PRP-H groups unveiled significant (P<0.05) increases in the number of normal follicles in comparison to control an IO group (PRP-L=38.65±2.26; PRP-H=45.541.54, Control= 90.41±5.63; D-galactose=0±0), respectively (Fig. 3,4,5 and 6 a-c). There was a significant decrease in the numbers of atretic follicles (29.87±2.71, 33.74±1.72 and 20.74±3.12) in control, PRP-L and PRP-H groups respectively than in D-galactose group (84.46±2.35) (Fig. 3, 4, 5, and 6 a-b). Moreover, we observed that D-galactose injection caused pathological remodeling such as hyperemia (as indicated by dilated vessel structures) and fibrosis.

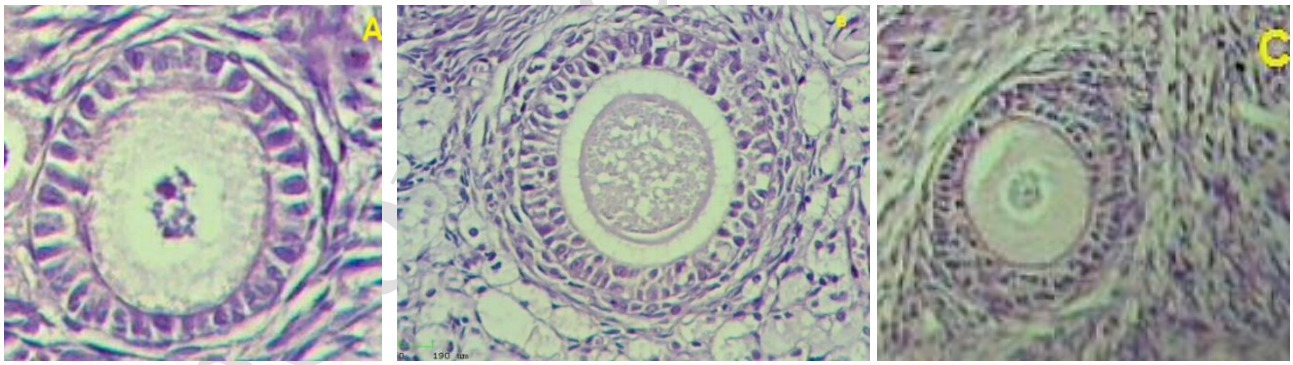

Fig. 1: control group showing (A) Primary (B) Secondary (C) Antral Follicles (H&E, 400X).

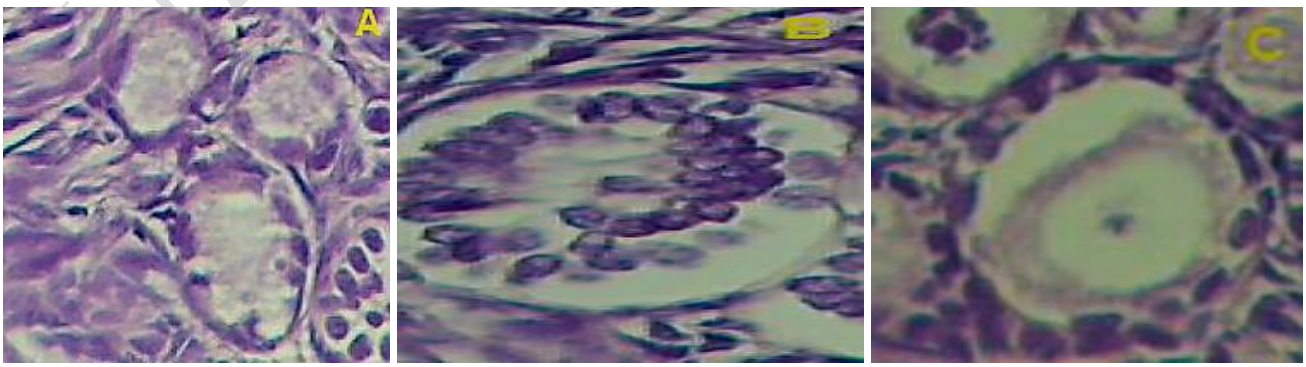

Fig. 2: D-galactose group showing an (A) Primary (B)Secondary (C) Antral Follicles (H&E; 400X).

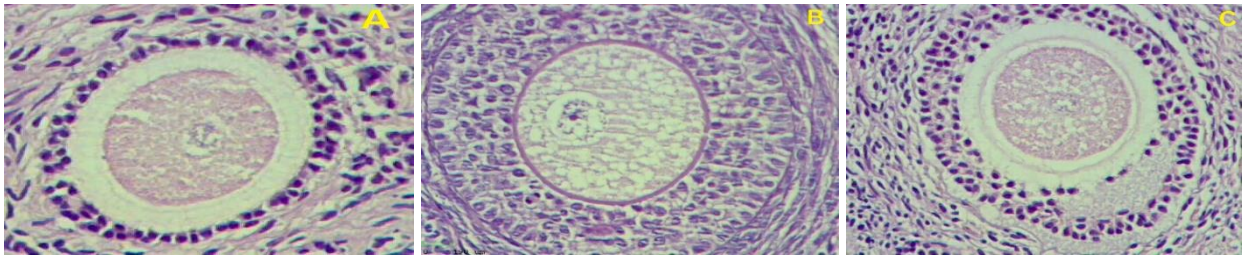

Fig. 3: Ovarian sections from control group showing (A) Primary (B)Secondary (C) Antral Follicles (H&E; 400X).

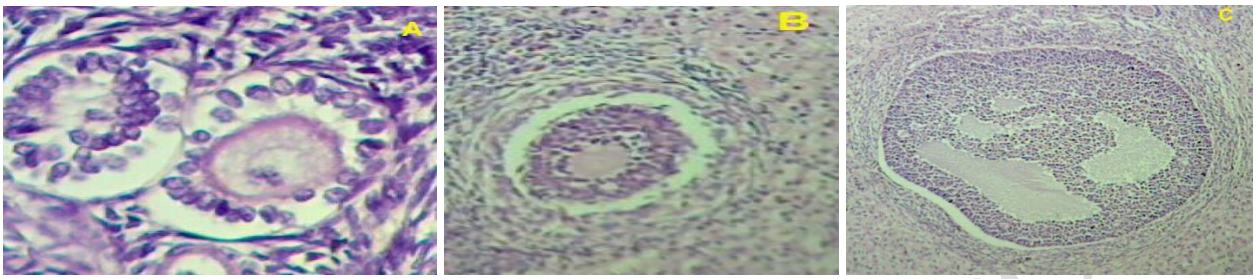

Fig. 4: Ovarian sections from the IO / D-galactose group showing an (A) Primary (B)Secondary (C) Antral Follicles (H&E; 400X).

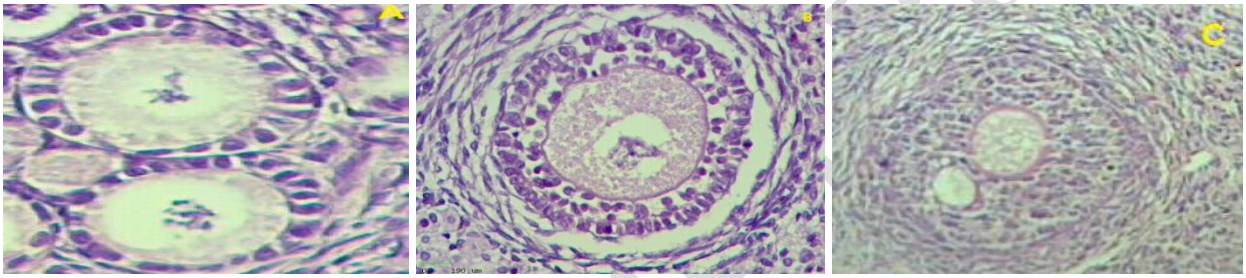

Fig. 5: Ovarian sections from PRP-L showing (A) Primary (B)Secondary (C) Antral Follicles (H&E; 400X).

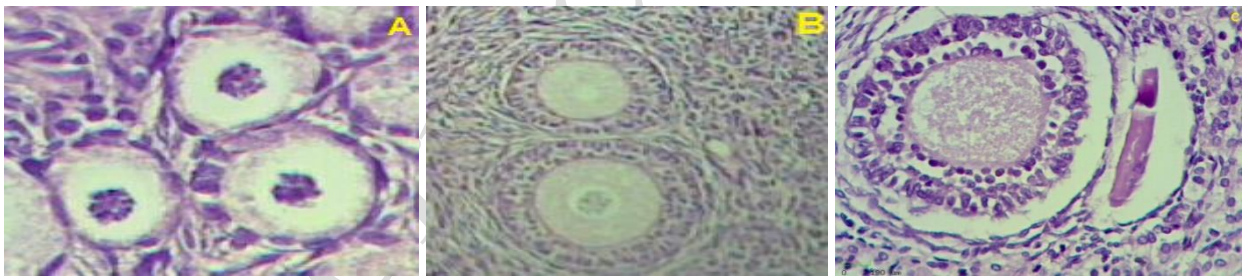

Fig. 6: Ovarian sections from PRP-H showing(A) Primary (B)Secondary (C) Antral Follicles (H&E; 400X).

DISCUSSION

Many studies demonstrated that IO considered as a symptom in female sever from galactose toxicity (Rubio-Gozalbo et al. 2010; Yan et al. 2018). Studies have indicated that ingested lactose is converted to glucose and galactose by the intestinal enzyme called lactase (Man et al. 2022; Lu et al. 2022). The further metabolism of galactose to UDP-glucose involves three major enzymes, galactokinase, galactose-1-phosphate uridyltransferase (GALT), and UDP-galactose-4-epimerase (Ma et al. 2022). There are three clinically significant disorders of galactose metabolism among which are seen in the ovaries (Dovom et al. 2022; Patel et al. 2022).

The galactose toxicity in ovaries leads to an increase in FSH levels and a decrease in estrogen levels due to galactose and its metabolite interference with gonadotrophin signaling (Liu et al. 2000; Abdollahifar et al. 2022). The dysfunction in IO group is caused by defect in the receptors signaling not by loss of FSH biopotency (Tucker et al. 2016). Two weeks after treatment, intraovarian administration of PRP at both low and high concentrations improved ovarian functions. This effect may be the result of a reduction in follicular atresia, thereby restoring fertility, this may be due to carrying growth factors to the ovaries. (Guevara-Alvarez et al. 2014). There was a significant decrease in the number of atretic follicles in the PRP-H group than PRP-L group; this value was because of negative feedback and follicular dominance on growing follicles (Fortune et al. 2004).

The regenerative properties of PRP because it contains growth factors like insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF),

transforming growth factor-β, basic fibroblast growth factor and epidermal growth factor (EGF) (Sánchez-González et al. 2012; Ramaswamy et al. 2018; Sfakianoudis et al. 2019). Many researchers reported that injection of PRP into the stroma of ovaries leads to an increase in the retrieved follicles in different stages of development and eggs (Sills et al. 2018; Sfakianoudis et al. 2018; Pantos et al. 2019; Keikha et al. 2022). These differences in follicular growth are due to the interaction between local factors produced by granulosa and theca cells with FSH and LH (Gougeon 2010; Yang et al. 2022). It was concluded that PRP can partially restore the function of IO ovaries in rabbits.

Conclusion

In conclusion therefore, the increase in follicles in PRP groups compared to galactose group indicated that PRP had a protective effect against galactose toxicity and improved follicular growth.

Author's contribution

All authors participated in the surgical procedure and intermittent examination.

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