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The effect of PMSG treatment on the ovarian histomorphometry of prepubertal rabbits

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Abstract

The study aimed to determine the effect of pregnant mare serum gonadotropin (PMSG) administration on ovarian immunohistomrphology and follicular growth in prepubertal female rabbits (*Oryctolagus cuniculus*). Sixteen animals, aged 75±5 days, were equally assigned into two groups, each containing eight animals. Animals in the control group were administered with normal saline (1 ml), while the treatment group received a single intramuscular injection of PMSG (100 IU). After 72 hours, the animals were euthanized, and their ovaries were dissected. Ovarian sections were stained with hematoxylin and eosin to determine ovarian features, follicle measurements, and classifications. The estrogen receptor beta (ERs-β) expression pattern was recognized by immunohistochemical staining. The diameter of the secondary, preantral, and antral follicles was significantly greater ($P<0.05$) than the control group. Interestingly, the matured large-sized follicles were exclusively detected in the treated group. However, relative to control, the proportion of preantral and antral follicles was significantly increased ($P<0.001$). In contrast, PMSG triggered a substantial reduction ($P<0.001$) in the percentage of secondary follicles. The control group demonstrated an intense expression of ERs-β in the nuclei of granulosa cells of preantral follicles, stroma, theca, and epithelial cells. However, in the treated group, the staining pattern of ERs-β was largely decreased in the growing and antral follicles. Collectively, a single PMSG treatment effectively induced ovarian hyperstimulation in prepubertal rabbits, significantly increasing the size and number of developing follicles. Further research is necessary to understand the marked decrease in ERs-β expression triggered by PMSG treatment.

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Introduction

Rabbits (*Oryctolagus cuniculus*) are economically significant small herbivorous mammals due to their short generation interval, high reproductive potential, fast growth rate, and breed diversity (1). Female rabbits reach puberty around 14-15 weeks and become sexually mature at 18-20 weeks. Hence, rabbits do not have a regular estrous cycle; they express estrus at 4-6 days intervals or 7-10 days without mating (2). In rabbits, ovarian follicle formation, activation, and development occur postnatally, with primordial follicle assembly completed between 2 and 4 weeks of age (3). During the prepubertal period, a proportion of the dormant follicles are triggered to grow; however, these follicles undergo atresia due to inadequate pituitary gonadotropins (4). Ovulation in female rabbits is induced approximately 10 to 13 hours after mating (2,5). Even though considerable progress has been made in the study of folliculogenesis, manipulation of ovarian function, and gonadotropin biochemistry, the application of ovarian hyperstimulation remains a challenge (6,7). Ovarian hyperstimulation involves administering gonadotropins to artificially induce

follicular growth and release more oocytes in a single reproductive cycle (8,9). Furthermore, reduced dosages or shorter therapeutic protocols with exogenous gonadotropin have become the most prevalent method for mild ovarian stimulation (7). For instance, in rabbits, ovarian stimulation by various exogenous hormonal treatments, e.g., follicle-stimulating hormone (FSH), Pregnant mare serum gonadotropin (PMSG), or gonadotrophin-releasing hormone GnRH, has been investigated (9-11). PMSG (also termed equine chorionic gonadotrophin, eCG) is a glycoprotein hormone produced by endometrial cups of pregnant mares (from the 35th day of gestation), which exhibits luteinizing hormone (LH)-like action. Nevertheless, it demonstrates LH and FSH-like activity in heterologous animal species (6,12). The incorporation of gonadotropins, notably PMSG, has become indispensable in assisted reproductive technology (ART), enabling the retrieval of a substantial number of oocytes in a single in vitro fertilization (IVF) practice (13). It has been suggested that ovarian hyperstimulation may be dose-dependent. For instance, the administration of a higher dose of PMSG (200 IU) significantly decreased the oocyte recovery rate compared to lower doses (50 IU) and the untreated control (11). In contrast, another work indicated that the number of recovered oocytes improved with the increased PMSG doses (14). It is well-known that the growth of multilayered follicles is controlled by the hypothalamic-pituitary-ovarian axis, where estrogen plays a crucial role not only in modulating the gonadotrophin secretion and secondary sexual traits but also in the process of granulosa cell differentiation (15,16). To express its function, estrogen signaling is mainly mediated through two types of nuclear receptors (ERs-α and ERs-β) and a third type named membrane estrogen receptors (17,18). Studies have indicated that the estrogen's nuclear receptors are differentially expressed in the ovaries according to the growth stage reviewed (19,20). In mice and rats, infertility disrupted follicle growth, and anovulation were the major characteristics of ERs-β knockout females, indicating the crucial role of ERs-β in preserving fertility (21,22).

Consequently, in addition to studying the implication of PMSG on follicle growth, the present work further hypothesized that the administration of exogenous PMSG might impact the expression of ERs-β. Only a few pieces of literature addressed the effect of the PMSG treatment on rabbit ovaries. Therefore, the present work exploited prepubertal rabbits as a model to investigate the impact of a single PMSG injection on ovarian morphological features, follicle growth, and its possible association with ERs-β expression.

Materials and methods

Ethical statement

The experimental protocols and animal welfare considerations were approved by the Institutional Animal Care and Use Committee, College of Veterinary Medicine/University of Mosul (approval number: UM.VET.2023.060).

Experimental animals

Prepubertal female rabbits (*Oryctolagus cuniculus*; n=16) aged 75± 5 days old and almost had comparable body weights (2±0.25 kg) were utilized in this study. Animals were purchased from rabbit breeders and maintained in the Animal House Unit (College of Veterinary Medicine/ University of Mosul) under the same conditions. Animals had free access to food (leafy greens, fresh fruits, grass hay) and water. Standard laboratory conditions were provided, including temperature 24±4ºC, humidity 55%, and exposure to 14 hours of light daily. All animals were frequently monitored throughout the experimental period and confirmed disease-free. Following a one-week acclimation period, the animals were randomly divided into two identical groups (n=8 each). As indicated in a previous investigation (11), conflicting outcomes were observed in follicle growth in response to doses of PMSG (200 and 50 IU). Therefore, the current study utilized a single intramuscular administration of PMSG (Follimag® Mosagrogen, Russia) at a concentration of 100 IU per animal in the treated group. At the same time, the control group received an intramuscular injection of normal saline (1 ml). Following 72 hours of treatment, all animals were euthanized with inhaled ether before undergoing cervical dislocation.

Ovary dissection

To access the organs located in the peritoneal cavity, the animals were placed in a dorsal recumbent position, and a midline abdominal incision was made from the xiphoid cartilage to the pubic symphysis in a cranial to caudal direction. The ovaries were exposed and pictured in situ. Then, ovaries were incised and dissected free from adjustment tissues (ovarian bursa and oviduct). The dissected ovaries were transferred into a Petri dish containing normal saline drops with BSA 4% (Sigma). The transferred ovaries were cleaned under a dissecting microscope (Gmbh Ref. 14900, Wiesbaden Germany) using a surgical blade and needles, gauge 18 (23).

Hematoxylin and eosin staining

In both groups, the left ovaries (n=8 each) were utilized for hematoxylin and eosin staining (Surgipath). The freshly cleaned ovaries were preserved in neutral buffered formalin 10% before being embedded in paraffin. The paraffin blocks were sliced at a thickness of 5µm using a manual processing microtome (Reichert-Jung). Three midsections from each ovary were selected for staining following a standardized staining procedure (24). Sections were pictured using a digital camera (OMAX, A35180U3, China) attached to an optical microscope (Kruuse, Primo phot 290205, Denmark).

Follicle count and classification

The hematoxylin and eosin-stained sections were used to evaluate ovarian morphology and estimate follicle size utilizing ImageJ software (Fiji 1.46). Dual measurements were taken for each counted follicle from the basement membrane of granulosa cells, and the average of the vertical and horizontal measurements provided the follicle diameter. As primordial and primary follicles are gonadotrophin independent (25), only the advanced stages of follicle development (gonadotrophin dependent) were included. Thus, the follicles were categorized into four groups based on their morphology. The first is the secondary follicles, where an oocyte is surrounded by more than two layers of granulosa cells without antrum formation. Secondly, the preantral follicles are where an oocyte is enclosed in multiple layers of granulosa cells and contains 2-4 small antrum. Thirdly, antral follicles presented by a multilayered follicle with a single large antrum (< 800 µm). Fourth, fully matured follicles that expressed a large antrum (> 800 µm) (12,26).

Immunohistochemical staining

To investigate the impact of PMSG treatment on the expression pattern of ERs-β, ovary sections from both groups (right ovaries, n=8 each) were used for the localization of ERs-β. According to the manufacturer (Novus Biologicals NB200-305) and a protocol from previous work (25), ovary sections were dewaxed in xylene and rehydrated through series of decreasing ethanol concentrations. After a single step of washing in distilled water, antigen retrieval was performed by dipping the sections in citrate buffer 0.01 M (pH 6.0) and heated in a microwave (4×5 minutes) before being double washed in phosphate-buffered saline. To minimize nonspecific binding, the sections were treated with blocking serum (BSA 4%; Vector Laboratories, SP-5050-500) at room temperature for 20 minutes. Subsequently, sections were incubated overnight at 4°C with a mouse monoclonal ERs-β antibody (1:400µl; NB200-305, Novus Biologicals USA). For negative control, sections were exposed to comparable concentrations of non-immune mouse IgG. Following three washes in PBS, sections were treated with goat anti-mouse IgG secondary antibody (1:200, HAF007) for 30 minutes at room temperature in a dark, humidified chamber. After a double wash in PBS, slides were incubated in diaminobenzidine substrate (DAB) until the brown color appeared. Following a double wash in PBS, sections were counterstained with hematoxylin and rinsed with tap water. Eventually, the sections were dehydrated in ethanol, cleared with xylene, and mounted for imaging.

Statistical analysis

The follicle size was estimated with ImageJ software (Fiji 1.46), and the data were expressed as mean (µm)±SEM. Statistical analysis of the mean follicle sizes (classified follicle groups) across groups was performed using One-Way ANOVA with a post hoc test to assess the variability. Significant differences in follicle proportions between the groups were evaluated using the Chi-square test. The assessments were performed using Sigma Plot 12.5 software, where the inconsistency between the treated and untreated groups was examined at $P<0.05$.

Results

Gross findings

The ovaries are positioned on either side of the uterine horns and fixed to the abdominal cavity by the mesovarium. Both ovaries are situated posteriorly to their respective left and right kidneys. The left ovary is slightly separated from its corresponding kidney, while the right ovary is located apart from the right kidney. The right and left ovaries were either elongated or somewhat triangular, displaying a color ranging from greyish to slightly yellowish. The ovaries of the untreated group appeared small, and the ovarian surface contained numerous small blister-like follicles without exhibiting any large antral follicles or corpora lutea. Interestingly, in the PMSG-treated group, the left and right ovaries demonstrated large antral follicles prominent from the ovarian surface. Remarkably, several antral follicles appeared with a dark red to pinky coloration (hemorrhagic), figure 1 similar to the control group, no ovulated nor corpora lutea were distinguished.

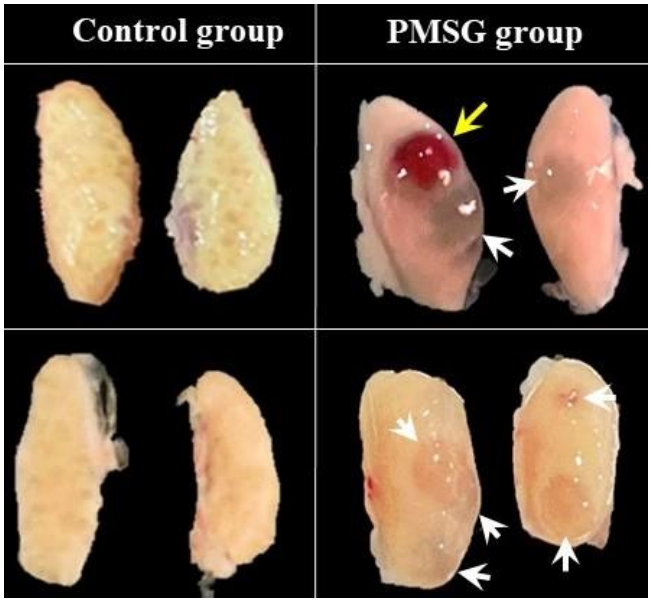

Figure 1: The dissected ovaries from the prepubertal untreated/ PMSG-treated rabbits. The ovaries in the control group appeared elongated and contained small follicles. Grossly, the right ovaries are slightly larger than the left ones. The ovaries of the treated group appeared larger, and large antral follicles (white arrows) were present. A treated female ovary reveals a large follicle with a dark red-pinky color (yellow arrow).

Microscopic examination

Examination of the untreated immature stained ovary sections reveals that the ovaries are covered with pseudostratified columnar epithelium. Beneath the surface epithelium, a layer of dense irregular collagenous connective tissue fibers is present (tunica albuginea). Ovary sections displayed two regions represented by the outer cortex and an inner medulla. In the cortical area, the predominant type of follicles consists mainly of primordial follicles, accompanied by a lesser number of primary follicles and minimal numbers of small secondary follicles. However, numerous small preantral follicles were identified in the central region of the ovary (Figure 2).

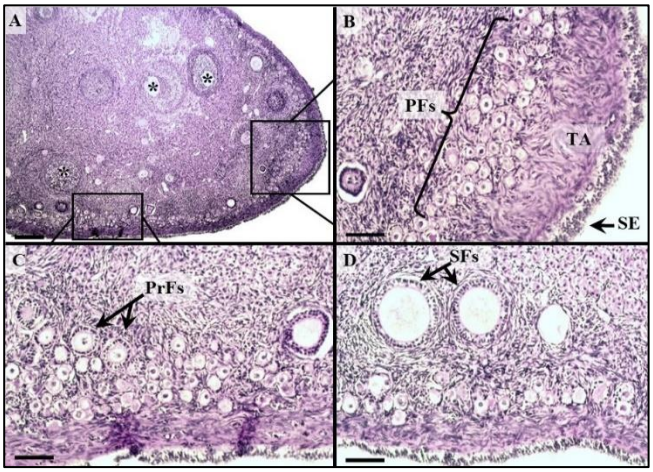

Figure 2: Ovary section of an immature untreated female rabbit. The hematoxylin-eosin-stained sections from the control group revealed the ovarian morphology and distribution of follicles. Small preantral follicles (starred) are presented at the central area of the ovary section (A, scale bar 200µm). The zoomed cortical region (B-D, scale bar 100µm) reveals the surface epithelium (SE) followed by tunica albuginea (TA). The cortex is enriched with primordial (PFs), numerous primary (PrFs), and numerous secondary follicles (SFs).

Relative to control, treatment with PMSG exposed an enormous distinction in the ovarian morphology and the associated ovarian structures. Interestingly, the ovarian response to the PMSG treatment varied among animals in the treated group. For instance, an ovary section showed the presence of three large mature antral follicles with numerous secondary follicles. Relative to control, the population of primordial and primary follicles was largely reduced. In addition, large follicles extending from the outer cortical region toward the interior (medullary region) made it challenging to determine the medulla (Figure 3). Another ovarian section in the treated group revealed the presence of a single large mature follicle. However, by comparison with the previous ovarian section, more cortically presented primordial and growing follicles were determined (Figure 4). Two other ovarian sections demonstrated a high number of preantral and antral follicles; however, unlike the two previous figures from the treated group, no large antral follicles were presented. Unlike the control, neither ovulation nor corpora lutea were distinguished in the ovarian sections (Figure 5).

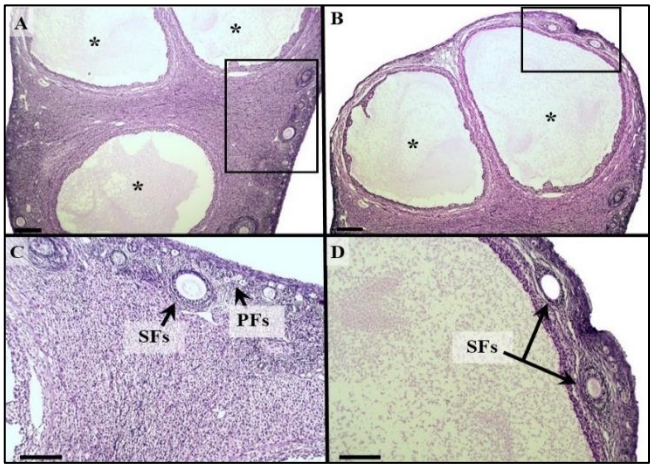

Figure 3: The effect of PMSG treatment on the ovarian structure. The PMSG treatment caused massive follicle growth; three large antral follicles (A-B, starred) were distinguished. The zoomed squares (C and D) demonstrated only several secondary follicles (SFs) accompanied by a sharply decreased number of primordial follicles (PFs); Scale bar 200µm.

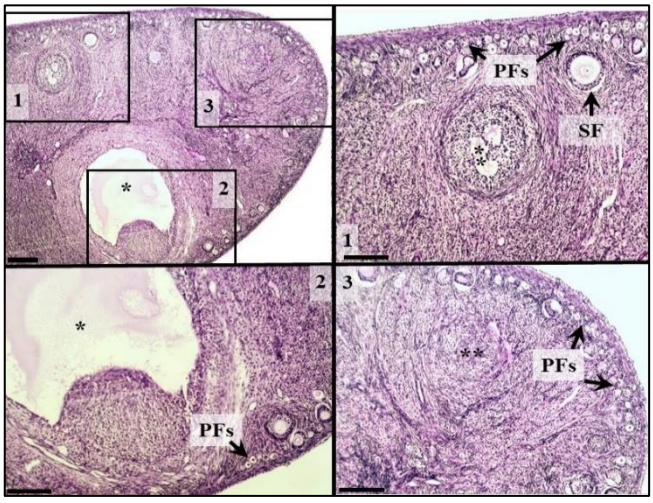

Figure 4: The effect of PMSG treatment on the ovarian structure. Another ovary section demonstrated the presence of a single large matured antral follicle (single star). High-power images (1-3) revealed a decline in the earlier follicle growth stages (Preantral follicles: two stars; primordial follicles: PFs; secondary follicles: SFs). Scale bar 100µm.

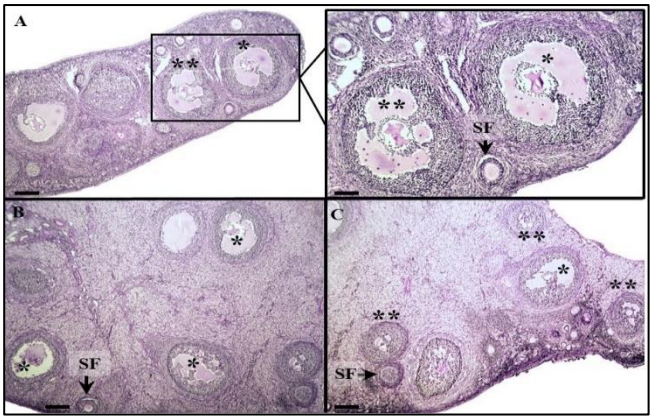

Figure 5: The effect of PMSG treatment on the ovarian structure. The ovary sections (A-C, scale bar 200µm and the boxed magnified area, scale bar 100µm) specified the presence of many preantral (two stars) and antral follicles (one star). However, large mature follicles are not presented. The upper ovarian panel (A) appeared elongated, demonstrating only numerous secondary follicles (SF). Both ovarian sections revealed a reduced population of primordial follicles.

Effect of PMSG treatment on follicle growth and proportion

To determine the impact of PMSG on follicle growth relative to control, follicle diameter was measured using ImageJ software. The mean diameter of the secondary, preantral, and antral follicles in the treated group (90.2± 0.6, 215.4 ±5.4, and 499.8± 5.9 µm, respectively) was significantly greater (P<0.05) than the relative follicular growth stages in control (73.1±0.7, 183.4±10.3, and 377.1±17.1 µm, respectively). Interestingly, the matured large-sized follicles (1053.6±14.7 µm) were only specified in the ovaries of the treated group. In the PMSG-treated group, there was a notable decrease in the proportion of secondary follicles compared to the control group (21% vs. 79.8%, respectively; P<0.001). Conversely, the percentages of preantral (34.1%) and antral follicles (38%) were significantly higher in the PMSG-treated group compared to the control (17.3% and 2.9%, respectively; P<0.001). However, fully matured follicles (6.9%) were only observed in the treated group (Table 1).

Table 1: The effect of PMSG treatment on follicle diameter and proportion

Groups	Classification	Number (%) counted follicles	Mean diameter (µm± SEM)
Control group	Secondary	138 (79.8) ^{a**}	73.1± 0.7 ^{a*}
	Preantral	30 (17.3) ^{a**}	183.4± 10.3 ^{a*}
	Antral	5 (2.9) ^{a**}	377.1± 17.1 ^{a*}
	Mature	-	-
	Total	173 (100%)	
Treated group	Secondary	82 (21) ^{b}	90.2± 0.6 ^{b}
	Preantral	133 (34.1) ^{b}	215.4± 5.4 ^{b}
	Antral	148 (38) ^{b}	499.8± 5.9 ^{b}
	Mature	27 (6.9)	1053.6± 14.7
	Total	390 (100%)	

The Chi-square test revealed significant variations (**P<0.001) in group follicle proportions. The proportion of secondary follicles in the control group was significantly higher than in the treated group. However, the percentage of the advanced growth stages was dramatically increased in the PMSG-treated group relative to the control group. The mean follicle diameter of the tested growth stages was significantly higher (*P< 0.05) in the PMSG-treated group than in the non-treated control. Fully matured follicles were not observed in the control group. Data were presented as Mean (µm) ± SEM and were analyzed using One-Way ANOVA with post hoc test. The varied letters (a and b) in both columns at the same growth stage represent significant differences.

Localization of ERs-β

The potential effect of the PMSG treatment on the ovarian expression of ERs-β protein was tested. In the untreated control, ERs-β was extensively expressed in the nuclei of the epithelial and stroma cells but not in the oocytes or the subepithelial tissue. Consistently, the stain was undetectable in primordial follicles; however, several nuclei of the granulosa cells were detected as positive in the activated and secondary follicles. Interestingly, a higher intensity of staining was detected in the nuclei of the granulosa cells of the preantral and small antral follicles. Theca cells expressed a weakened pattern of staining (Figure 6). Similar to the control group, the stained sections from the PMSG-treated group revealed positive staining for ERs-β protein in the stroma, epithelial cells, and theca cells. However, the most interesting finding is that the granulosa cells of the growing follicles (preantral and large antral follicles) were detected negative. Moreover, several granulosa cells of the atretic follicles were also detected negative for ERs- β staining (Figures 7 and 8).

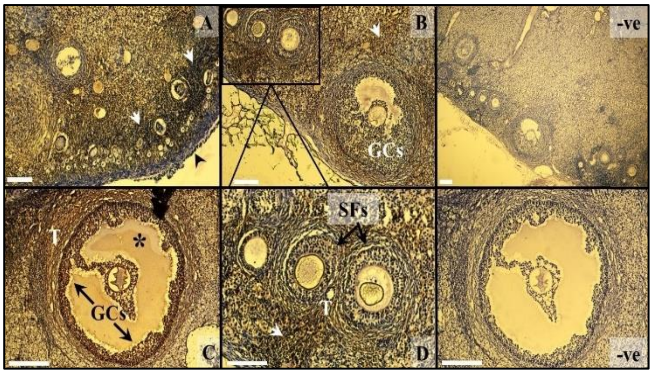

Figure 6: Expression of ERs-β protein in the ovaries of untreated rabbit. ERs-β protein was localized in the nuclei of epithelial cells (A, black arrow) and stroma cells (white arrow); however, the staining is undetectable in subepithelial tissue, oocytes, and primordial follicles. In small antral follicles, a strong stain is evident in nuclei of granulosa cells (GCs) (starred, B and C). However, the zoomed area (D) revealed that only numerous GCs of secondary follicle expressed staining. Theca cells (T) expressed a weakened pattern of staining. The negative controls (-ve) do not express any staining; Scale bars 100µm.

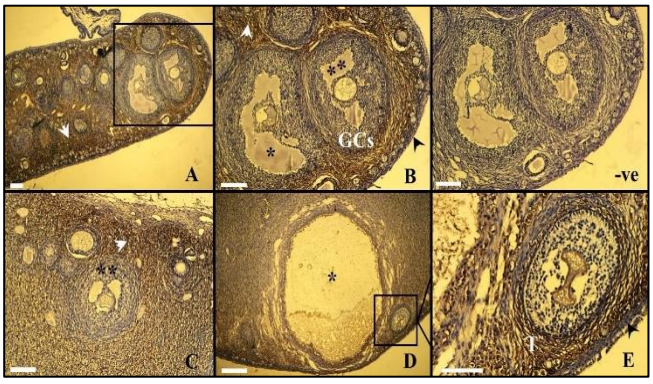

Figure 7: Expression of ERs-β protein in the ovaries of the PMSG-treated rabbits. The low-power image (A) demonstrated strong staining for ERs-β protein in the stroma (white arrow). However, the majority of the presented growing follicles were detected as stain-negative. The zoomed area (B-D) revealed that ERs-β was undetectable in the granulosa cells (GCs) of preantral (double stars) and antral follicles (starred) as well as in the primordial follicles located in the peripheral area. The zoomed area (E) demonstrated an atretic follicle where the granulosa cells and subepithelial tissue were detected negative for ERs-β stain. However, epithelial cells (black arrow) and theca cells (T) expressed a strong staining pattern. The negative control (-ve) does not express any staining; the Scale bar is 100µm.

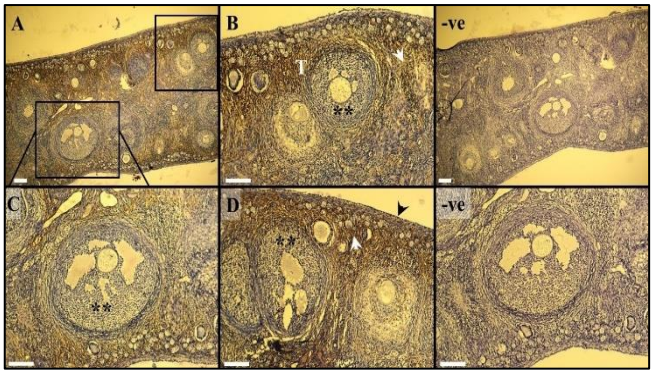

Figure 8: Expression of ERs-β protein in the ovary of the PMSG-treated rabbit. The same expression pattern, ERs-β staining, is confirmed in another treated rabbit. The zoomed images (B and C) and D show strong staining for ERs-β protein in the stroma (white arrow), epithelial cells (black arrow), and theca cells (T). However, ERs-β was undetectable in preantral follicles' granulosa cells (GCs) (starred). The negative controls (-ve) do not express any staining; the Scale bar is 100 µm.

Discussion

Despite extensive research investigations in various animal species, ovarian response to hyperstimulation and superovulation remains controversial (7-9,27). PMSG alone or with an ovulation inducer (hCG) has been widely used to obtain many oocytes from a single dosage (6,8,13,28). Therefore, the present study utilized prepubertal rabbits as a model to study the efficiency of single PMSG treatment on ovarian follicle growth. Prepubertal female rabbits serve as a valuable experimental model for studying the impact of hormonal treatment due to the diverse developmental stages of follicles in their ovaries, excluding the fully antral follicles and corpora lutea. Importantly, immature rabbits have likely not yet engaged in reproductive cycles or encountered hormonal fluctuations that could potentially influence the growth of ovarian follicles.

Consequently, this diminishes variability in research outcomes and facilitates more precise analysis. Moreover, rabbits have served as a pivotal animal model in various experimental research areas, including surgery (29), medicine (30), and stem cell investigations (31), and not limited to female reproductive studies but also extending to male research (32-34). In this study, the location and gross morphology of prepubertal female rabbits' ovaries confirmed the observations indicated in previous studies (2,35). Consistent with other studies (35,36), the histological examination demonstrated that the prepubertal ovaries in the control group are composed of two areas: the outer region (cortex) is covered by the surface epithelium and consisting mainly of primordial, primary, and secondary, rarely numerous small preantral follicles, and lacks for fully mature

antral follicles. The second region is the interior (medulla), which consists of somatic cells and connective tissue enriched with blood vessels. By comparison with the control, the gross examination of the dissected ovaries from the treated group revealed the presence of both the preantral and large antral follicles prominent from the ovarian surface. Two ovaries from this group displayed the presence of large hemorrhagic antral follicles. A similar observation in rabbits was indicated in previous studies stating that ovarian hyperstimulation using PMSG or FSH successfully increased follicular growth accompanied by an increased number of hemorrhagic follicles (10,37). Previous work on ewes indicated that the half-life of PMSG is longer (several days) than FSH, a few hours (38). Another investigation in cows specified that a single dose of FSH was ineffective in significantly impacting the number and viability of the collected oocytes (39). Several hemorrhagic antral follicles might be due to the prolonged effect of PMSG accompanied by ovulation failure because rabbits are induced ovulators.

Conversely, another related study (11) reported that the administration of 200 IU of PMSG resulted in a considerable reduction in oocyte retrieval rate as opposed to the injection of 50 IU or without treatment (28.8% vs. 47.7% and 48.7%, respectively). In the present study, a dose of 100 IU was used, indicating that PMSG might exert its effects in a dose-dependent manner. In addition, even though the prepubertal rabbits were approximately the same age and received the same dose of PMSG, animals responded differentially to the treatment. In other words, not all ovaries in the treated group expressed fully matured antral follicles; however, all treated animals demonstrated large preantral/ antral follicles relative to the control. This observation aligns with findings from numerous prior studies indicating that the ovarian response to super-stimulation can vary significantly based on various factors, For instance, inheritances, age, breeds, parity, the biological condition of the animal (27), the dosage of hormones, and the timing of treatment or sacrifice (11). Another previous study indicated that PMSG treatment revealed a significantly higher ($P<0.05$) follicle count on the right side than left ovaries (9).

Similarly, due to the increased number of antral follicles, female rabbits treated with PMSG had heavier ovaries (0.60 g) than those injected with the saline solution (28). The present study demonstrated that treatment with PMSG caused a significant increase ($P<0.05$) in the mean diameter of the secondary, preantral, and antral follicles relative to the relevant follicular growth stages in the untreated control. These results agreed with many previous associated studies (9,13,28). Notably, PMSG treatment led to a noticeable decrease in the proportion of secondary follicles compared to the control group. This outcome might have accounted for the follicle growth-accelerated impact of PMSG on the secondary stage, which eventually progressed into more advanced growth stages. Consistent with the gross examination and relative to the untreated group, the proportion of preantral and antral follicles was dramatically elevated in response to the PMSG treatment.

In contrast to the treated group, the ovaries of the control group exhibited an absence of large-sized matured follicles. Therefore, the assessment of ovarian morphology, follicle measurements, and proportions distinctly pointed towards the positive impact of PMSG on stimulating the growth trajectory of developing follicles that consequently progress into antral follicles. These effects can be attributed to the fact that PMSG exerts the functional role of both the pituitary FSH and LH on the ovaries (6,12). This observation aligns with previous research findings that demonstrated a markedly higher number of antral follicles in immature rabbits treated with PMSG than in untreated rabbits (28).

In both groups, the gross and microscopic examination revealed no evidence of ovulation or luteogenesis, where such a finding is expected as all of the utilized animals were prepubertal, and the type of ovulation in rabbits is induced. This work provides a crucial and preliminary insight into the implication of gonadotropins on the ovarian expression pattern of ERs-β. In the control group, a weak staining intensity was specified in the nuclei of granulosa cells of small growing follicles; However, the multilayered, preantral, and small antral follicles revealed intense staining. A similar observation was indicated in earlier works in rats (40,41), mice (42), hamsters (43), monkeys and humans (44), and bovine (45). In addition, numerous granulosa cells of atretic follicles demonstrated weak positive staining for ERs-β. However, a previous study indicated that ERs-β was undetectable in atretic follicles (40); this variation might be associated with the stage of the follicle's degenerative changes. The elevated expression of ERs-β in the granulosa cells is essential for the modulation of follicle development and ovulation-associated genes, such as a steroidogenic enzyme (Cyp19a), LH, and progesterone receptors (46).

Interestingly and relative to control, the PMSG treatment triggered a significant decline in the expression pattern of ERs-β in the granulosa cells of preantral and large antral follicles. In rats, a previous study reported a high expression level of ERs-β mRNA in granulosa cells of immature ovaries. However, in mature ovaries, the ERs-β level, but not ER-α, was significantly downregulated in the preovulatory follicles along with the increased gonadotropins secretion at the onset of proestrus (47). Another interesting finding was specified in hamster ovaries where the elevated LH surge or administration of human chorionic gonadotrophin triggered a significant upregulation in the ER-α convoyed with a downregulation of ERs-β expression. Moreover, the expression of ERsβ, but not ER-α, was dramatically reduced in the antral follicles, suggesting that such a scenario is essential for granulosa cell differentiation and follicle selection (43). Another study attributed the downregulation of ERs-β in the granulosa cells of the preantral/antral follicle to the increased LH surge, which triggers the disruption of the preexisting ERs-β mRNA (48). Conversely, another work

on bovine ovaries indicated that both estrogen receptors are detectable in the granulosa cells of developing and fully matured follicles, suggesting that both receptors are required for follicle growth (45). The variation in the expression pattern of estrogen receptors might credited to the species distinction, where rabbits are poly-ovulators while cows are classified as mono-ovulators.

Several limitations are addressed in connection with this work; for instance, administering PMSG at various dosages along with different time points of the ovary collection is required. Comparing the effectiveness and safety of PMSG with other gonadotropins such as FSH, LH, or a combination of both can offer valuable perspectives on the varying advantages and restrictions of distinct hormonal stimulation approaches. In addition, ovulation/ pregnancy rates are to be considered in ovarian hyperstimulation works by administering LH or exposing the PMSG-treated females to male rabbits. Moreover, the recovered oocytes will be tested for quality and validity for in-vitro fertilization (IVF). Furthermore, the rate of follicle atresia is to be considered in both the treated and untreated groups.

Another critical point that needs to be addressed in future work is that ovarian tissues should be utilized to express follicle growth-associated proteins/ genes. Lastly, the effect of PMSG treatment on the ERs-α expression will be tested along with ERsβ localization. Thus, further work is required to understand the processes and consequences of ERs-β modulation in response to PMSG treatment.

Conclusions

A single PMSG treatment demonstrated a significant increase in the diameter and proportion of the gonadotrophin-dependent follicular growth stages, except for the secondary follicle count. Despite variations in the ovarian response among prepubertal rabbits, PMSG treatment proves effective in stimulating follicle growth in prepubertal female rabbits. Treatment with PMSG effectively decreased the staining pattern of ERs-β, especially in the advanced stages of follicle growth, suggesting a potentially pivotal role in follicular development.

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Conflict of interest

The author declares no conflicts of interest.

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تأثير العلاج بالهرمون المحرض للقند لمصل الفرس الحامل على القياس النسيجي والشكلي للمبيض في الأرانب قبل البلوغ

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الخلاصة

هدفت الدراسة إلى الكشف عن تأثير حقن الهرمون المحرض للقند لمصل الفرس الحامل على التركيب النسيجي المناعي للمبيض والنمو الجريبي في إناث الأرانب ما قبل البلوغ. قسمت الحيوانات (عدد:١٦؛ بعمر ٧٥ ± ٥ يوم) إلى مجموعتين متساويتين (٨ لكل منهما). حقنت حيوانات مجموعة السيطرة بمحلول الملح الفسيولوجي (١مليلتر)، في حين تلقت مجموعة العلاج حقنة عضلية واحدة من الهرمون المحرض للقند (١٠٠ وحدة دولية). بعد ٧٢ ساعة، تم القتل الرحيم للحيوانات وعزل المبايض. صبغت المقاطع النسيجية للمبايض الهيماتوكسيلين والايوسين لتحديد مظهر المبايض، قياس قطر الجريبات وتصنيفها، كذلك تم الكشف عن تموضع مستقبلات هرمون الأستروجين بيتا بالصبغ النسيجي المناعي الكيميائي. أظهرت مجموعة المعالجة جريبات ما قبل الغارية والغارية كبيرة الحجم مع اختلاف معنوي بحجم الجريبات الثانوية وقبل الغارية والغارية نسبة الى مثيلاتها في مجموعة السيطرة. شخصت الجريبات الناضجة والكبيرة الحجم فقط في المجموعة المعالجة. أحدث العلاج بالهرمون انخفاض معنوي في النسبة المئوية للجريبات الثانوية؛ في المقابل، ارتفعت نسبة الجريبات قبل الغارية والغارية وبصورة معنوية مقارنة بالسيطرة. ومن المثير للاهتمام، أظهرت مجموعة السيطرة تصبغا مكثفًا لمستقبلات هرمون الأستروجين بيتا في نوى الخلايا الحبيبية للجريبات ما قبل الغارية والسدى والقراب والخلايا الظهارية. في حين، انخفض نمط الاصطباغ وبخاصة في الجريبات النامية والغارية إلى حد كبير في مجموعة المعالجة. بالخلاصة، اظهر العلاج بالهرمون المحرض للقند وبجرعة واحدة كفاءة في فرط تحفيز المبيض في الأرانب الغير بالغة حيث اظهر ارتفاع كبير في حجم وعدد الجريبات في مراحل النمو المتقدمة. من الضروري إجراء المزيد من الدراسات لتوضيح الاختزال الكبير للاصطباغ لمستقبلات هرمون الأستروجين بيتا في الجريبات الناضجة بالاستجابةً للعلاج بالهرمون المحرض للقند.