Acta Entomology and Zoology

E-ISSN: 2708-0021
P-ISSN: 2708-0013
www.actajournal.com
AEZ 2024; 5(2): 213-220
Received: 28-08-2024
Accepted: 05-10-2024

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Efficacy of CIDR alone and in combination with PMSG on reproductive performance of local Iraqi ewes

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DOI: https://doi.org/10.33545/27080013.2024.v5.i2c.173

Abstract
The aim of study ability of CIDR alone and in combination with PMSG in improving the fertility potential of the ewes, Determine the concentration of progesterone between difference period. Eighteen multiparous local Iraqi ewes aged between 3-4 years were randomly divided into three groups (n=6/ group). Ewes were synchronized by CIDR The total number of animals used in the experiment was Eighteen ewes, The animals were subdivided into three groups; Group1 (n=6) ewes treated with CIDR for 12 day and treated with a single dose of intramuscular injection of PMSG 500 IU at the time of CIDR removal. Group 2 (n=6) non pregnant ewes treated only by CIDR, which applied by intra-vaginal route for (12 days), then withdrawal. Group3 (n= ***6***) ewes control without treatment throughout the study. The estrus response was significantly ($P<0.05$) for the treatment groups compared to the control group, with a significant superiority $P\leq0.05$ for the first treatment group, G1, compared to the second treatment group, G2, in both the response rate to the estrus synchronization programs, the rate of onset of estrus, and the duration of estrus, at 100%, 66%, 0%, and 35.50, 51, and 0 hours and 31.67, 29.50, and 0 hours for the three study groups G1, G2 and the control group for the three fertility. The results showed a clear significant superiority ($p<0.05$) in progesterone levels in the 12-day period after treatment with progesterone in the G1 and G2 treatment groups compared to the control group, which did not show any change in progesterone levels in all three study periods. In addition to the convergence of progesterone levels in the first and last periods of the study (before treatment and at day of estrus). The results also showed a clear significant superiority ($p<0.05$) in the level of progesterone in the 12-day period after treatment with progesterone in pregnant and non-pregnant ewes compared to the periods before treatment and after the end of the treatment period, which showed convergence in progesterone levels, and although the 12-day period showed an increase in the level of progesterone in non-pregnant ewes, but it was lower significantly ($p<0.05$) than in pregnant ewes during the same aforementioned period. Regarding for the length of pregnancy and the length of labour, no significant differences ($p<0.05$) were observed between the two treatment groups, which recorded very close values that did not exceed 149 days and 41.8 minutes for the periods of pregnancy and labour, respectively.

Keywords: CIDR, Sheep, Iraq, Progesterone, PMSG

Introduction
Ewes are animals that exhibit seasonal polyestrous behavior. The breeding season is characterized by regular intervals of 16-17 days of estrus behavior and ovulation (Hussain *et al*., 2017, Hatif and Younis, 2018a) [38, 35]. Also Younis *et al*. (2019) [73]. However, during the mid-anestrus period, there is a cessation of cyclic ovulatory activity (Dehkordi *et al*., 2022) [16]. These animals are influenced by seasonal fluctuations in environmental conditions, with changes in day length playing a crucial role in controlling the breeding season (Hatif and Younis 2018a) [35]. reproductive activity occurs during shorter days, while it diminishes during winter and spring as the day length increases (Al-Mutar, 2017 and Younis et al., 2020) [6, 72]. The length of the breeding season varies between two to three estrus cycles (Taher, 2014) [63]. and in certain tropical breeds, cyclic activity occurs throughout the year, with ewes going into estrus approximately 21 times a year due to the effect of latitudes (Abdul Hussain *et al*., 2017) [38]. In sheep, the experimental modification of photoperiod, without a change in other factors can shift the timing of the breeding season. The reversal of the annual photoperiodic cycle causes the reproductive season to shift by six months (Rosa and Bryant, 2003) [57].

In addition, within six months, ewes exposed to light regimes that provided a natural annual variation in day length, have two breeding seasons per year (Cameron *et al*., 2010) [13] during the Spring photoperiod that nocturnal MLT concentration is higher than diurnal concentrations in seasonal and non- seasonal local Awassi ewes at spring (Hatif and Younis, 2018b) [34]. It is clear that short days are stimulatory and long days are inhibitory at short time (Forcada and, 2006) [21]. Modifications to the activity of the hypothalamic-pituitary axis through changes in pulsatile gonadotrophin releasing hormone (GnRH) and luteinizing hormone (LH) control the seasonal changes in ovine reproductive condition. Such modifications reflect differences in sensitivity to the negative feedback of circulating oestradiol (Smith *et al*., 2010) [59]. Estrous synchronization is manipulation of the bovine estrous cycle to result in the majority of animals exhibiting standing estrus in a short period of time (Tamer and Al-Hamedawi, 2013) [64]. It is a very effective method to increase the proportion of animals that are bred at the beginning of the breeding season (Larson and Randle, 2008) [44]. One of the advanced managemental processes through which the humane errors and management costs could be minimized is synchronization of estrus (Al-Hamedawi *et al*., 2016, Al-Hamedawi *et al*., 2020) [4, 5]. It is predominantly useful in sheep, where timely heat detection is difficult due to exhibitions of less external heat symptoms and also in large herd of cattle. It helps in fixing the breeding time within a short predefined period and thereby scheduling the parturition time at the most favorable season in which newborns can be reared in suitable environment with ample food for augmenting their survivability. As timely breeding of the animals is possible with this technique, fertility in farm animals may be expected toward the upper side (Al-Zubaidi, 2017) [1]. By improving the production efficiency of animals, estrus synchronization provides more economic returns to the owner (Chaudhari *et al*., 2018) [13]. The primary benefits of utilizing a synchronization protocol containing P4 are its ability to induce estrus out of breeding season, along with its high pregnancy rates and litter size (Yu *et al*., 2022) reported that the conception rate was 74-80% and the twining rate was 55-70% after cervical or intrauterine fixed-time artificial insemination. The P4 regimens mainly composed of P4 treatment for different days with equine Chorionic Gonadotropin (eCG) and/or Prostaglandin F2alpha (PGF2α) (Kadhim and Hussain, 2024) [42]. The PGF2α enables the removal of the luteal effect, leading to a decrease in serum P4 level that exposed the gravian (preovulatory follicle) for a long time (14 days), which promote a healthy oocyte-containing younger follicle to ovulate after the P4 device withdrawal (Kadhim and Hussain, 2014) [41]. Inserting of an intravaginal 0.3 g P4-containing device caused elevation in serum P4, which blocks follicular dominance and stimulates follicular turnover that allows a new follicular wave to emerge within 3 or 4 days (when P4 level fall) in goats and sheep (Vilariño *et al*., 2013) [68]. In doe and ewes the growing stage of every follicular wave generally lasts approximately 5 days. Therefore, when the intravaginal device is taken out, the newest and largest follicle typically undergoes ovulation within 60 hrs later (Menchaca *et al*., 2004) [52]. The ovulatory follicle emerged at day 2.9 days after device insertion and the lifespan of the ovulatory follicle was 5.4 days, while the onset of estrus after 42.0 hrs from device withdrawal and ovulation occurs after 68 hrs (maximum follicle diameter at ovulation 5.6 mm) with pregnancy rate 80% for this program, the insemination conducted 54-56 hours following the removal of P4 device (Vilariño *et al*., 2013) [68]. Ewe synchronisation can be achieved by inserting intravaginal progesterone sponges or the progesterone containing CIDR Ovis intravaginal devices. Once the sponge or CIDR is removed, the ewe's progesterone levels fall rapidly, which leads to oestrus activity within 36-48 hours. Depending on the specific aims of ewe synchronisation, a PMSG injection may also be used (*et al*., 2011) [2]. The most used hormonal treatments in estrous syncronization protocols for sheep are those based on progesterone or its analogues (Gonzalez-Bulnes *et al*., 2020) [24]. Controlled internal drug release (CIDR) is an intravaginal device impregnated with 0.3 g of natural progesterone. (Swelum *et al*., 2015) [60] designed for use between 12 and 14 days in sheep (Boscos et al., 2002) [12]. The device inhibits GnRH secretion and consecuently prevents the release of gonadotropins, especially luteinizing hormone (López-García *et al*., 2021b). Once the device is removed, an injection of equine chorionic gonadotropin (eCG) is applied (Hussain, 2007 and Kadhim and Hussain, 2014) [41]. Which has an effect of follicle-stimulating hormone (FSH) and LH to enhance ovulation (Al-Zubaidi, 2017) [1]. The average level of peripheral P4 increased gradually throughout the gestation in ewes. In gravid ewes, the P4 level started to rise above 1 ng/mL on days 0-6 and reached more than 4 ng/mL on days 14-16. However, the P4 level in ewes that returned to estrus fall to less than 1 ng/mL (Mendoza *et al*., 2009) [53]. The level increased steadily and significantly in days 10, 20,112,119,126 and133, then declined in the last two weeks until lambing. Progesterone levels ranged from 0.01 to 0.16 ng/mL (basal level) along anestrous months of years (Arsoy and Sağmanlıgil, 2018) [10].

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.

Experimental Animals and Management

Experimental design

The animals were subdivided into three groups

Group1 (n= ***6***) ewes treated with CIDR for 12 day, and treated with a single dose of intramuscular injection of PMSG 500 IU at the time of CIDR removal. Group 2 (n=6) non pregnant ewes treated only by CIDR, which applied by intra-vaginal route for (12 days), then withdrawal. Group3 (n= ***6***) ewes control without treatment throughout the study; Animals were monitored on a daily basis, and the time of estrus and mating was recorded. Pregnancy was monitored, as well as the time of delivery. The gender of the offspring, the number of embryos and the status of the embryos were also recorded.

Estrus synchronization protocol, estrus detection, and breeding

The total number of animals used in the experiment was Eighteen ewes, out of which twelve were taken non-

pregnant ewes were synchronized by CIDR, they were subdivided equally into two categories were inserted through the intravaginal root by a special applicator. It persists for 12 days, then withdrawal. At the time of CIDR removal, the eCG (500 IU) one groups.

Estrus detection and mating after synchronization

The heat was detected by observing the mating of the breeding ram, and also by examining the external and internal genital organ's softness and the presence of mucus. During the estrus period, sexual activity was checked every half hr, from 08:00 am to 01:00 pm, and day 0 was considered a day of breeding. Each ewe was considered in estrus when observed to accept a service from a ram, the day of breeding was considered day 0.

Blood collection for hormonal assay

The blood were collected 10ml from the jugular vein by vacuum gel tubes on days (0) before treatment and (12 days and during estrus) to determine hormonal concentration (Progesterone). Serum was collected after centrifugation with 3000 RPM for 10 minutes. Serum kept in epindroff tube at -18 ºC until analysis of hormonal concentrations by Abbott TECTplus immunoassay analyzer.

Results and Discussion

The result of Reproductive Performance of local Ewes of Estrous Synchronization

The results showed a clear significant superiority P≤0.05 (Table 1). for the treatment groups compared to the control group, with a significant superiority P≤0.05 for the first treatment group, G1, compared to the second treatment group, G2, in both the response rate to the estrus synchronization programs, the rate of onset of estrus, and the duration of estrus, at 100%, 66%, 0%, and 35.50, 51, and 0 hours, and 31.67, 29.50, and 0 hours for the three study groups G1, G2, and the control group for the three fertility parameters above, respectively.

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

Groups	No.	Estrus response %	Duration of response (estrus onset) (hrs)	Length of estrus phase (hrs)
G1	6	100% a	35.50±2.06 b	31.67±1.05 a
G2	6	66% b	51.00±1.29 a	29.50±1.04 a
Control	6	0% c	0.00±0.00 c	0.00±0.00 c
LSD value	---	11.074 *	6.175 *	5.447 *
Means with different small letters in the same column are significantly different.* (P≤0.05).				

The findings of this study suggest that using CIDR-based protocols with or without the addition of eCG can induce estrous behavior in most treated sheep after device removal. Additionally, administering eCG upon device withdrawal can enhance estrus induction and promote estrous behavior and subsequent ovulation. These results are consistent with those of (Martinez-Ros and Gonzalez-Bulnes, 2019) [66]. Who observed a higher percentage of females displaying estrous behavior in the CIDR+ eCG group compared to the CIDR-only group (83% vs. 100%) after 6 and 7 days of CIDR insertion, and also nearly similar to results of (Ungerfeld and Rubianes, 2002) [64]. who recorded a response to estrus by using CIDR+eCG was 96% and results of (Gungor *et al*., 2009). Who recorded an estrus rate was 86% by using CIDR only for 12 days. Additionally (López-García *et al*., 2021a). However, these findings disagreed with (Uriol *et al*., 2019) [66]. Who found that both the CIDR+eCG and CIDR-only groups exhibited a 100% occurrence of estrus, indicating estrous behavior and subsequent preovulatory LH surges and ovulation in response to treatment. The results of the present study support previous knowledge on the beneficial role of eCG treatment on ovulatory success and subsequent fertility, and emphasize the negative consequences of eCG unavailability for sheep productivity (Martinez-Ros *et al*., 2018). The interval between CIDR removal and estrus onset in the present study were 35.50±2.06 and 51.00±1.29 hrs in G1 and G2 groups with a significant differences (P≤0.05) between the two groups. These results were agreed with (Gardón *et al*., 2015) [22] who recorded a significantly low average time to estrus onset with the addition of eCG in comparison with ewes treated with progesterone only (46.93±12.44 h vs 60.60±20.46 h). In our study, When eCG was not administered after a prolonged duration of CIDR treatment (12 days), the onset times for estrus delayed, whereas the timing of estrus was influenced by eCG injection, resulting in a prolonged onset of estrous behavior. These observations are consistent with the study by (Uriol *et al*., 2019) [66], which found that the onset of estrus occurred earlier in the CIDR+eCG group compared to the CIDR-only group. Additionally, the study agrees with the findings of (Martinez-Ros and Gonzalez-Bulnes, 2019) [66] who reported that the time of estrus onset after CIDR removal was longer in the CIDR+normal saline group than in the CIDR+eCG group throughout all experimental periods (5, 6, 7, and 14 days post CIDR removal). Furthermore, (Ungerfeld and Rubianes, 2002) [64] reported that the estrus interval, from eCG injection to estrus appearance, was 39 hours, with a response rate of 96% when using CIDR+eCG. The same results also reported by (Gungor *et al*., 2009) in that the interval from CIDR removal to estrus (h) was longer in ewes that treated with CIDR only compared with ewes that treated with a combination of CIDR+ eCG. (Van Cleeff *et al*., 1998) [67] determined that estrus occurred about 36 h after the end of progesterone treatment, and (Godfrey *et al*., 1999) [23] reported estrus 34-40 h following progesterone treatment. In brief, eCG administration advanced and grouped the appearance of estrous behavior and induced ovulation in all the ewes that exhibited estrus (Martinez-Ros and Gonzalez-Bulnes, 2019) [66]. The length of estrus phase in our study was 31.67±1.05 and 29.50±1.04 in G1 and G2, respectively with non-significant differences between length of estrus phase at P≤0.05. The estrus duration is important parameter to determine the time of ovulation. Same finding was reported by (Hashemi *et al*., 2006) [33] who recorded an estrus duration 31.87 hrs when using the synchronization protocol CIDR+eCG in Karakul ewes. And also agreed with (Hameed *et al*., 2020) [30] results, which showed that the duration of estrus was longer in eCG vs. non-eCG groups (32 vs 22.9 hrs). According to, The oestrus in progesterone+eCG ranged between 32-39 hrs depending on time of progesterone treatment. These results were in

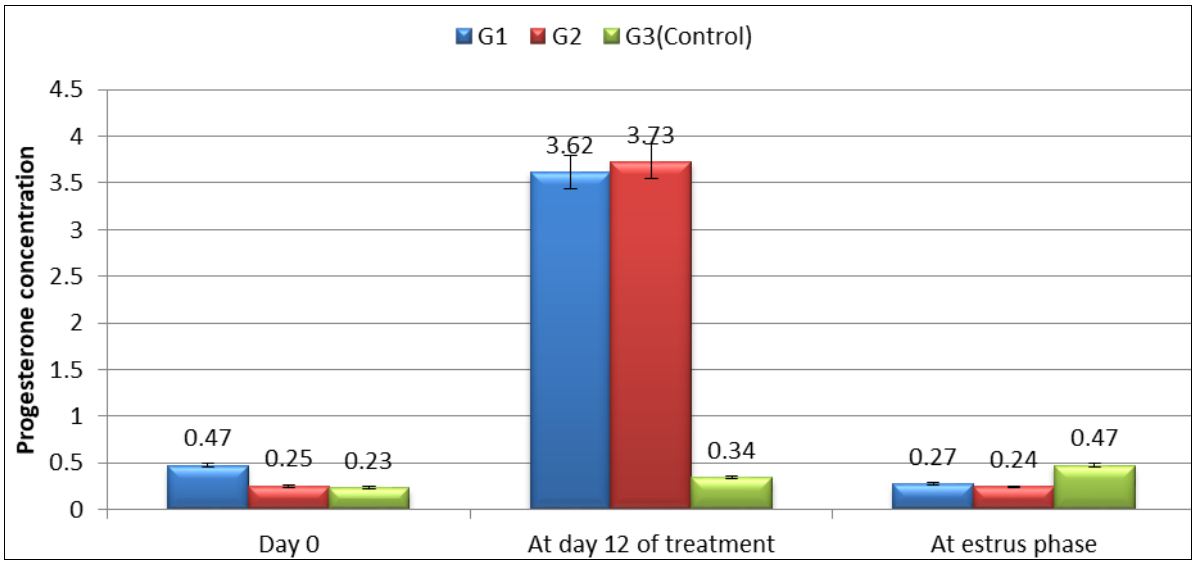
agreement with our findings. On the other hand, (Eldomany *et al*., 2023) [20] showed that estrus duration not different significantly between the groups that treated with progesterone only and progesterone with eCG. The observed longer duration of estrus in eCG vs non-eCG treated ewes may be due to an increased estradiol production by rapid follicular growth following eCG administration (BF *et al*., 2016). Similar longer duration of estrus following eCG administration has been documented in previous study (Greyling and Van der Nest, 2000). In contrast, none of the control group ewes showed estrus.

Progesterone levels

The results showed a clear significant superiority (p<0.05) in progesterone levels in the 12-day period after treatment with progesterone in the G1 and G2 treatment groups compared to the control group, which did not show any change in progesterone levels in all three study periods. In addition to the convergence of progesterone levels in the first and last periods of the study (before treatment and at day of estrus) (Table 2).

Table 2: Levels of progesterone throughout different periods of study in different groups (ng/ ml).

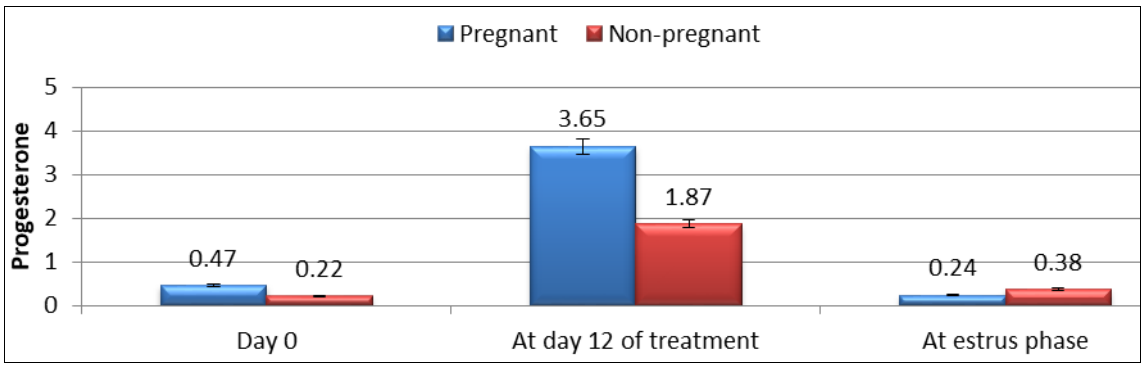
Groups	Mean± SE		
	Day 0	At day 12 of treatment	At estrus phase
G1	0.477±0.06 ^{Ab}	3.62±0.15 ^{Aa}	0.270±0.03 ^{Ab}
G2	0.257±0.06 ^{Ab}	3.74±0.19 ^{Aa}	0.244±0.04 ^{Ab}
Control	0.224±0.03 ^{Aa}	0.341±0.03 ^{Ba}	0.468±0.06 ^{Aa}
LSD value = 0.294 *. Means with different capital letters in the same column and small letters in the same row are significantly different. *(P≤0.05)			



Our results close to results of (Dias *et al*., 2015) [18] who demonstrated that mean serum progesterone values rise after device insertion. In support of our results (López-García *et al*., 2021a) also recorded that serum progesterone value was higher in sheep from the 10 day of CIDR treatment, which also agree with. (Harl, 2014) [32] who found that progesterone level increased rapidly in maternal blood follow CIDR insertion and decreased rapidly after CIDR withdrawal. The CIDR provides high blood progesterone concentrations immediately after its insertion, due to its efficient release kinetics. (De Graaff and Grimard, 2018) [15]. High plasma progesterone concentrations reduce the secretion of LH at the pituitary. (Adams, 1999) [3] and the maintenance of large follicles at the ovary is highly dependent on LH support. (Hsueh *et al*., 1994) [37] hence, such large follicles become atretic in case of shortage of the hormone, giving way to the recruitment of a new follicular wave. (Leyva *et al*., 1998). Mention the high levels of progestagens affect viability of large follicles and increase follicular turnover. (Gonzalez-Bulnes *et al*., 2008) [25] recorded after maintained exposure to progesterone/progestagens for several days, but also after the administration of a single progesterone injection. The induces high plasma progesterone concentrations for only 20–24 h (Martinez-Ros *et al*., 2018). In contrary (Swelum *et al*., 2018) [61] found that progesterone values did not differed before and after CIDR or sponge insertion. This result is not scientifically logical because CIDR is highly progesterone content. So its normal to increase serum level of progesterone. (Al-Rawi and Hussain, 2024) [42]. 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. (Dias *et al*., 2015) [18] showed that maintain blood progesterone levels which released from new CIDR device more than 2 ng /ml after 7 days of treatment. The results also showed a clear significant superiority (p<0.05) in the level of progesterone in the 12-day period after treatment with progesterone in pregnant and non-pregnant ewes compared to the periods before treatment and after the end of the treatment period, which showed convergence in progesterone levels, andalthough the 12-day period showed an increase in the level of progesterone in non-pregnant ewes, but it was lower significantly (p<0.05) than in pregnant ewes during the same aforementioned period (Table 3)

Table 3: Progesterone levels (ng/ml) throughout different periods of study in pregnant compared with non-pregnant tested ewes.

Groups	Day 0	Day 12 of treatment	At estrus phase	Day 0
Pregnant	0.47±0.05 ^{Ba}	3.65±0.13 ^{Aa}	0.24±0.03 ^{Ba}	0.47±0.05 ^{Ba}
Non-pregnant	0.22±0.02 ^{Ba}	1.87±0.54 ^{Ab}	0.38±0.04 ^{Ba}	0.22±0.02 ^{Ba}
LSD	0.81			
Means with different big letters in the same row and small letters in the same column are significantly different.				



The increased level of progesterone in pregnant ewes in compare to the non-pregnant once may refer to delay of CL regression in non-pregnant as compared to pregnant ewes (Wiltbank *et al*., 2018) [69]. These results may suggest that P4 and PMSG administration had negative effect on ovulation of ewes (Quintero-Elisea *et al*., 2011) [55]. The blood P4 concentration values in ewes carrying single or twin fetuses are comparable to those reported by researchers (Deligiannis *et al*., 2005) [17]. According to these results measurement of P4 concentration during pregnancy can be used to diagnose pregnancy with single or multiple fetuses (Al-Sobaiyl, 2010) [8] demonstrated that P4 level is higher significantly in pregnant ewes than those non pregnant. As mentioned by (Singh *et al*., 2018) [57] the mean P4 concentration of ewes proven pregnant was significantly higher than that of non-pregnant ewes in days 19 after sponge removal (day 17 post insertion). There is satisfactory agreement between the present study and (Younis and Akram, 2023) [74]. which showed that P4 concentrations in pregnant ewes were found significantly higher ($p< 0.001$) than in non-pregnant ewes (2.8 Vs 5-9 ng/ mL). the increased level of P4 in pregnant ewes comparing to non-pregnant, may be attributed also to multiple pregnancies within the treated groups of study which may increase in order to meet increasing need to maintain pregnancy. Regarding for the length of pregnancy and the length of labour, no significant differences ($p<0.05$) were observed between the two treatment groups, which recorded very close values that did not exceed 149 days and 41.8 minutes for the periods of pregnancy and labour, respectively.

Table 4: Gestation length and duration of labour in different groups of study.

Groups	Gestation length	Duration of labour
G1	148.80±0.86	41.80±2.15
G2	149.00±1.00	41.50±2.50
P-value	0.89 NS	0.93 NS

The result of current study demonstrated that used of vaginal sponge for ovarian control with similar effectiveness to the CIDR alone and in combination with single dose of PMSG at the time of device removal 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. The length of gestation in sheep ranges from 140 to 159 days (mean 149 days) (Jainudeen and Hafez, 2000); 148.00±3.22 days (Press, 2022); and 147±4.45 days (Mathius *et al*., 2002) [51]. The length of gestation in Palu fat-tailed sheep varied up to 5 days, with a range of between 145-150 days (mean 147.76±1.54 days). The length of gestation differs between breeds and individuals within breeds can also vary up to 13 days (Press, 2022). The shorter length of gestation in this study was thought to be due to differences in breeds, which affect both body weight and birth weight. Ewes having higher body weight have a longer gestation period (Handarini *et al*., 2016) [31] while younger animals have a shorter gestation period (Jainudeen and Hafez, 2000). The length of gestation is also influenced by the age of the ewes, birth weight, type of pregnancy (single or multiple) and sex of the off-springs. The range of duration of the current study was within the normal range, which give an impression that the synchronization programs used in this study were well designated (Gowane *et al*., 2014) [26]. On the other hand, the short duration of labour in this study ensure that the synchronization programs were well designed. The short duration also may be attributed to the low weights of fetuses that the tested ewes carried single or multiple pregnancies. These results encourages the application of synchronization and superovulation in ewes reproduction because are safe and efficient.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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