Original Research Article

ISSN (Print) 2786-0272 ISSN (Online) 2786-0280

Effect of Letrozole-treatment on luteal activity and reproductive performance in non-lactating ewes during breeding season

Abdel Dayem M. A. [1], Hassan M. [1] Senosy W. [1]

[1]Theriogenology Department, Faculty of Veterinary Medicine, New Valley University, Al kharga,72511, Egypt.

*Corresponding author: abdeldayem128@yahoo.com

Received at: 2024-11-12 Accepted at: 2024-12-11

ABSTRACT: This study aimed to compare estrus synchronization in ewes using nonsteroidal aromatase inhibitor (letrozole, for five days), combined with PGF2α injection at day of removal of sponges with the standard GPG (GnRH-PGF2α- GnRH) protocol. A total of twenty healthy pluriparous, cyclic and non-lactating Farafra ewes were randomly divided into two equal groups each containing ten ewes. Ewes in the first group received intravaginal sponges containing 7.5 mg letrozole for five days followed by intramuscular injection of PGF2 α at time of removal. The second group were treated with standard GPG protocol (involving injection of GnRH at day zero followed by injection of PGF2 α at seventh day and another GnRH injection at nineth day). Fertile ram were introduced to both groups for estrus detection and natural insemination. The percentage, onset and duration of estrus, conception and pregnancy rates as well as lambing rate were recorded for each group. Pregnancy diagnosis was performed at 15thDay after natural mating using ultrasonic device (detection of un-echoic embryonic vesicle in uterine horn ipsilateral to corpus luteum; echoic floating fetal membranes and echoic fetal structure adjacent to endometrium). The results revealed that estrus responses (detected by ram and confirmed by ultrasound findings) were 100% in both groups, while onset of estrus was significantly shorter (54.00 ± 0.40 hrs.) in letrozole treated group than in GPG treated group (72.64 ± 1.34 hrs.). Duration of estrus in letrozole treated group (25.17 ± 0.24 hrs.) were significantly shorter than GPG treated group (46.52 ± 1.70 hrs.). Non return to estrus were significantly higher (60%) in letrozole treated group compared to 50% GPG treated group. Pregnancy rates were significantly higher (60%) for letrozole treated group compared to 50% for GPG treated group. While the number of lamb born/ ewe lambed were significantly higher in letrozole (1.83) group compared to (1.2) for GPG group. Percent of ewe lambed twin were significantly higher in letrozole treated group (80%) compared to (20%) for GPG treated group. In the letrozole treated group, after sponge removal concentration of estradiol begin to rise in association to synchronous follicular recruitment and selection then it decreased again just before ovulation and continued to decrease along post ovulatory period. Serum concentrations of progesterone (ng/ml) decreased after removal of sponges and injection of luteolytic agent, also serum concentration of progesterone secreted from five days corpora lutea were significantly higher in letrozole treated group compared to GPG treated group. Concerning measured biochemical parameters, there were no significant differences between letrozole treated group and GPG treated group. It could be concluded that synchronization of estrus using Letrozole sponges+ PGF2α protocol has a better conception rate and reproductive performance than standard GPG protocol and further study should be applied on large flocks to confirm these findings.

KEYWORDS: Embryonic mortality, luetotrophic effect, Estrus synchronization

1. Introduction

A great overload on animal production industry is brought by increasing human welfare levels which in-turn increased demands for animal protein [1]. Researchers of animal production communities facing the challenges of keeping the current levels of animal proteins for daily increasing demands [2]. Improving reproductive management by applying hormonal treatment is main solution, frequently used hormones are e CG (equine chorionic gonadotropin) and progesterone aren't frequently available [3]. Regarding ewe estrus management, a novel protocol was recently described by researchers [4]. Involving use of letrozole as vaginal sponge for five days followed by injection of prostaglandin at day of removal, resulted in uniformity in onset of estrus. Letrozole is one of aromatase inhibitors generations. which are substances capable of suppression of aromatase enzyme activity. Aromatase enzyme activity is a unique requirement for final aromatization of esterones into estrogen. Suppression of aromatase activity result in decreased estrogen concentration in blood. Hindering preovulatory LH surge, which in turn will arrest follicular development after state of

dominance [4, 5]. Also delay emergence of next follicular wave. Synchronous large number of follicles with synchronous ovulation is responsible for increased concentration of estradiol in plasma [4, 6]. Also after ovulation this large number of follicles will yield in increase number of corpora lutea that is responsible for high diestral progesterone concentrations[4, 5]. Which will increase blood perfusion to corpora lutea. Letrozole could be future main product for ovarian function synchronization in farm animals [4, 7], the use of letrozole for estrus synchronization in cyclic ewes could decrease the variation in onset of estrus which is useful specially when timed insemination protocols are implicated. Moreover, it can increase the number of ovulatory follicles which is favorable for increased twining and therefore a profitable synchrony of ovulation. This experiment were set to evaluate the effect of novel letrozole based synchronization protocol during breeding season on conception rate, as well as estrus activity and reproductive traits pregnancy, lambing and twining rates compared with standard GPG protocol. Letrozole was previously used in ewe estrus synchronization [4]. But unfortunately, without testing its role on conception or fecundity rates. Therefore, without a trusted findings concerning conception, fecundity rates and early embryonic development, it is still not widely adopted in sheep reproductive management.

2. Materials and Methods

This study was approved by the Institutional Review Board of the Faculty of Medicine, Assiut University, Assiut, Egypt (approval no. 04-2024-100239).

2.1. Animals and experimental design

This study was performed during the breeding season (September-October) at the veterinary teaching hospital Faculty of Veterinary Medicine, New Valley University, Al Kharga city (latitude 25° 30"53" N - 30°""35 4" E). A total of number of twenty cyclic (ewes were monitored for ovarian activity by ultrasound beside hormonal analysis) non-lactating pluriparous Farafra ewes (3 to 5 years old and 35-40 kg/body weight). Ewes were apparently healthy and kept indoors with outdoor availability with concrete floor. Animals were provided with balanced ration; good quality hay add labitum in addition to concentrate mixture $\frac{1}{2}$ kg per head mixed with mineral mixture. They were divided randomly into two equal groups each containing ten ewes. The first group were letrozole treated group which received vaginal sponges containing 7.5mg letrozole for five days, then 250 µg of Prostaglandin F2α were given intramuscularly at onset of sponge removal, while the second group named GPG treated group and treated with standard GPG protocol (involving injection of GnRH at day zero followed by injection of PGF2α at seventh day and another GnRH injection at nineth day), as it considered standard protocol for fixed time insemination in most ruminants.

2.2. Intravaginal administration of Letrozole

Using locally made vaginal sponges which contain two-centimeter-wide, two-centimeter-thick, three-centimeter-long pieces supplied with 15 centimeters of silk. The vaginal sponges were sterilized through autoclaving then allowed to dry and stored in tightly closed, sterilized glass containers until needed. Before being inserted into a vagina, each sponge was soaked with 7.5 mg of letrozole powder dissolved in 3ml water (Femara® Tablets, 2.5 mg; Novartis Pharmaceuticals Corporation). A suitable, sterilized, 2-centimeter glass tube was opened from both sides and the loaded sponge was pushed through the tube until it settled inside the vaginal lumen. To prevent sponge removal, the glass tube, metal rod, and silk were all carefully removed, and the silk was cut one centimeter below the level of the vulva. At the 5th day just after removing the sponges from the vagina, ewes were injected intramuscularly with 250 µg of PGF2α / cloprostenol (Estrumate; Mallinckrodt Vet GmbH, Friesoythe, Germany). Ewes were observed and examined for estrus signs through the introduction of ram (Single ram previously tested

NVVJ., Vol. 5, Issue (1), 2025

for its fertility) and observing whether ewes refused ram or searched for its accompany and stand to be mounted. These signs were confirmed through ultrasound examination of ovaries and through disappearance of dominant follicles.

2.3. Ultrasonographic examination of female genitalia

The ewes were examined using ultrasound scanner (ECM80, Exago, France) outfitted with a 5-10 MHz transrectal linear probe (Lv513) with extension. The lateral recumbent ewe was examined using rectal probe. The urinary bladder served as a guide to locate the uterine horns. The probe was moved laterally 90° clockwise and 180° anticlockwise to scan the ovaries and investigate all regions of the genitals. The animals were evaluated daily using ultrasonography to determine the number and diameter of ovulatory follicles, confirm estrus signs detected by rams and to examine the size, shape, and typical echographic appearance of the corpora lutea. Then to early diagnosis of conception based on ultrasonic findings of uterus up to 15th day. Ultrasound scanning in letrozole treated group performed at onset of sponge removal and every day till ovulation. Then every other day for two weeks. In the group treated with GPG protocol, ultrasound scanning was performed after PGF2α (Estrumate; Mallinckrodt Vet GmbH, Friesoythe, Germany) injection and daily till ovulation with subsequent detection of CL, then every other day for two weeks. the findings (images and videos) were saved on a USB flash drive (Adata, Adata technology Co., Ltd, Taiwan).

2.4. Blood Sample collection

Blood Samples were collected from Jugular vein puncture into 5ml plain vacuum tubes (vaccutainer system) at onset of removal the sponge (Data about hormonal and biochemical parameters while keeping letrozole sponges on vagina were sufficiently obtained in our previous work), and one day interval till ovulation then every other day for two weeks. The collected samples centrifuged at 2500 rpm for 15 minutes (419XG). The serum was separated and placed into 1.5 ml epindorff tubes, labeled, and stored at -20 until assayed. For Ovsynch protocol (involving injection of GnRH at day zero followed by injection of PGF2α at seventh day and another GnRH injection at nineth day) sampling were every other day after PGF2α. Injection.

2.5. Hormonal assay

Serum P4 and E2 concentrations were determined using a commercial ELISA Kits (Chemux Bioscience, Inc.U.S.A). (Tietz, [8]) as described by the producer.

2.6. Biochemical parameters

Blood glucose was determined using GODPAP enzymatic colorimetric method [34]. Total proteins were determined using colorimetric method (Biuret reagent; [34]. Determination of triglycerides were carried out using GPO-PAP enzymatic colorimetric method [8]. Total cholesterol was measured by using CHOD-PAP enzymatic colorimetric method [8]. High density lipoproteins were determined using the precipitation method. Low-density lipoproteins were Calculated according to the following

(LDL = cholesterol-(HDL+Triglycerides/5).

2.7. Reproductive performance of ewes:

Estrus activity

The following measurements were calculated for all ewe groups: - Estrus response (%): The number of ewes showed standing estrus/ total number of ewes in each treatment group X 100.

- Onset of estrus: days interval from end of treatment to the time when ewes expressed standing estrus (heat).
- Estrus duration (heat duration): time (hours) between the first and last accepted mount.
- Non- return to estrus. - Time to conception: time of non-return to estrus and confirmed at lambing.

Reproductive traits

The following measurements were calculated for all ewe groups:

Table 1: Number and mean diameter of ovulatory follicles before ovulation (Mean ± SEM) in Letrozole treated (n= 10) and GPG treated ewes (n=10) during the study period.

	G1 (Letrozole-treated)	G2 (GPG-treated)
Number of ovulatory follicles.	3.67 ± 0.88^{a*}	1.00 ± 0.58^{b}
Diameter of ovulatory follicles.	6.25 ± 0.029^{a*}	5.90 ± 0.029^{b}

*Letters with different superscripts in the same raw are significantly different ($p<0.05$).

- Pregnancy rate: Number of ewes lambed/ number of ewes mated X100. - Lambing rate: Number of ewes lambed/ number of pregnant ewes in each groupX100.
- Twining rate: Number of total lambs/ number of lambing ewes in each group.

2.8. *Statistical analysis*

Statistical analysis was performed using Microsoft excel computer program (2019), and the significant difference between means were analyzed by T-Test, data expressed as (Mean±SEM).

3. Results

Table 2: Estrus activity in letrozole- treated (n= 10) and GPG-treated ewes (Control; n=10) during the study period.

Items	G1 (letrozole treated)-	G2 (GPG treated)-
Estrus response number-%	(10/10)-100%	(10/10)-100%
Onset of estrus (hrs)**	54.00 ± 0.40^{b*}	72.64 ± 1.34^{a}
Duration of estrus (h)	25.17 ± 0.24^{b}	46.52 ± 1.70^{a}
Non-return to estrus number-%	(6/10)-60%	(5/10)-50%

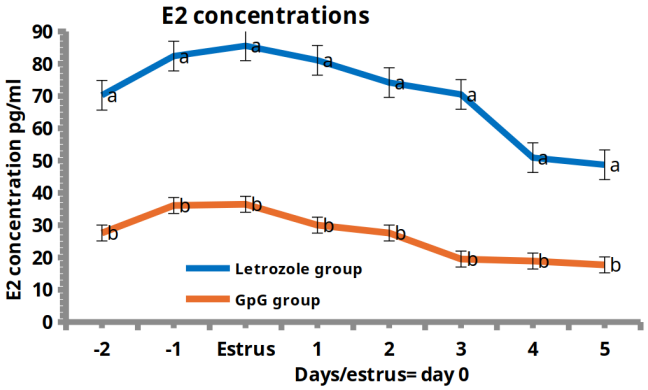
*Letters with different superscripts in the same raw are significantly different ($p<0.05$) **Post-removal or post- PGF2α injection.

Table 3: Reproductive traits in letrozole-treated (n= 10) and GPG-treated ewes (control; n=10) during the study period.

Items	G1 (letrozole-treated)	G2 (GPG-treated)
Pregnancy rate (%)	60%	50%
Lambing rate (%)**	100%	100%
No. of lamb born/ ewe lambed	1.83^{a*}	1.2^{b}
Ewes lambing twins (%)	80 ^{a}	20^{b}

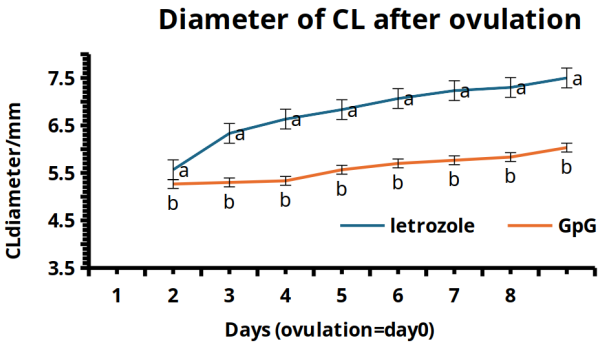
*Letters with different superscripts in the same raw are significantly different ($p<0.05$). **calculated from the pregnant ewes.

Figure 1: Concentrations of Estradiol (E2) before and after ovulation (pg/ml; Mean ± SEM) in Letrozole-treated (blue line; n= 10) and GPG-treated ewes (Orange line; n=10) during the study period



Letters with different superscripts in the same day are significantly different ($p<0.05$).

Figure 2: Diameter of post ovulation CL (mm; Mean ± SEM) in Letrozole-treated (Blue line; n= 10) and GPG-treated ewes (Orange line; n=10) during the study period.



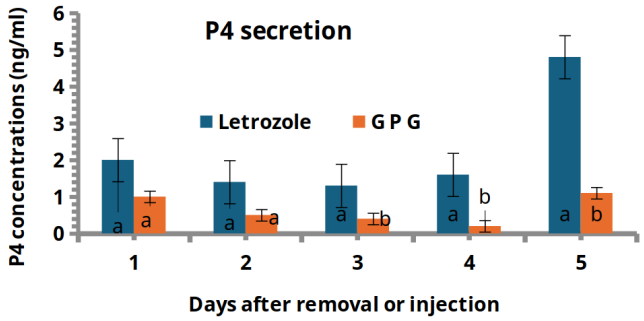
Letters with different superscripts in the same day are significantly different ($p<0.05$)

1. **Number of ovulatory follicles** Number of ovulatory follicles (ranged from 1.00 ± 0.58 to 3.67 ± 0.88). Moreover, In Table 1 and Fig. 6, number of ovulatory follicles differed significantly between letrozole group and GPG group during the period of the study where letrozole group significantly recorded a higher number.

2. **Diameter of ovulatory follicles:** Diameter of ovulatory follicles ranged from (5.90 ± 0.029 to 6.25 ± 0.029 mm). Moreover, diameter of ovulatory follicles differed significantly between letrozole

NVVJ., Vol. 5, Issue (1), 2025

Figure 3: Concentrations of Progesterone (P4) after ovulation (ng/ml; Mean ± SEM) in Letrozole-treated (n= 10) and GPG-treated ewes (n=10) during the study period



Letters with different superscripts in the same day are significantly different ($p<0.05$)

group and GPG group during the period of the study where letrozole group recorded a higher ovulatory follicle diameter as showed in Table 1 and Fig. 6.

3. **Diameter of corpus luteum** Diameter of corpus luteum (mm) from letrozole group during early metestrus were higher ($p<0.05$) in letrozole group than in GPG group (7.5 ± 0.05).as showed in Figs. 2 and 7.

4. **Reproductive parameters** All ewes showed estrus (100%) in both groups, while onset of estrus were significantly shorter (54.00 ± 0.40 hrs.) in letrozole treated group than (72.64 ± 1.34 hrs.) GPG treated group. Duration of estrus in letrozole treated ewes (25.17 ± 0.24 hrs.) was significantly shorter than GPG treated ewes (46.52 ± 1.70 hrs.). Non return to estrus were significantly higher 60% in letrozole treated group compared to 50% GPG treated group. The pregnancy rate was significantly higher 60% for letrozole treated group compared to 50% for GPG treated group. While the number of lamb born/ ewe lambed was significantly higher in letrozole treated (1.83) group compared to (1.2) for GPG treated group. The percentage of ewe lambed twin was significantly higher in letrozole treated group (80%) compared to (20%) for GPG treated group. As shown in Tables 1 and 3

5. **Steroid concentration** In letrozole treated group, after sponge removal concentration of estradiol begin to increase significantly (compared to GPG treated ewes after injection of PGF2α) in association with synchronous follicular recruitment and selection then it decreased again just before ovulation (but still significantly higher in letrozole treated ewes than GPG treated Ewes) and continued to decrease along post ovulatory period Fig. 1 . Serum concentration of progesterone (ng/ml) decreased after removal of sponges and injection of luteolytic agent in letrozole treated ewes and GPG treated ewes but still significantly higher in letrozole treated ewes than GPG treated ewes, also serum concentration of progesterone secreted from four days corpora lutea were significantly higher in letrozole treated group compared to GPG treated group. as showed in Fig. 3.

6. **Biochemical parameters** Biochemical profile including total proteins concentration. Blood glucose concentration. Total cholesterol concentration, triglycerides concentrations, HDL concentrations, LDL concentrations were shown in Fig. 4 and there were no significant changes during the experiment.

4. Discussion

Letrozole was recently prescribed for control of ovarian functions [7, 9, 10], either alone or in combination with other gonadotropins [11], Instead of being only adjuvant anti-estrogenic drugs in treatments [12]. Observed positive effects of nonsteroidal inhibitors of letrozole on reproductive health inspired its application in animals [3, 13, 14, 15]. Administrating letrozole vaginally allows its action to be kept for five days inside the vagina and leads to the continuity of the drug's action [16]. In the

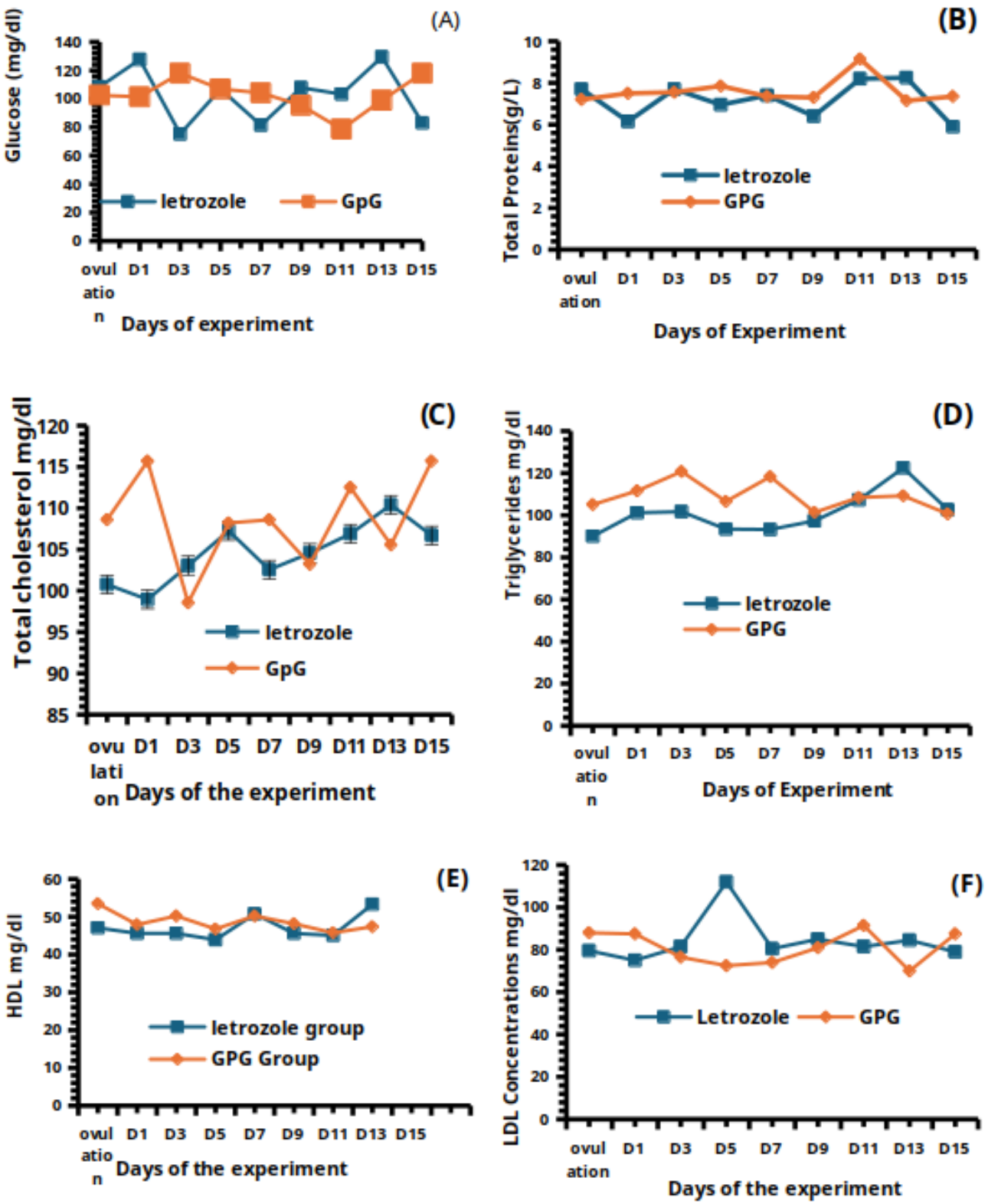


Figure 4: Concentrations of Blood glucose (A), Total proteins (B), Total cholesterol (C), Triglycerides (D), High density lipoprotein (E), and low density Lipoprotein (F) in letrozole (Blue opened diamond; n= 10) and GPG ewes (Orange opened squares; n= 10) during the study period. No significant difference between groups (P<0.05

NVVJ., Vol. 5, Issue (1), 2025

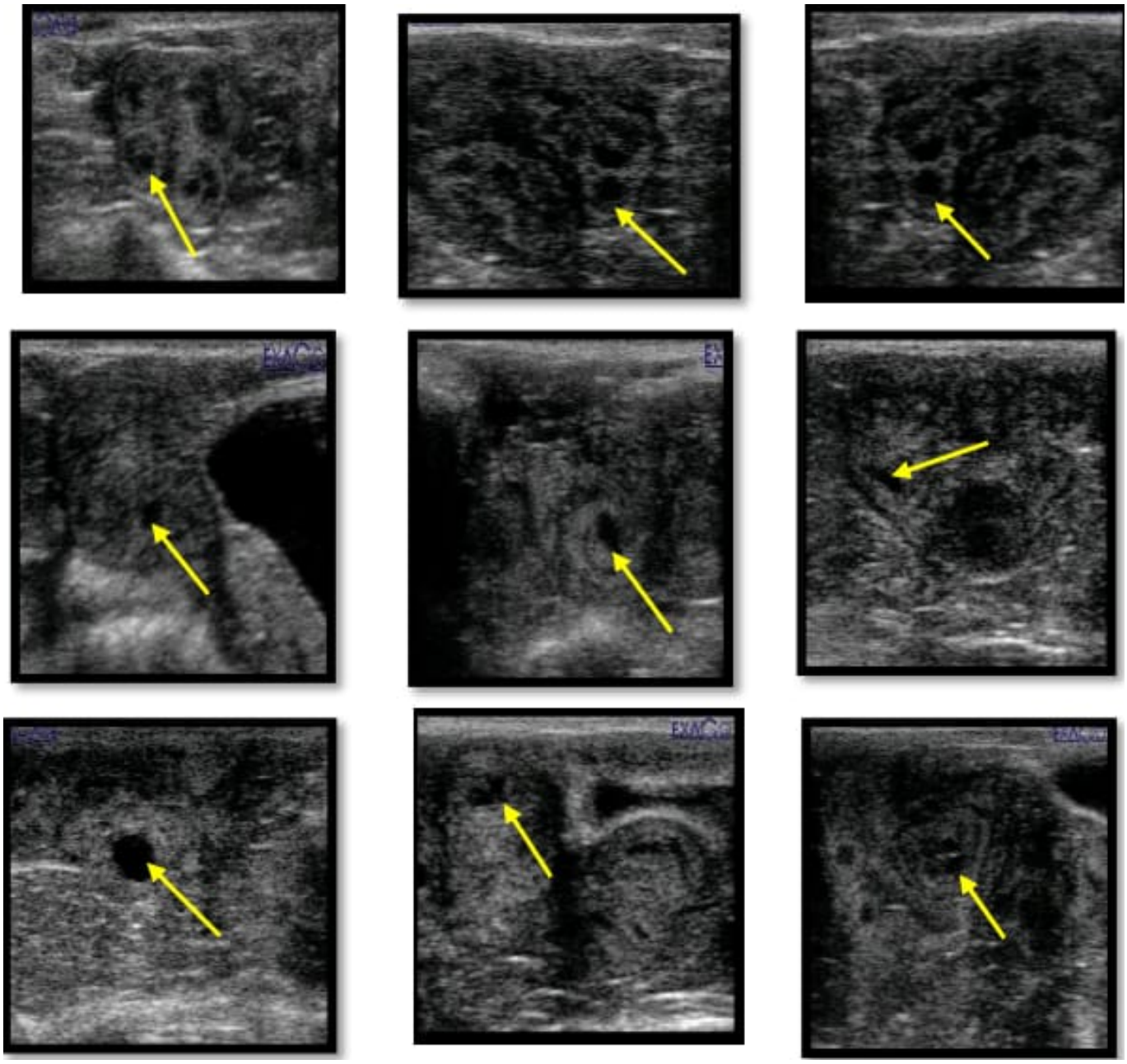


Figure 5: Ultrasound findings of uterus During early luteinization in nonpregnant ewes, of letrozole treated ewes showed fluid accumulation (yellow arrows) on uterine lumen

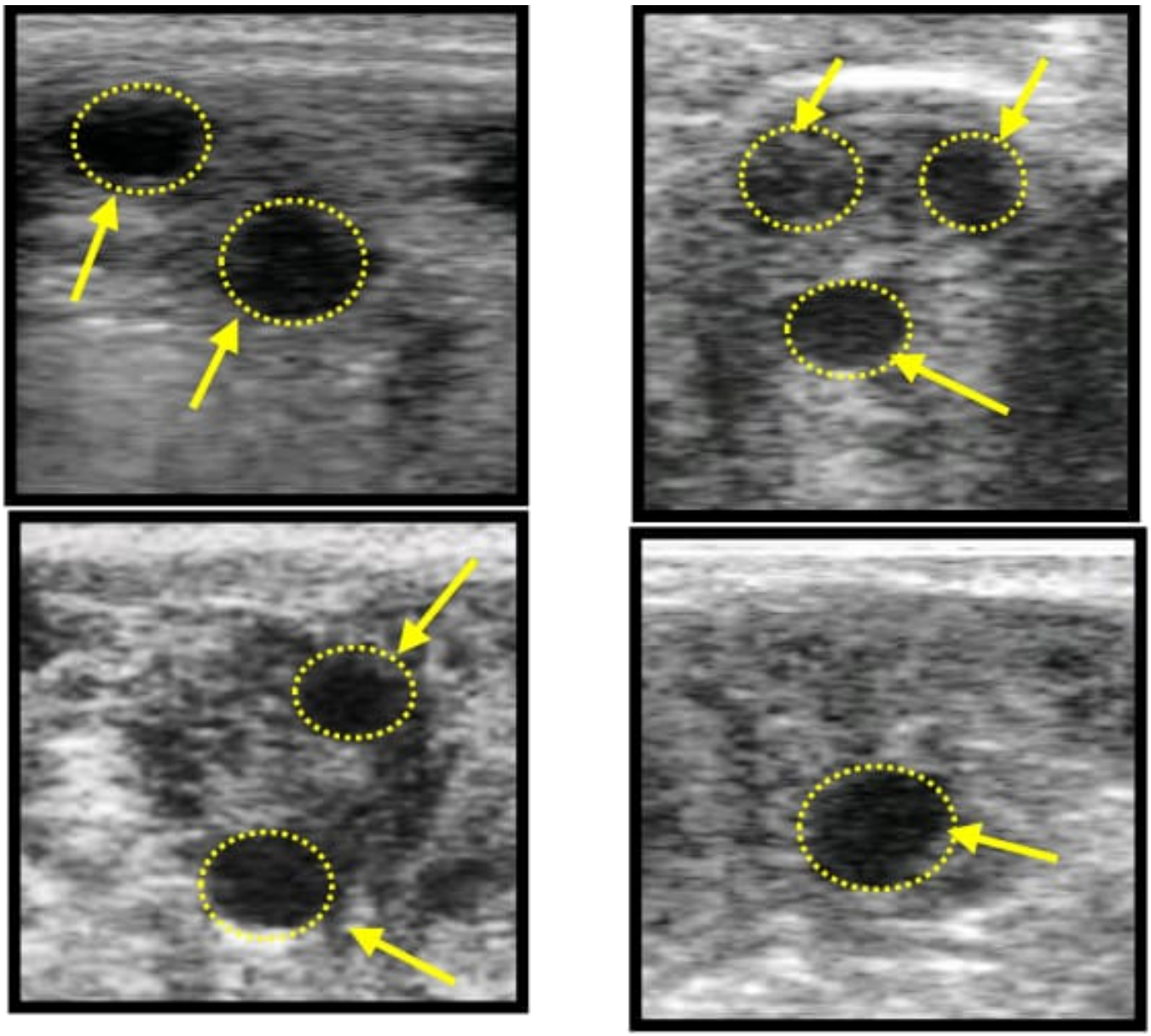


Figure 6: ultrasonographic images for preovulatory follicles (yellow arrows and dashed yellow circles) in letrozole treated Group

NVVJ., Vol. 5, Issue (1), 2025

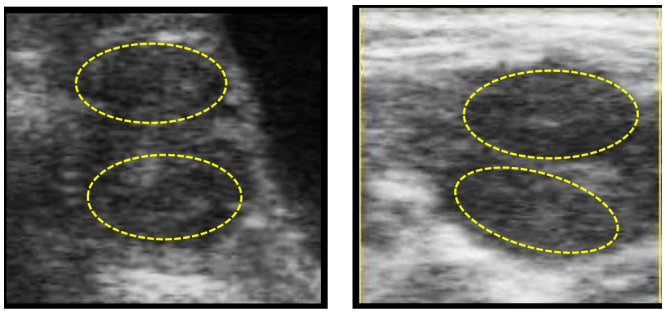


Figure 7: Ultrasound picture of newly formed CLs after ovulation in Letrozole treated ewes during early luteinization

current study letrozole exerted a positive action on number and diameter of preovulatory follicles in addition to met-estral corpora lutea. This result is compatible with what was observed on cattle [14], and sheep [4]. super ovulatory brought gonadotrophic response of letrozole on recruited selected follicles which is the main reason for an increased number and diameter of ovulatory follicles, and therefore corpora lutea in letrozole-treated [3, 4]. The increased number of corpora lutea was attributed to an increased number of ovulatory follicles which formed the corpora lutea under a potent luteotropic action of luteinizing hormone. The diameter of corpora lutea from letrozole-treated ewes was greater than non-treated ones, these findings are like what was described [4]. It was explained that luteinization of a larger diameter of the ovulatory follicle could result in an increase in the initial diameter of the newly formed corpus luteum [3, 4]. Distribution and growth of lutein cells within initially large cavitation post-ovulatory follicular antrum, result in initially large diameter of ovarian cortex occupied by corpus hemorrhagic. This cavity when examined by ultrasound has different echogenicity delineating it from occupying ovarian echotexture [17]. This description ideally explains the findings in this experiment, thus large-diameter corpora lutea originated from large-diameter ovulatory follicles [18]. Increased estrogen concentration in letrozole group over GPG group during preovulatory period is brought by increased number and diameter of ovulatory follicles in Letrozole ewes over GPG ewes [15]. While increased progesterone secretions from post ovulatory corpora lutea of letrozole ewes over GPG ones is considered normal result of increased number and diameter of newly formed corpora lutea of letrozole group over GPG group [4]. In the current study metabolic parameters measured during the experiment did not differ between letrozole and GPG group. This is an indicator that the used treatment did not affect the metabolism of the treated animals. five days letrozole treatment in cyclic ewes did not show any unfavorable changes on animal health. This is similar to what was previously explained [4]. However, other reports [19] indicated an alteration in lipid metabolism over long term use of letrozole if compared with short term use in the present study. Variation in onset of estrus were diminished between members of letrozole group. Differences in aromatase activity recorded in letrozole group is a strong indicator for the effect of letrozole on ovarian function which is brought up by its inhibitory effect on follicular dynamics [14]. The short onset of estrus after letrozole sponge removal is attributed to rapid emergence of synchronous follicular wave just 36 hrs after removal of letrozole inhibitory effect [4], The removal of circulating estradiol by means of aromatase inhibition will relieve the suppressive effects of estradiol permitting a surge in FSH, which would in-turn induce the recruitment of a new wave of follicular development, drive follicular growth, and even trigger the development of more than one ovarian follicle to a pre ovulatory size [15]. For GPG group the differences in onsets are attributed to the stage of follicular development. Higher pregnancy and lambing rate in letrozole group were mainly attributed to high rates of heat expression [9], and good post mating steroidal and endometrial environment. While slight lower pregnancy and lambing rates in GPG group were mainly attributed to low heat expression rate and low oocyte quality of lower developmental competence, and lower progesterone secreted from smaller and decreased

number corpora lutea hence disturbed endometrial environment impairing conception. The increased number of lambs born (litter size) in Letrozole group is attributed to increased number of ovulatory follicles and potency of corpora lutea produced [4], increased percent of twins in letrozole group over GPG group is mainly attributed to increased number of ovulatory follicles developed under good met-estral steroidal and endometrial environments. This theoretical ideal vascular and steroidal endometrial environment should yield ideal implantation and early embryonic development, translated by conception rates. It could improve early embryonic survival; however clinical findings suggest that other factors has negatively affected early conception events as following. Increased uterine lumen diameter in nonpregnant ewes and increased thickness of uterus observed in nonpregnant ewes of letrozole group, is mainly attributed to leakage of fluids from vagina over a period when sponges were kept into uterus. this fluid containing letrozole which exerts a direct action on endometrial cells [20], Inhibition of estrogen production of letrozole is proposed to decrease normal peristaltic movement of endometrial, however it was no negative correlation between peristaltic inhibition and letrozole [21]. Successful implantation events require synchronicity between endometrial development and blastocyst attachment. Also many authors claimed the role of high levels of E2 [22, 23] during early metestrus on fluid accumulation in uterus. and decreased endometrial peristaltic movement in supraphysiological levels [21]. However, endometrial peristaltic movement is necessary for implantation events in normal range. Inhibition of aromatase activity led to a significant reduction in decidual mass, and the majority of the implanted embryos failed to develop properly and were resorbed by d 10 This result indicated that the aromatase activity and local Estrogen production is essential for endometrial functions that support embryonic development during early pregnancy [20]. In female mice letrozole induced delay of implantation for one day caused failure of decidualization and pregnancy were compromised in about half of the females [21]. In addition to time elapsed from sponge removal to mating is not enough to complete evacuation of uterine contents. The most critical point is that leaked fluids not sterile, and should be contaminated from vaginal inflammatory fluids resulting from keeping sponges in vagina [24]. As long standing of sponges into vagina causes vaginitis [26] and offensive exudates appear naturally on vulval lips and fatty tail [9]. The subsidence of such inflammation and clearance of vagina rely mainly on time of keeping sponges and its placement in vaginal compartments. and texture and consistency of intravaginal sponge [25], technique of sponge insertion [26], management system[27], and size of intravaginal implant [28]. No doubt that movement of sponges [29] through inner third of vagina towards cervical portion increase severity of inflammation and increase opportunity to transmission of inflammation to cervix and uterus in turn impairing conception [30]. The factor of short duration between sponge removal and onset of estrus in this protocol, favors the unsanitary vaginal conditions to interfere with conception [24]. In comparison between protocols using sponges for estrus synchronization in ewes, This protocol has shortest duration between sponge removal and natural mating [5]. The lack of secretion of ecbolic after sponge removal together with increased estrogen over met-estrus hinders mechanical evacuation of intrauterine fluids. And these fluids may remain over many estrus cycles. In addition to the quality of oocytes obtained after five days ovarian exposure to letrozole may be affected, however it wasn't tested in this experiment, but in human experiments, tested quality of oocytes obtained after letrozole stimulation protocols revealed that oocytes collected have high percent of immature ones [31]. As it was concluded that embryos originating from immature oocytes have lower cleavage and blastulation rates compared that originating from mature ones [32].This may suggest that

NVVJ., Vol. 5, Issue (1), 2025

obtained oocytes from first follicular wave after letrozole synchronization is compromized in quality. This can support findings, in other experiment on postpartum delay of insemination to later estrus induced by PGF2α injection ten days after first estrus improved conception rates.

Conclusions

We concluded that the use of intravaginal sponges containing letrozole, had a positive effect on estrus response, synchronicity of estrus and luetotrophic effect on corpus luteum, however further researches are required to develop more sanitary method for intravaginal route of delivery to avoid inflammation on vagina and uterus that may decrease conception rates.

Declaration of Competing interest

Authors declare that no conflict of interest may interfere with the publication of the manuscript.

References

[1] K. sigrid, *International Journal of Environmental Research and Public Health*, 2020, 4005 10 3390 17114005.

[2] C. Mayberry and D. Hatcher, *Animal Production Science,2020*, **61**, 201–207.

[3] M.-b. A, M. A and Martin, *Reproduction Fertility Development*, 2020, 441–452.

[4] M. A.Abdeldayem, M. M.Hassan and W. Senosy, *Journal of applied Veterinary Science*, 2020, **5**, 1–9.

[5] M. J, *Reproduction Fertility Development.2012*, **24**, 631–640.

[6] C. G, *Biotech. Journal of Reproductive Fertility,1984*, **72**, 153–163.

[7] M. Yapura, E. Zwiefelhofer, R. Pierson and G. Adams, 2018.

[8] N. TIETZ, *Clinical guide to laboratory tests*, 1995.

[9] P. Surappa, A. Sahadev, G. Jayanna, S. Bajanthri and V. M, *Pharmacology Innovation Journal*, 2023, **12**, 1225–1229.

[10] S. Gavimath, N. Murthy and A. Sahadev, *Pharmacology Innovation Journal*, 2023, 3167–3170.

[11] A. Gowri, A.-A. V., A. A. and S. Khaduri, *International Journal of Reproductive Medicine*, 2022.

[12] E. Requena, H. A., L. J., N. J., C. Neyro, S. J.L., T. C., C. R., J. and M. Farré, *Human Reproduction Update.2008*, **14**, 571–582.

[13] P. Cohen.M.H, N. Johnson and C. C. Research, 2002.

[14] M. Mitwally and R. F, 2001.

[15] R. A, H. J, L. J and N. E, *N. E*, Human Reproduction Updates, 2008, p. 71–82.

[16] B. A, *Breast Cancer Research and Treatment.2007,105*.

[17] J. Kastelic, D. Bergfelt and O. Ginther, 1990.

[18] A. Evans and A. R. Science, 2003.

[19] K. Wasan, *Annals Of Oncology .2005,16,707-715*.

[20] A. Das, S. Raju, A. Kannan, D. Evans and M. Bagchi, 2009.

[21] F. Wang, L. Meng, Y. Kuang, Z. Liu and X. Ma, *Reproduction Sciences.2022*, **29**, 2864–2875.

[22] F. Ziegler, M. D., S. C., I. C., F. V. and N., *Annual NY Academic Sciences*, 2004, 262–277.

[23] M. Mitwally and C. M.F., *American Journal of Obstetrics and Gynecology*, 2005, **192**, 381–386.

[24] A.-U. Al-Hamedawi.TM and Khammas, *Iraqian Journal of Veterinary Science*, 2003, **16**, 73–79.

[25] M.-R. P, M. L, F. H, A. T, A. R and M. A. CL, 2018.

[26] J. Romano and S. R. Research, 1998.

[27] R. BO, R. PI and A. I. Makun.HJ, *Sokoto Journal of Veterinary Science*, 2012, **10**, 21–25.

[28] A. Swelum, I. A.F.Moumen, H. M.A.Ali and Awadh, *Reproduction in Domestic Animals.2018.53*, 1114.

[29] U. J, C. C, H. F and A. R, *Reproduction in Domestic Animals,2015*.

[30] E. Ribeiro, G. Gomes, L. Greco and R. Cerri, *Dairy Sci*, 2016, 99,2201–2220.

[31] M. Ludmila, M. C. Bercaire and A. Eliana, *JBRA Assisted Reproduction.2020,24,2-264*.

[32] S. Rachel, S. Mandelbaum and A. Ali, *Assisted Reproductive Technologies,2021,38.2291-2299*.