RESEARCH
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IMPACT OF CIDR-PMSG AND MAP SPONGES-PMSG TREATMENT OF REPRODUCTIVE PERFORMANCE OF EWES WITH ASSESSMENT OF PROGESTERONE CONCENTRATION DURING TREATMENT AND PREGNANCY.

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Abstract

The aims of present study to estimate the effect of CIDR-PMSG and MAP sponges- PMSG on reproductive performance of adult ewes and to evaluate blood progesterone profile during pregnancy as a biomarker for pregnancy detection and litter size determination. Eighteen multiparous local cross Iraqi ewes aged between 3-4 years were randomly divided into three groups (n= 6/ group). In CIDIR treated group (G1), the animals were insertion with the devices impregnated with 300mg of progesterone for 12 days then i/m injection with 400 IU PMSG at devices withdrawal. The MAP sponges group (G2), the animals were insertion with sponges impregnated with 60 mg medroxyprogesterone acetate (MAP) for 12 days then i/m injection with 400 IU PMSG at sponges withdrawal, while in third group (G3) control group. The estrus response was significantly (P<0.05) higher in the treated groups (G1 and G2), compared to control group (G3) (100±0.00%, 100±0.00% and 17±0.52%respectively). The duration of response and estrus phase length were significantly (P<0.05) longer in G3 compared to G1 and G2 (duration of response was 39.17 ±2.74, 43.50 ±3.19, 48.00 ±0.75 respectively and estrus phase length was 31.67 ±1.97, 26.33 ±2.85, 38.00 ±0.52 respectively). Also pregnancy rate, lambing rate and fecundity rate were significantly (P<0.01) higher in G1 and G2 compared to G3 (pregnancy rate was 83%, 100%, 17% lambing rate was 117%, 117%,17% and fecundity rate was 140%, 117% and 100% respectively. The progesterone values no significant difference between three groups before treatment and at estrus phase while significantly (P<0.05) higher in G1 and G2 compared to G3 after 12 days of treatment and at day 30 and 60 of pregnancy. Also progesterone level depending on litter size significantly (P<0.05) in twin pregnancy compared to single pregnancy at day 30 and 60 of pregnancy. In conclude the current study illustrated that application of CIDR-PMSG and vaginal sponges-PMSG regimes was effective in estrus synchronization and achievement of high pregnancy rate in ewes with approximately similar results. The serum progesterone level via sheep progesterone ELISA kit on day 30 after insemination can be successfully used for diagnosis of pregnant and non pregnant sheep and the number of fetus with high accuracy.

Keywords: Iraq ewes, CIDR, MAP sponge, PMSG, Estrous synchronization, progesterone , liter size.

INTRODUCTION

Iraqi ewes can be considered seasonal polyestrous sheep and the activity of their reproductive aligns perfectly the ideal timeframe of a year where available food resources during spring (Hussain et al., 2017, Hatif and Younis, 2018a). Also Younis et al. (2019) observed estrus activity in some ewes in April. Generally sheep considered seasonal polyestrous animals and short day breeders (Younis and Hatif 2017 and Ajafar et al., 2022). The breeding season started in autumn and continued until med or late winter, while anestrous period happening through spring and summer (Hatif and Younis 2018a and b).The beginning and period of breeding season may be effected via interaction between genetic factors and daylight (Al-Mutar, 2017 and Younis et al., 2020).

Estrus synchronization in ewes may be achieved via regulating the activity secretion of corpus luteum and ovulation (Aqwaan, 2023). Synchronization of estrus in ewes can be achieved by administration the exogenous

hormones such as progesterone (Tamer and Al-Hamedawi, 2013 and Al-Zubaidi, 2017) also by adding equine chorionic gonadotropin (Hussain, 2007, Al-Zubaidi, 2017 and Kadhim and Hussain, 2014). The administration of equine chorionic gonadotropin with progesterone during both breeding and non breeding season can improve estrus response and gestation rate (Hussein et al., 2007). Synchronization of estrous can be achieved via different methods including daylight manipulation, ram effect and exogenous hormones administration (progesterone, eCG, prostaglandins, melatonin, kisspeptin and Bromocriptine) during both breeding and non breeding season in sheep (Tamer and Al-Hamedawi, 2013, Al-Hamedawi, et al., 2016, Al-Hamedawi, et al., 2020, Abas et al., 2022 and Kadhim and Hussain, 2024a) and in goat (Kadhim et al ., 2014). The ram effect may be improve the effectiveness of progesterone and serve as substitution for eCG in sheep (Al-Mutar, 2017 and Hameed et al., 2021). During both breeding and non breeding season the administration of progesterone has demonstrated to effect on estrus induction and synchronization in ewes (Abdul Hussain et al., 2017). Estrus response and lambing rate were higher significant in ewes using CIDR+Kisspeptin injection than those used CIDR alone (Abdul Kareem et al., 2021), but other authors show no significant difference between two groups when used same protocols (Kadhim and Hussain, 2024a).

Progesterone therapy has been shown to effectively synchronize estrus in sheep throughout both breeding and nonbreeding seasons. Progesterone (P4) therapies are often administered by controlled internal drug releasing (CIDR) devices, intravaginal sponges, or injectable formulations. Both short (5–9 days) and long (14 days) durations of P4 therapies are administered in conjunction with gonadotropins (Texeira et al., 2016; Martinez-Ros et al., 2019). Additionally, various quantities of fluorogestone acetate (FGA) and medroxyprogesterone acetate (MPA) have been used in the utilization of sponges. The extended use of intravaginal sponges has been often associated with the development of vaginitis and the production of purulent discharge (Martinez-Ros et al., 2018). According to Al-Hamedawi et al. (2003), the use of intravaginal sponges successfully blocks the secretion from the vagina, which in turn promotes the growth of microorganisms such as Salmonella spp. and Staphylococcus aureus (Swartz et al., 2014). According to Bragança et al. (2017), S. aureus is recognised as a significant etiological factor in vaginitis in sheep. According to Suárez et al. (2006), CIDR devices demonstrated a lower occurrence of vaginitis when compared to sponges. The improved design and structure of CIDR may be attributed to its ability to facilitate the drainage of vaginal secretions (Martinez-Ros et al., 2018). During the non-breeding season, Merino ewes who were subjected to FGA sponges for varying periods (7 days, 10 days, and 14 days) exhibited higher estrus response and lambing rate ($P < 0.05$) compared to ewes treated for shorter durations. Nevertheless, the improved response might be attributed to the administration of (eCG) during the removal of sponges, leading to enhanced follicular turnover and ovulation in ewes treated with FGA for an extended period of time (Altincekic and Koyuncu 2019).

In a study conducted by Iida et al. (2004), the estrous response of different sheep breeds treated with a 12-day long regimen for estrous synchronization was compared using MAP sponges (60 mg), CIDR (300 mg). The results indicated that there were no significant differences in estrus response across the treatments at non-breeding seasons. In addition, for other study during the non-breeding season, a comparison was conducted between CIDR (300 mg) and MAP sponges (60 mg) to determine their effectiveness in inducing estrus in Karakul ewes. Both groups had a comparable estrus response (Hashemi et al., 2006). According to Swelum et al. (2015), the estrus response of ewes was found to be similar between the treatments of long-term insertion of FGA sponges and CIDR, along with 600 IU of eCG treatment on day 14 (insertion = Day 0). Nevertheless, it was observed that the rate of gestation was significantly higher ($P < 0.05$) in ewes treated with CIDR compared to those treated with sponge. The usual dosage of MAP is 60 mg, therefore it may be more suitable to use 60 mg MAP sponges for estrous synchronization. According to Yu et al. (2019), the efficacy of a CIDR device with 300 mg progesterone is similar to that of a 60 mg MAP Sponge.

During the mating season, when daylight hours are short, sheep and goats undergo spontaneous estrus and ovulation; however, this does not occur during non breeding season, when daylight hours are long (Habeeb and Anne Kutzler, 2021). Ovulation induction during non-estrus cycles has been achieved by the use of several techniques, such as progesterone, gonadotropins, and male sex pheromone. Nevertheless, when the ovaries are overstimulated, the size of the follicles increases and the concentration of estradiol-17β also increase, leading to a decline in fertilization rates (Habeeb et al., 2019). It is recommended to use male sex pheromones in conjunction with progestogen (CIDR) and PGF2a in ewes and does throughout the breeding season to synchronize estrus with best results in terms of reproduction (Habeeb and Anne Kutzler, 2021). Sheep treated with eCG demonstrated final follicular growth and the beginning of estrous behaviour at 48 hours and nearly all of them (95.2%) ovulated approximately 70 hours after CIDR removal and had good-quality corpora lutea, as assessed on Day 14 after CIDR removal. Subsequently, the percentage of pregnancies resulting from a single mating approached 70%.(Braun-Galarraga et al., 2021; Uriol et al., 2019; Martinez-Ros and Gonzalez-Bulnes, 2019; Martinez-Ros et al., 2019). A study conducted by Bruno-Galarraga et al. (2021) found that injecting sheep with human chorionic gonadotropin (hCG) or human menopausal gonadotropin during estrus synchronization caused a high rate of abnormal follicular growth patterns, ovulation disturbances and retardations, and the concurrent formation of follicular cysts, although the lambing rate was found to be higher in the hCG group compared to the control group. In a study that examined

the effects of injecting prostaglandin and gonadotropins (GnRH and hCG) along with the ram effect on progesterone concentrations and reproductive performance during the non-breeding season, during the first cycle, the injection of prostaglandin and hCG, together with the ram effect, shortened the lambing duration while increasing the lambing rate compared with control group (87.1% versus 58.1%; P < 0.01) (Ayaseh et al., 2021).In order to facilitate out-of-season and/or synchronized lambing, the majority of sheep reproduction management methods focus on inducing and synchronizing estrus and ovulation involve either administering prostaglandin F2 (which induces the lysis of the corpora lutea and subsequent estrus and ovulation) or progesterone or its analogues (which mimic the activity of the corpus luteum) with single intramuscular dose of equine chorionic gonadotrophin (eCG). This hormone simulates the surge of preovulatory luteinizing hormone (LH) and ovulation during seasonal anoestrous periods, which can increase the frequency of twin pregnancies and help with fixed-time artificial insemination (TAI) by adjusting the interval between ovulation and semen deposition (Abecia et al., 2012; Gonzalez-Bulnes et al., 2020).

The primary hormone responsible for getting the uterus ready for implantation and keeping the myometrium quiescent is progesterone, and called "the hormone of gestation" (Rahman, 2006). According to Mesiano (2007), P has many actions, such as (a) promoting an acrosomal reaction, (b) meiosis resumption, and (c) reducing neural excitability and anesthesia. It is usual practice to detect the number of foetuses in pregnant animals by analyzing their blood progesterone levels (Medan et al., 2004). According to research by Halasz and Szekeres-Bartho (2013) and Grazul-Bilska et al. (2018), During ovulation, progesterone causes the oviduct to secrete more fluid, which also in turn encourages the closing of the cervical opening and the expansion of the endometrial glands via cell proliferation and angiogenesis. As well as P4 plays a crucial role in establishing pregnancy by promoting blastocyst endometrial receptivity and trophoblast attachment by suppressing of deleterious maternal immune responses.

According to Field and Taylor (2008), the corpus luteum synthesizes the hormone progesterone (P4) in female sheep so that the endometrium may proliferate in preparation for an embryo. In all animals, it is crucial for establishment and keeping of pregnancy. P4 is essential for early embryonic development, but low levels can cause pregnancy failure and early embryonic loss (Fernandez et al., 2017). According to Ortega-Mora (2007), most embryos lost before 13-day after fertilization, before maternal recognition happens, so P4 doesn't affect the ewe's cycle length.

Boscos et al. (2003) found no significant variations in blood progesterone in sheep carrying one or more foetuses during the 19th day of pregnancy. However, Müller et al. (2003), found that the number of foetuses could be determined on the 19th day of pregnancy via the progesterone concentration with 78% accuracy. The CIDR has rapid release kinetics, resulting in elevated blood progesterone concentrations upon insertion. Elevated levels of progesterone in the bloodstream decrease the release of luteinizing hormone (LH) from the pituitary gland (de Graaff and Grimard, 2018).

Materials and Methods

Ethical Approval

Before any experiment performing, the experimental protocol and design used in present study were examined and approved by the Committee of Ethics in College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq (Number P. G/995 at 15/5 2024).

Experimental animals

This study conducted at the farm belong to the College of Veterinary Medicine /University of Baghdad. Eighteen cross adult Iraqi ewes aged between 3-4 years determined by breeding record and verified by dental formula with 3 fertile rams used in this study. The animals subjected to careful clinical examination and ultrasonography examination to determine that they are non-pregnant/healthy and free from diseases. Preventive health measures applied such as vaccination against enterotoxaemia (against clostridia infection) at dose of 2 ml S/C and treatment against internal &external parasites (Rafoxanide and Ivermactin).

Experimental design

The animals were randomly divided into three groups (six ewes each), group A, B and C, group A was received CIDR-PMSG for12 days (Kadhim and Hussain 2024b), with i/m injection 400 IU of PMSG at withdrawal and group B was received vaginal sponge for 12 days with i/m injection 400 IU of PMSG at withdrawal of the sponges, while group C was not treated and served as control group. The animals kept at semi-opened shade shelter supplemented with drinking water ad libitum , provided with 1kg concentrated diet Balanced diet of grains (barley 40%, wheat 51%, soya been 5%, limestone 2%, Nacl 1%, minerals and vitamins 1%) (Abood, 2012, AbdulKareem et al., 2014, Hussain et al., 2016 and Alrawy and Hussain 2025) daily per ewe. The experiment extend from January 2023 until September 2023.

Clinical study

Confirmation of non- pregnancy by application of ultrasonography, estrus detection by aproned rams for 2 successive cycles to monitoring estrus activity in experimental animals, estrus synchronization by CIDR-PMSG for 12 days with i/m injection 400IU of PMSG at withdrawal and vaginal sponge for 12 days with i/m injection 400IU PMSG at withdrawal of the sponges, estrus detection in order to determine (estrus response, percentage of responded animals, and duration of estrus

phase), the responded animals served by fertile rams, detection of pregnancy by (ultrasonography estimation of blood progesterone, follow up pregnancy until parturition, and after parturition recorded the Length of gestation, number of birth, weight of new born and type of birth (Normal or Dystocia).

Estrus detection

Current study started in mid of January and before application of CIDR and vaginal sponge , all ewes were exposed to aproned ram two hours in the morning and two hours in the evening daily for two successive cycle from mid of January until end of February. At this period of detection show low number of ewes in estrus. After treatment the detection continue on the control group of ewe until the end of experiment but did not show any cyclic activity.

Blood collection

Blood was collected from jugular vein using vacutainers gel tubes and numbered by hand approximately 5ml, the lower part of the neck of the ewe was held firmly by the left hand so that jugular vein can be visible. The skin on the jugular vein was cleaned by 70% alcohol. The needle was inserted in jugular vein and collected the blood for estimation of progesterone hormone at the following days: before treatment, at withdrawal of CIDR in G1 and sponges for G2, estrus phase and at following days of pregnancy 30 and 60. Serum was harvested following centrifugation of samples at 3000 RPM for 10 minutes and then stored at -20ºc until the assay (Hussain et al., 2017).

Hormonal study

Determination the concentration of progesterone before and during gestation, at following days : At day or CIDR and sponge insertion, at the time of CIDR and sponge withdrawer, at estrus and at days 30,60 of pregnancy. Blood samples were collected at different physiological stages by jugular vein puncture into vacutainer tubes without anticoagulant to test the efficiency of the visual ELISA-PROG in diagnosing diagnose pregnancy and non-pregnancy in sheep. The tubes were transported to the laboratory of college veterinary medicine –university of Baghdad in a cool box containing ice bags, centrifuged at 3000 x g for 10 min, aliquoted in 1.5ml Eppendorf tubes and stored at -20 C until analysis.

Progesterone were measured using Sheep PROG ELISA KIT. All collected serum samples were frozen and kept at -20°C until assayed. For this assay, added 50 µl in first column of kit plate as standers for test and added 10 µl of (collected serum) and assay controls (positive and negative) were pipetted into 88-well, anti-PAG antibody coated plates along with 40 µl of sample diluent, sealed, and 100µl of conjugate reagent enzyme (anti-IgG-horseraddish peroxidase) was added for 96-well with standers and incubated for 60 min at 37ºC in a forced air incubator. After incubation, plates were washed four times with 350 µl of wash solution. The detector solution (chromogenic A and chromogenic B) 100 µl was added to each well, covered, and incubated for 15 min at 37ºC. The plates were washed (4x) and 100 µl of substrate solution (tetramethylbenzidine) added into the wells and incubated for 15 min at room temperature. Finally, 100 µl of stop solution (foric acid)was added and the absorbance was determined at 450 nm and 630 nm) determined on a microtiter plate spectrophotometer. Sample values were reported as serum sample minus negative controls after subtracting the mean. . The interpretation of results was based on the color of the sample at the end of the test. When the pregnancy test was positive, the well was stained blue; in a negative test, well color remained transparent.

Statistical examination

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study. Receiver operation characteristic curve (ROC curve)was used to identify the validity of markers as an indicator of the pregnancy and type of offspring. The markers were compared according to area under curve. The analysis was submitted by using MedCalc (2016) Software. $P < 0.05$ is considered statistically significant.

Results

Table 1: Values of estrus response(%),duration of response (hrs) and estrus phase length (hrs) in animals of study

Groups	Number	Estrus response %	Duration of response (hrs)	Length of estrus phase (hrs)
G1	6	100 ±0.00 A	39.17 ±2.74 B	31.67 ±1.97 B
G2	6	100 ±0.00 A	43.50 ±3.19 B	26.33 ±2.85 C

G3 (Control)	6	17 ±0.52 B	48.00 ±0.75 A	38.00 ±0.52 A
	LSD value	9.437 *	4.902 *	3.283 *
Means with different letters in the same column are significantly different. , * (P≤0.05).				

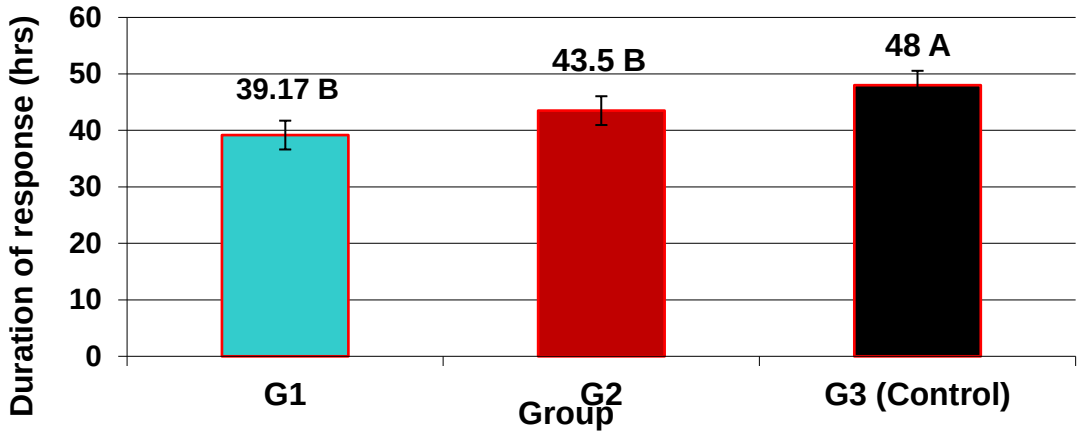

Figure 1: Values of duration of response in animals of study.

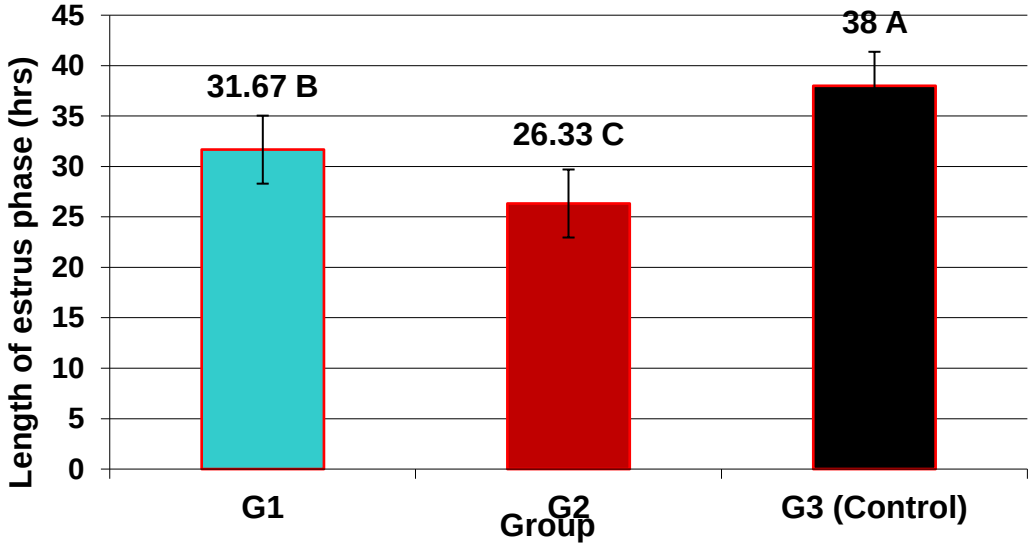

Figure 2: Values of estrus phase length in animals of study.

Results of reproductive parameters (estrus response % , duration of response (hrs) and estrus phase length (hrs)) of estrus synchronized ewes of present study are depicted in (table 1). The results indicate highest values of estrus response % was recorded in G1 and G2, which differed significantly ($p<0.05$) from result of G3, which recorded lowest value. Regarding duration of response (hrs) the result proved highest values was recorded in G3, which differed significantly ($p<0.05$) from G1 and G2, on the other hand the length of estrus phase (hrs) shows highest values in G3 which differed significantly ($p<0.05$) from G1 and G2.

The result of current study demonstrated that used of vaginal sponge for ovarian control with similar effectiveness to the CIDR in combination with single dose of PMSG at the time of divice withdrawal resulting in an advancement of the onset of estrus and time of ovulation, because serum P4 levels greater than 1 ng /ml are enough to control LH pulsatility and ovulation in ewes, thus concentration of P4 in sponge does not or slightly effect to reproductive performance, this results supported by Vilarino et al., (2010). In fact, evaluation of fertility changes demands large number of animals, while evaluation of estrus onset and response demands a high number of successive response which are obtained from a small number of animals, this fact is supported by (Martinez-Ros et al., 2019). The duration of response (from progesterone removal until beginning of estrous

symptoms) in current study varies from 39 to 48 hrs this results close to some authors Gungor et al (2007);(2009) and Kacar et al., (2008), they show estrus response varies from 34.5 hrs to 74 hrs. Kaya et al., (2013) show the onset of estrus symptom was from 50.3 hrs to 59 hrs this variation depending on many factors one of them breed of ewes also show close to our results in percentage of estrus response in treated and control groups. Blaschi et al. (2014) illustrated that the period from vaginal sponge removal and onset of estrus was 34.2± 8.9 hrs shorter than our detection and this period increase when decrease the duration of sponges insertion (9 days and 5 days). Also the onset of estrus detection by Salehi et al. (2010) and Martemucci and D'Alessandro, (2010) in synchronized ewes with CIDR and vaginal sponge were 36 hrs and 33.1± 4.3 hrs after device withdrawal also this period observed slightly shorter than our results . Texeira et al. (2016) proved that when estrus synchronized ewes for 12 days progesterone treatment with injection of300 IU of PMSG at the end of progesterone treated obtained onset of estrus 32.5±15.4 hrs estrus response 80% and duration of estrus 42.0±20.3 hrs. Godfrey et al. (1999) show that when administration CIDR (300 mg) for 12 days combination with ram introduction obtain estrus response 100%. Letelier et al. (2009) proved that decreasing progesterone amount in vaginal sponges or decreasing treatment period from 12 to 6 days did not negatively affect follicular growth and ovulation. Kaylan et al. (2016) found that estrus response was 83.85% in ewes after treated with vaginal sponges for 12 days then 200 IU injection of PMSG at sponge withdrawal. deNicolo et al. (2008) and Mulvaney et al. (2013) detected slightly lower estrus response during non breeding season than those in breeding season which was 75% and 100% respectively. It possible because ewes in non breeding season were deep anestrus which reduced estrus response slightly , even with P4 and PMSG treated (deNicolo et al., 2008). Ataman et al. (2006) recorded that estrus rate is 100% during breeding season and 93.3% during non breeding season and estrus onset after vaginal withdrawal 42.9± 1.3 hrs during breading season and 45.6± 1.5 hrs during non breeding season after treated ewes with vaginal sponges for 12 days and 400 IU injection of PMSG, this results in agreement with our results. Naderipour et al. (2012) recorded that estrus response was 85% in sponge treated ewes and 90% in CIDR treated ewes. Current study was very closed to and in agreement with Ozyurtlu et al. (2010) recorded that estrus response after CIDR , sponge treatment for 12 days and 400IU of PMSG at device withdrawal and control was 90% , 87.5% and 16.7% respectively, which higher significantly (P<0.05) in treated groups than control group also show that the onset of estrus (35.22±1.6, 38.48± 1.5 and 49.33±3.5 respectively and duration of estrus (30.17±0.7, 29.43±0.8 and 36.67±0.7 respectively which lower significantly (P<0.05) in treated groups than control group in Awassi ewes . Gardon et al. (2015) observed that duration of estrus depending on ewes age which recorded estrus onset in treated ewes ranged between 24-31hrs and extended to 70-77 hrs while treated lamb ewes ranged between 24-31 hrs and extended to 62-69 hrs. In support of our results Vilarino et al. (2013) found that estrus onset was 30-40 hrs and estrus response 100% after new CIDR –PMSG treatment. Iida et al. (2004); Hashemi et al. (2006); Yu et al. (2019) and Hameed et al. (2021) in agreement with present study which illustrated that CIDR device containing 300mg progesterone is similar effective to 60 mg MAP sponge with 12 days long protocol on reproductive performance. In support with our results Sefidbakht et al. (1978) fond that mean estrus duration no difference between treated progesterone ewes (27.2 hrs) but lower than that detected for natural estrus in Karakul ewes (35.2hrs) .

Table 2: Changes in progesterone concentration in estrus synchronized ewes during CIDR and vaginal sponges treatment (ng/ ml

Groups	Day 0	At day 12 of treatment	At estrus phase
G1	0.884 ±0.16 A b	5.27 ±0.44 A a	0.435 ±0.12 A b
G2	0.771 ±0.18 A b	5.87 ±0.22 A a	0.589 ±0.10 A b
G3 (Control)	0.817 ±0.19 A a	0.965 ±0.17 B a	1.05 ±0.14 A a
LSD value= 0.781 *			
Means with different capital letters in the same column and small letters in the same row are significantly different. , * (P≤0.05).			

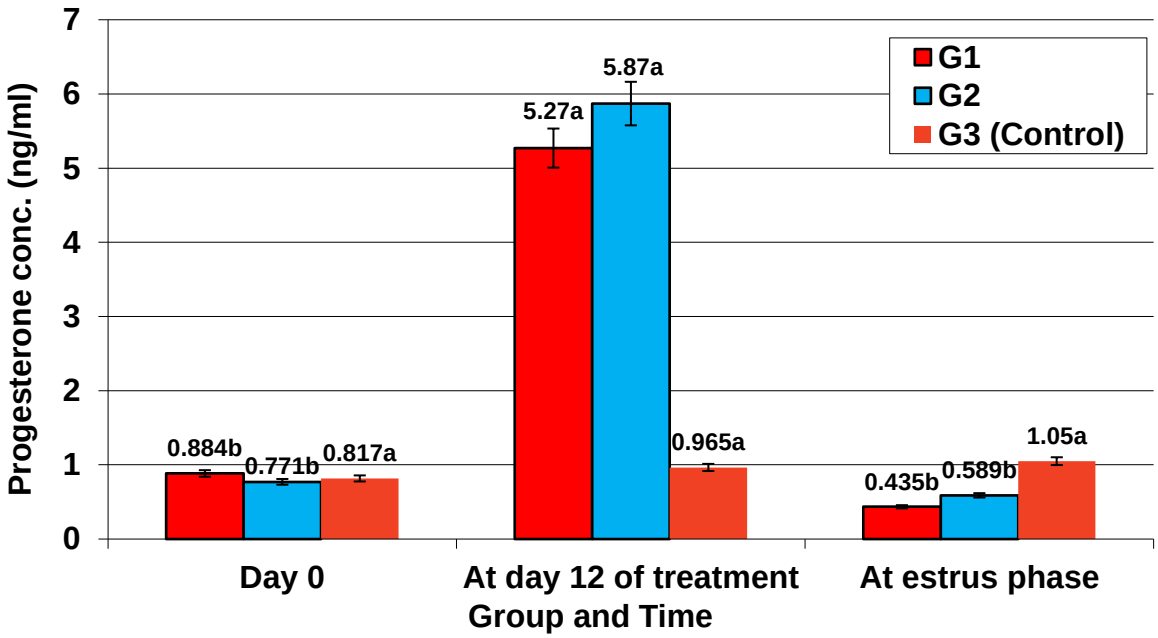

Figure 3: Changes in progesterone concentration in oestrus synchronized ewes during CIDR and vaginal sponges treatment

Results of progesterone level (ng/ml) before treatment and after 12 days of application different hormonal regimes for estrus synchronization in ewes are presented in (table2 and figure3). The results proved non significant difference ($p<0.05$) between the values of P4 level before treatment and during estrus in G1, G2 and G3, while after 12 days of treatment the results indicate that highest values in G1 and G2, which differed significantly ($p<0.05$) from G3, which show lowest significant value.

Concerning the differences in P4 concentration before treatment , after 12 days of treatment and at days of estrus ,these results revered that in G1 treatment the concentration of P4 recorded highest value after12 days of treatment which differed significantly ($p<0.05$) from other periods of study (before treatment and at day of estrus) and the lowest significant value was found in day before treatment and also at estrus period, also in G2 treatment result of current study regarding P4 concentration recorded the same trend in which highest significant value found after 12 days of treatment and lowest value at day before treatment and estrus phase, while results of G3 non significant differences ($p<0.05$) among all period (before treatment, after 12 days of treatment and estrus phase).

Our results close to Vilarino et al., (2010) they demonstrated that mean serum progesterone values rise after device insertion. In support of our results Lopez-Garcia et al. (2021) recorded that serum progesterone value was higher in sheep from the 10 day CIDR groups, this agree with Wheaton et al. (1993) which found that progesterone level increased rapidly in maternal blood follow CIDR insertion and decreased rapidly after CIDR withdrawal. In contrary Alhimaidi et al. (2023) found that progesterone values did not differences before and after CIDR or sponge insertion. Naderipour et al. (2012) and Turk et al.(2008) found that progesterone absorption in CIDR treated ewes faster than those treated by vaginal sponges, which detected after 24 hrs and reach peak values within 72 hrs in CIDR treated ewes. Cox et al. (2012) show that maintain blood progesterone levels which released from new CIDR device more than 2 ng /ml after 7 days of treatment. De Graaff and Grimard, (2018) proved that CIDR provides increase serum progesterone level soon after its insertion because its efficient release kinetics which reduce LH secretion from pituitary and maintain of large follicles which depended on LH at the ovary .

Table 3: Progesterone values in estrus synchronized ewes (CIDR, vaginal sponges) during first and second month of pregnancy (ng/ml).

Groups	Day of estrus	Day 30 pregnancy	Day 60 pregnancy
G1	0.435 ±0.12 A c	10.61 ±1.29 A b	14.50 ±1.34 A a
G2	0.589 ±0.11 A c	8.86 ±1.01 A b	12.01 ±1.30 A a
G3 (Control)	1.068 ±0.17 A a	0.782 ±0.08 B a	0.999 ±0.16 B a
LSD value= 3.068 * Means with different capital letters in the same column and small letters in the same row are significantly different. , * (P≤0.05).			

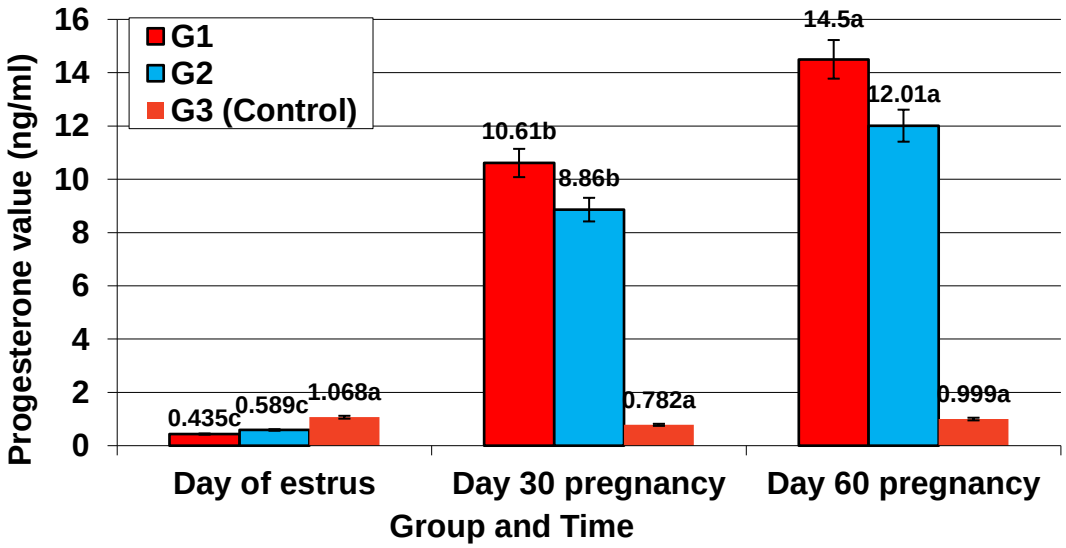

Figure 4: Progesterone values in estrus synchronized ewes (CIDR, vaginal sponges) during first and second month of pregnancy.

Results of progesterone level (ng/ml) during estrus and different stage of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in (table3 and figure4). The results proved non significant difference ($p<0.05$) between the values of P4 level during estrus in G1, G2 and G3, while during pregnancy the results indicate that at day 30 of pregnancy highest values in G1 and G2, which differed significantly ($p<0.05$) from G3, which show lowest significant value, the same trend of P4 have been proved at day 60 of pregnancy, means highest values was recorded in G1 and G2 in comparison with G3 in all above mentioned period.

Concerning the differences in P4 concentration at days of estrus and different period of pregnancy (day30 and day 60), these results revered that in G1 treatment the concentration of P4 recorded highest value at day 60 of pregnancy which differed significantly ($p<0.05$) from other pregnancy periods of study and the lowest significant value was found at estrus period, also in G2 treatment result of current study regarding P4 concentration recorded the same trend in which highest significant value found at day 60 of pregnancy and lowest value at estrus phase, while results of G3 non significant differences ($p<0.05$) among all period of pregnancy and estrus phase.

Our results in agreement with De Carolis et al., (2020) who proved that P4 values can early detect and distinguish between pregnant and non pregnant ewes with sensitivity and specificity 100%.Boscos et al. (2003) spported our results , they recorded that P4 level on day 19 after mating can be used as a reliable method for pregnancy detection. . Nawito et al. (2015) and Al-Sobaiyl, (2010) demonstrated that P4 level is higher significantly in pregnant ewes than those non pregnant .

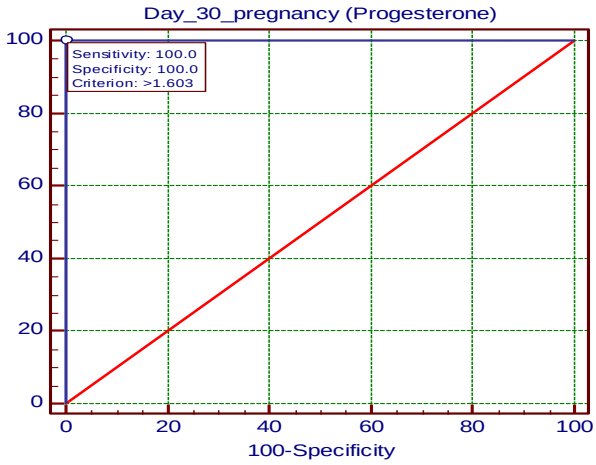

Figure 5 : The Roc curve of the progesterone at 30 days of pregnancy as a marker for pregnancy diagnosis

Table 4: Parameters of Roc curve at day 30 of pregnancy of ewes

Associated criterion (cutoff point)	>1.603
Sensitivity %	100.00
Specificity %	100.00
Area under the ROC curve (AUC)	1.000
Standard Error a	0.000
95% Confidence interval b	0.912 to 1.000
Significance level P (Area=0.5)	<0.0001

Results of receiver operation characteristic (ROC) curve and cutoff point of progesterone level (ng/ml) at day 30 of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in (table4 and figure5). The results proved ROC curve was conducted to evaluate the validity of progesterone as biological marker of pregnancy at day thirty of pregnancy with sensitivity and specificity 100% and the cut of point of progesterone is greater than 1.603 ng/ml which confirm that the ewe is pregnant at day thirty

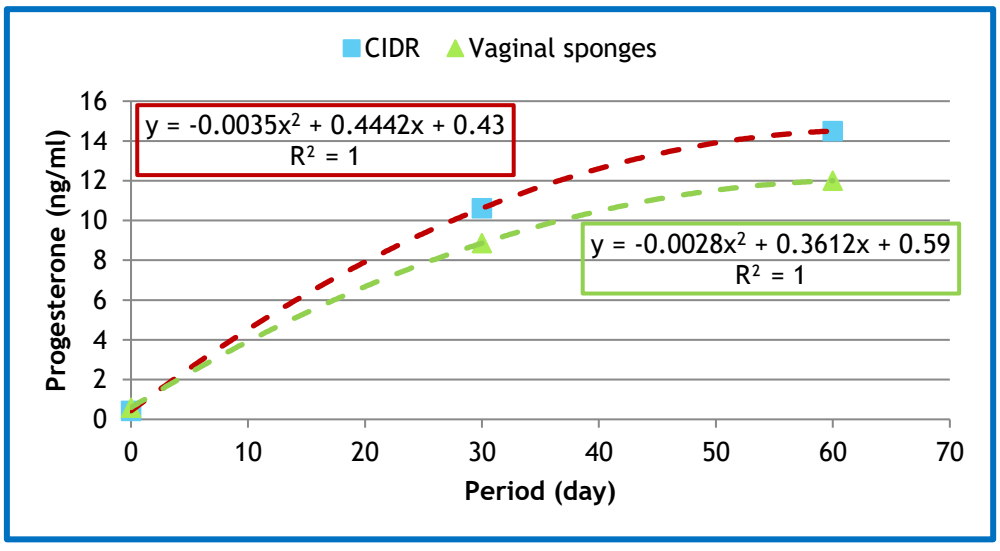

Figure 6: Quadratic predictor equation for estimation progesterone level during pregnancy

Results of prediction equations of progesterone at different day of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in (figure6). The results proved the prediction equations was to evaluate the validity of progesterone to predict P4 level in any day of pregnancy in studied ewes.

Table 5: Impact of single and twin pregnancy on progesterone values (ng/ml) at different stages of pregnancy in studied animals.

Groups	Day of estrus	Day 30 of pregnancy	Day 60 of pregnancy
P4 level in single pregnancy	0.546 ±0.10 A c	8.18 ±0.39 B b	11.29 ±0.45 B a
P4 level in twin pregnancy	0.547 ±0.16 A c	14.09 ±0.46 A b	18.48 ±0.32 A a
LSD value= 2.971 *			
Means with different capital letters in the same column and small letters in the same row are significantly different. , * (P≤0.05).			

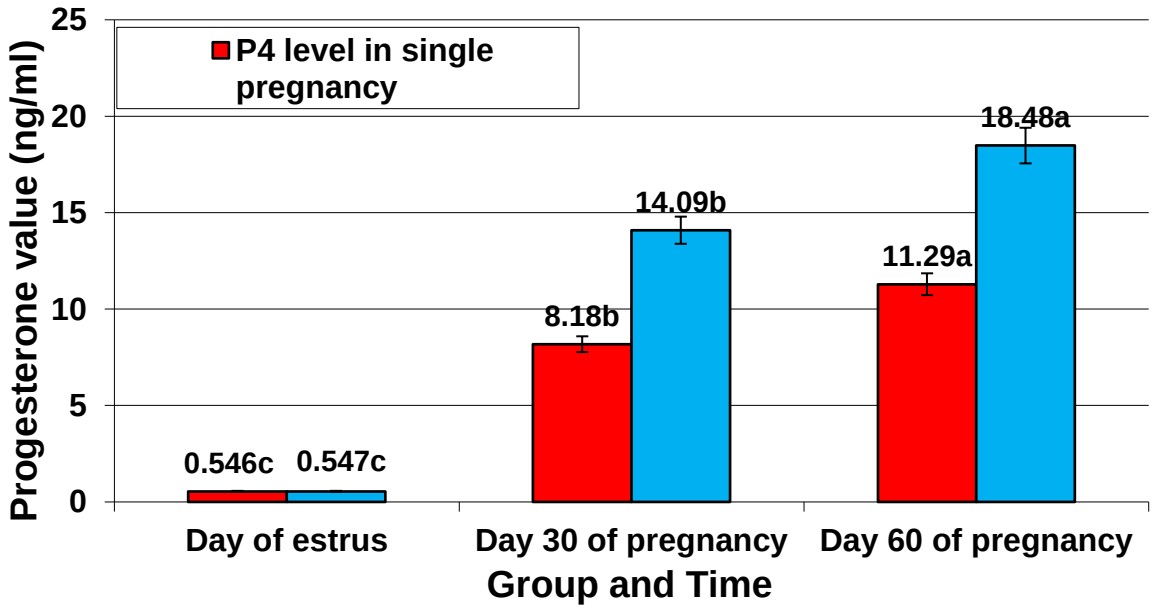

Figure 7: Impact of single and twin pregnancy on progesterone values (ng/ml) at different stages of pregnancy in studied animals.

Results of progesterone level (ng/ml) during estrus and different stage of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in (table5 and figure7). The results proved non significant difference ($p<0.05$) between the values of P4 level during estrus in ewes which after that become pregnant with single or twin , while during pregnancy the results indicate that at day 30 of pregnancy highest values in ewes with twin pregnancy , which differed significantly ($p<0.05$) from ewes with single pregnancy, which show lowest significant value, the same trend of P4 have been proved at day 60 of pregnancy, means highest values was recorded in ewes with twin pregnancy comparison with ewes with single pregnancy .

Concerning the differences in P4 concentration at days of estrus and different period of pregnancy (day30 and day 60), these results revered that in ewes with single pregnancy the concentration of P4 recorded highest value at day 60 of pregnancy which differed significantly ($p<0.05$) from other pregnancy periods of study and the lowest significant value was found at estrus period, also in ewes with twin pregnancy result of current study regarding P4 concentration recorded the same trend in which highest significant value found at day 60 of pregnancy and lowest value at estrus phase.

In the current study, proved that P4 level effected by the number of fetus at day 30 and 60 of pregnancy. Similarly Yazici et al., (2018) observed that significant difference in serum progesterone level in does that bearing twin pregnancy and does bearing a single pregnancy. Our study show of higher level of P4 in twin pregnant ewes in comparison with those pregnant with a

single, this result confirm by several authors in ewes and does (Manalu and Sumaryadi, 1998; Boscos et al., 2003 Yotov, 2007; Barbato et al., 2009; Haldar et al., 2013 and Singh et al., 2019). There are significant positive correlation between corpus luteum diameter and number of fetus which described by Gur et al., (2011) and Manalu and Sumaryadi,(1998) larger total luteal tissue size with twin pregnancy can be attributed to higher serum progesterone level in twin than single pregnancy also P4 level affected by birth weight in sheep and goat. Nawito et al. (2015) demonstrated that P4 level is higher significantly in ewes and does bearing twin fetuses than those bearing single fetuses . In support of our demonstration Muller et al. (2003), they can distinguish between ewes with single and twin pregnancy on day 19 of pregnancy in sheep. In contrary Kalkan et al., (1996); Boscos et al (2003) and Yotov, (2007) there are evidencing no significant difference in the same period but, they showed that can be detected the number of fetuses in sheep with significant difference in P4 concentration between ewes with single or twin fetuses at day 60 of pregnancy. Shabankareh et al. (2009) proved that total volume of corpus luteum differed in single and double ovulate ewes, in double ovulate ewes the volume of the individual corpus luteum was smaller than single ovulate ewes, but total volume of CL was higher in ewes with double ovulation lead to high serum progesterone levels.

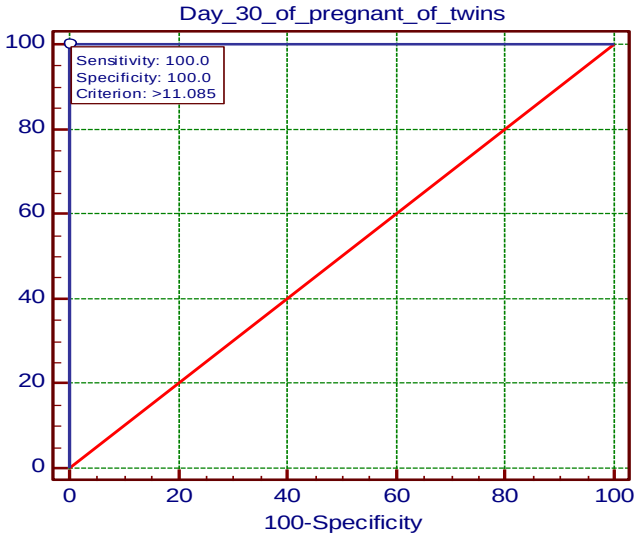

Figure 8: The Roc curve of progesterone at 30 days of pregnancy as a marker of twins pregnancy

Table 6: Parameters of Roc curve at day 30 of twin pregnancy of ewes

Associated criterion	>11.085
Sensitivity	100.00
Specificity	100.00
Area under the ROC curve (AUC)	1.000
Standard Error a	0.000
95% Confidence interval b	0.753 to 1.000
Significance level P (Area=0.5)	<0.0001

Results of receiver operation characteristic (ROC) curve and cutoff point of progesterone level (ng/ml) at day 30 of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in (table6 and figure8). The results proved ROC curve was conducted to evaluate the validity of progesterone as biological marker of twin pregnancy at day thirty of pregnancy with sensitivity and specificity 100% and the cut of point of progesterone is greater than 11.085 ng/ml which confirm that the ewe is twin pregnant at day thirty

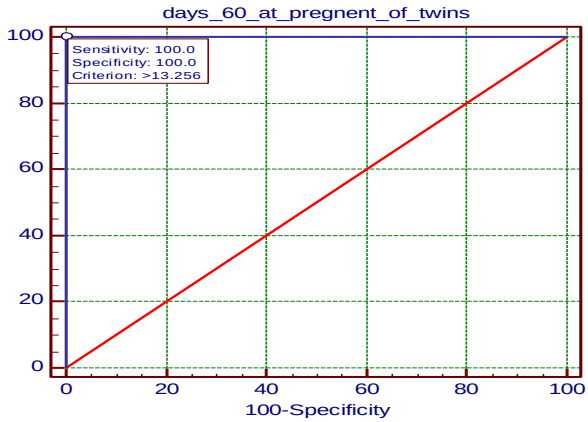

Figure 9: The Roc curve of progesterone at 60 days of pregnancy as a marker of twins pregnancy

Table 7: Parameters of Roc curve at day 60 of twin pregnancy of ewes

Associated criterion	>13.256
Sensitivity	100.00
Specificity	100.00
Area under the ROC curve (AUC)	1.000
Standard Error a	0.000
95% Confidence interval b	0.753 to 1.000
Significance level P (Area=0.5)	<0.0001

Results of receiver operation characteristic (ROC) curve and cutoff point of progesterone level (ng/ml) at day 60 of pregnancy after application different hormonal regimes for estrus synchronization in ewes are presented in (table7 and figure9). The results proved ROC curve was conducted to evaluate the validity of progesterone as biological marker of pregnancy at day sixty of pregnancy with sensitivity and specificity 100% and the cut of point of progesterone is greater than 13.256 ng/ml which confirm that the ewe is twin pregnant at day sixty

Table 8: Cut off point of twin pregnancy at 30 days and 60 days of pregnancy

	Progesterone at 30 days (pregnant twins)	Progesterone at 60 days (pregnant twins)
Cut off point	>11.085	>13.256

Table 9: Change in birth weight related to litter size and sex of fetus.

Sex	Birth weight/kg
Singleton /Male	4.50±0.04a
Singleton/Female	4.16±0.05b
Twins/Male	3.80±0.05c
Twins/Female	3.45±0.05d
LSD	0.17
Means with a different letter are significantly different (P<0.05)	

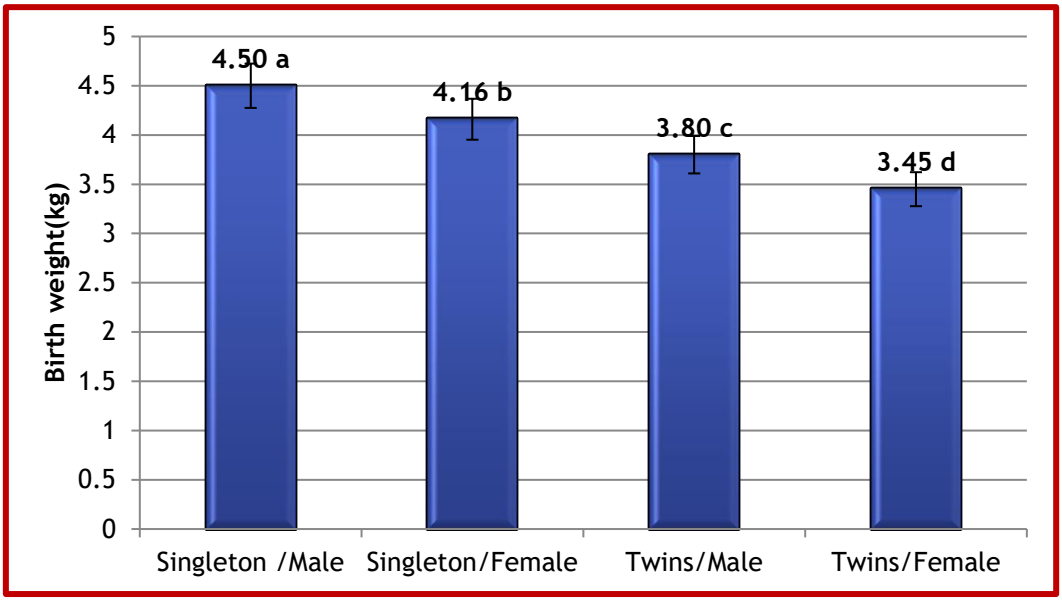

Figure 10: Change in birth weight related to litter size and sex of fetus

Results of birth weight(kg) during parturition after application different hormonal regimes for estrus synchronization in ewes are presented in (table 9 and figure10). The results proved significant difference ($p<0.05$) between the birth weight is depending on litter size and fetal gender , which show the highest values in male singleton parturition, which differed significantly ($p<0.05$) from female singleton , male and female twin parturition, which show lowest significant value in female twin.

Table 10: Reproductive performance of the ewes during CIDR and progesterone vaginal sponges in ewes of study

Groups	Number	Pregnancy rate %	Lambing rate %	Fecundity rate %
G1	6	83a (5/6)	117a (7/6)	140a (7/5)
G2	6	100a (6/6)	117a(7/6)	117ab (7/6)
G3 (Control)	6	17b (1/6)	17b(1/6)	100b (1/1)
	P-value	0.0001 **	0.0001 **	0.0001 **
				** (P≤0.01).

Results of reproductive performance (pregnancy rate%, lambing rate% and fecundity rate%) after parturition of estrus synchronized ewes of present study are depicted in (table 10). The results indicate highest values of pregnancy rate %(number of ewes lambing / total number of ewes x100) (Ozyurtlu et al., 2010) was recorded in G1 and G2 (83% and 100% respectively), which differed significantly ($p<0.01$) from result of G3 (17%), which recorded lowest value. Regarding lambing rate %(number of lambs born / total number of ewes x100) the result proved highest same values was recorded in G1 and G2 (117%), which differed significantly ($p<0.01$) from G3 (17%), on the fecundity rate % (number of lambs born / number of ewes lambed x100) shows highest values in G1 (140%) and no significant from G2 (117%), but, differed significantly ($p<0.01$) from G3 (100%).

In current study the incidence of fetal losses from day 30 of pregnancy until parturition is obviously lower (1/13) approximately 7.7%. This possible attributed to the lower incidence of twining for studied ewes. This results in agreement with Dixon et al., (2007) and Hayder and Ali, (2008), they showed that fetal losses from day 25 of pregnancy to parturition is (6.8-11.8%) because decrease the incidence of twining recorded in Farafra breed and they proved that embryonic death in ewes occurs when increase in ova released number. In support of our study Quintero-Elisea et al. (2011) proved that giving 400IU of PMSG under tropical conditions can be obtained high pregnancy rat (81.8%) and high fecundity rat (liter size, 177.2%) in synchronized haired sheep. The similarity of the protocol of 12 days with physiological events of luteal phase can provide cause explanation for relatively high

gestation rates obtained in current study. It is known that regression of CL in ewes occurs between 12 to 13 days after oocyte release and decrease in progesterone rapidly is essential for estrus onset and ovulation (Blaschi et al., 2014). This possible explain why ewe treated with 12 or 14 days progesterone obtained highest pregnancy rate. Ataman et al. (2006) obtain high level of gestation rate, lambing rate and fecundity rate which recorded 86.6%, 80.0% and 150% respectively after treated ewes with vaginal sponges for 12 days and 400 IU injection of PMSG, this results are close to our results. Swelum et al., (2015) and Alhimaidi et al. (2023) found that fertility rate , lambing rate and fecundity rate were significantly (P <0.05) higher in CIDR treated ewes than in FGA vaginal treated group. In contrary Kaylan et al., (2016). found that lambing rate ranged from 66.67% in breeding season to 57.57% in non breeding season in estrus ewes with fixed time artificial insemination. In support of current study Ozyurtlu et al. (2010) found that, after ewes treated with CIDR and vaginal sponges for 12 days and 400 IU of PMSG injection at the time of CIDR and sponge withdrawal, pregnancy rate, lambing rate and fecundity rate did not differ between CIDR and sponge groups but differed from control group, which they indicate that using CIDR devices and intravaginal sponges with PMSG injection at devices or sponges withdrawal to be similar effective in estrus induction and fertility in Awassi ewes during non breeding season.

Table 11: Mean gestation length in studied animals (days)

Gestation period/day	149.60±0.52

Results of mean gestation length in studied animals(days) during parturition after application different hormonal regimes for estrus synchronization in ewes are presented in (table11). The results proved the mean gestation period in ewes of study 149.6± 0.52 days. Similar results proved by De Carolis et al., (2020) in which the average gestation length was 149.5 days. Regueiro et al. (2020) found that gestation length ranged from 147-149 in primiparous ewes and ranged from 148-151 in multiparous ewes.

Table 12: Values of gestation length, duration of labour, delivery assistance and lamb weight in Iraqi cross breed ewes in different treated groups

Groups	Gestation length (day)	Duration of labour (min)	Delivery assistance %	Lamb weight			
				Single birth		Twin birth	
				Male	Female	Male	Female
G 1	148.60 ±1.36	41.40 ±1.87 b	0 ±0.00	4.50 ±0.05	4.15 ±0.02	3.83 ±0.11	3.50 ±0.08
G2	150.67 ±1.55	42.03 ±2.05 b	0 ±0.00	4.55 ±0.13	4.16 ±0.10	3.70 ±0.08	3.40 ±0.07
G 3 (control)	153.00 ±1.08	50.00 ±0.12 a	0 ±0.00	4.40 ±0.16	--	--	--
LSD value	3.219 NS	4.026 *	0.00 NS	0.359 NS	0.466 NS	0.302 NS	0.297 NS
Means with different letters in the same column are significantly different. , * (P≤0.05).							

Results of gestation length(day), duration of labour (min), delivery assistance(%) and lamb weight (kg) during parturition after application different hormonal regimes for estrus synchronization in ewes are presented in (table12). The results proved significant difference (p<0.05) between the duration of labour (min) in G1, G2 and G3, which show the highest values in G3 and lowest values in G1 and G2, while the results of current study proved non significant differences (p<0.05) between the value of gestation length, delivery assistance and lamb weight (male single birth, female single birth, male twin birth and female twin birth) in G1, G2 and G3.

The result of current study demonstrated that used of vaginal sponge for ovarian control with similar effectiveness to the CIDR in combination with single dose of PMSG at the time of divice withdrawal resulting in an advancement of the onset of estrus and time of ovulation, because serum P4 levels greater than 1 ng /ml are enough to control LH pulsatility and ovulation in ewes, thus concentration of P4 in sponge does not or slightly effect to

reproductive performance, therefor, the duration of gestation length in control group is longer than those in treated groups, in spite of no significant difference between groups, this results supported by Vilarino et al., (2010). Regueiro et al. (2020) found that gestation length ranged from 147-149 days in primiparous ewes and ranged from 148-151days in multiparous ewes, also found duration of labour slightly longer (50±1.8 hrs) in primiparous ewes than those of multiparous (42.9±1.7 hrs) and observed that delivery assistance percentage slightly higher (9.1%) in primiparous ewes than those of multiparous (6.8%) and show lamb weight percentage slightly heavier (5.1 kg) in primiparous ewes than those of multiparous (5.6 kg) this results was close to our results. In support of our result regarding lamb weight Fernandes et al., (2013). Illustrated that lambing weight significantly higher in single birth (3.7±0.13 kg) than in twin birth (2.6±0.13 kg) but no significant difference between female and male lambs in both single and twin in spite of slightly higher in male than in female.

Table 13: Change in pregnancy length, related to litter size and sex of fetus

Sex	Gestation period (day)
Singleton /Male	149.50±1.04ab
Singleton/Female	151.60±0.51a
Twins/Male	148.25±0.47b
Twins/Female	147.50±0.50b
LSD	2.38
Means with a different letter are significantly different (P<0.05)	

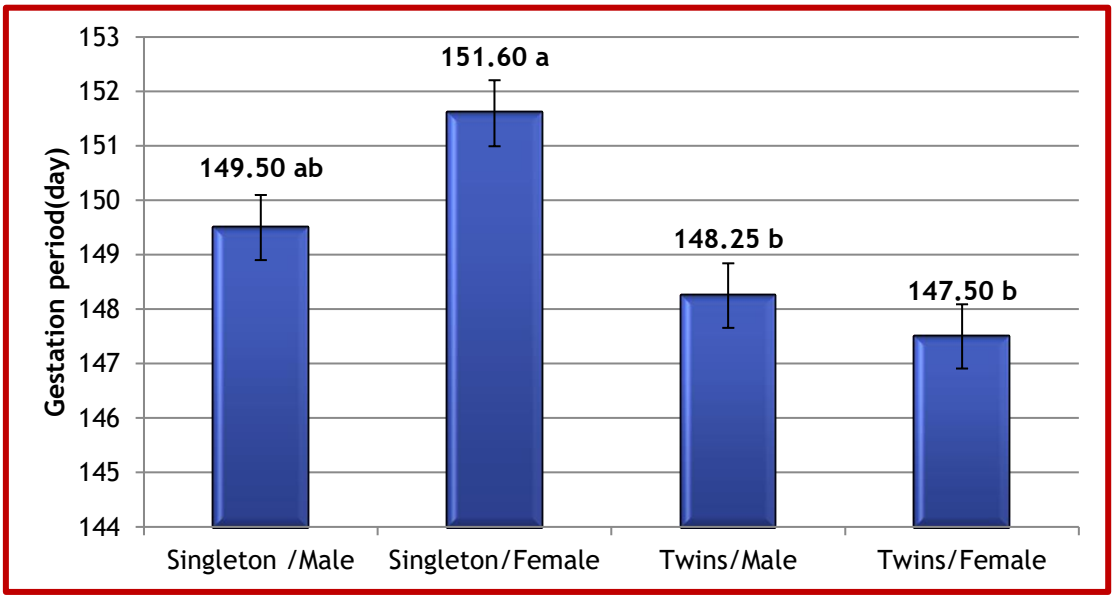

Figure 11: Change in gestation length, related to litter size and sex of fetus

In present study results of gestation length(day) during parturition after application different hormonal regimes for estrus synchronization in ewes are presented in (table13 and figure11). The results proved significant difference (p<0.05) between the gestation length, which depending on litter size and fetal gender, the results show the highest values in female singleton parturition, which non significantly differed (p<0.05) from male singleton , while significantly differed from male twine and female twin parturition.

Current study proved similar results of authors, in which the mean birth weight of single pregnancy was greater than that for twin pregnancy. This mean that the litter size affects birth weight of lambs and kids (Khanum et al., 2001; Juengel et al., 2018; Yazici et al., 2018 and Singh et al., 2019). These results can be explained by the fact that the space in uterine is of impacted capacity to accommodate offspring through pregnancy and the mother possible does not possess physiological capacity to adequately supply twin pregnancy when the demand of nutrient increase for fetus development and growth in comparison with single pregnancy, lead to decrease in

individual birth weight when increase liter size (Mellado et al., 2011). De Carolis et al ., (2020) show gestation length vary with litter size which were 149.1 days in single pregnant ewes and 148.1 days in twin pregnant ewes. Regueiro et al. (2020) show lamb weight percentage slightly heavier (5.1 kg) in primiparous ewes than those of multiparous (5.6 kg) . In support of our result regarding lamb weight Fernandes et al., (2013). Illustrated that lambing weight significantly higher in single birth (3.7±0.13 kg) than in twin birth (2.6±0.13 kg) but no significant difference between female and male lambs in both single and twin in spite of slightly higher in male than in female.

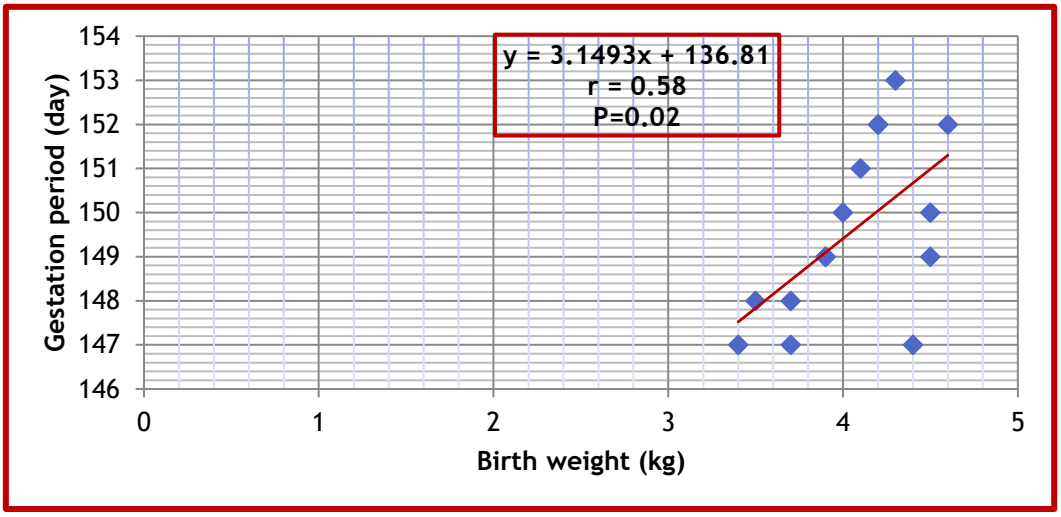

Figure 12: Prediction equation and correlation coefficient between body weight and gestation length

Results of gestation length(day) with body weight during parturition after application different hormonal regimes for estrus synchronization in ewes also prediction equation of birth weight depended on gestation length are presented in (figure12). The results proved significant positive correlation between body weight and gestation length. It can concluded that current study illustrated that application of CIDR-PMSG and vaginal sponges-PMSG regimes was effective in estrus synchronization and achievement of high pregnancy rate in ewes with approximately similar results. The serum progesterone level via sheep progesterone ELISA kit on day 30 after insemination can be successfully used for diagnosis of pregnant and non pregnant sheep and the number of fetus with high accuracy.

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