The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2023

Volume 11, Pages 30-39

ICVALS 2023: International Conference on Veterinary, Agriculture and Life Sciences

A Comparison of Two Estrus Synchronization Protocols Utilizing Progesterone Supplement 24 Hours before CIDR-G Removal in Sheep

Mustafa Q. Husein
Jordan University of Science and Technology

Hassan A. Ghozlan
Jordan University of Science and Technology

Abstract: Two experiments investigated the effect of progesterone administration 24 h prior to intravaginal CIDR-G withdrawal using two protocols in two geographical locations and different breeds of sheep. Ewes in both experiments were administered with intravaginal CIDR-G either for 8 days (Expt. 1, n=36 thin tail mixed-breed ewes) in early April or for 12 days (Expt. 2, n=28 fat tail Awassi ewes) in late May. Half of the ewes in each experiment were assigned randomly one day before CIDR-G removal and given 25 mg P4 (P4-treated) and the second half were given saline solution and served as controls. Fertile rams were allowed with the ewes of both experiments immediately following CIDR-G removal (day0, 0h) and estrus was monitored at 6-h intervals for 4 days. Blood samples were collected for analysis P4 and LH levels. Estrus responses differed between the two experiments. P4 profiles during the period of CIDR-G insertion behaved the same way among ewes of both experiments, but levels differed ($P < 0.05$) on day 0 in response to P4 supplement. The preovulatory LH surge was greater among groups treated with P4 supplement than those that did not. In both experiments, the percentage of ewes exhibiting estrus was greater ($P < 0.05$) and intervals to estrus were longer ($P < 0.05$) in P4-treated than control ewes. In the 12-day protocol in Expt. 2, ewes treated with P4 supplement produced greater ($P < 0.05$) incidence of estrus, longer ($P < 0.05$) intervals to onset of estrus and LH surge and had higher ($P < 0.01$) magnitude of LH surge and pregnancy rate than controls and those of the 8-day protocol. In conclusion, P4 supplement 24 h prior to CIDR-G removal in a 12-day protocol can be used successfully to improve reproductive performance of ewes.

Keywords: Progesterone supplement, Sheep, LH surge

Introduction

Reproductive performance is one of the major factors having an impact on sheep industry that will ensure future success and long-term sustainability. In sheep, estrus synchronization protocols implemented for 12 to 14 days increase reproductive efficiency through hormone administration (Kuru et al., 2022). Incorporation of additional injection of P4 in an estrus synchronization protocol produced superior reproductive performance in Awassi ewes (Husein and Ababneh, 2008). Such a protocol was based on an injection of 25 mg P4 administered 24 h before intravaginal inserts removal in a 12-day CIDR-G scheme (Husein & Ababneh, 2008). These authors reported 100% pregnancy and lambing rates in non-prolific Awassi ewes treated with P4 supplementation out-of-season.

There have been concerns that results reported by Husein and Ababneh (2008) are repeatable and may produce similar outcomes despite changes in latitude, season, nutrition, breed and duration of the synchronization protocol. The intention, therefore, was to compare the effect additional P4 supplement in two different estrus synchronization protocols using prolific breeds of sheep raised in non-tropical areas and non-prolific sheep raised under subtropical environment. Effectiveness of P4 supplement may be altered by any of these factors.

The aims of this study were to compare the effectiveness of an 8-day CIDR-G-P4 with a 12-day CIDR-G-P4 supplement protocols in two different geographical locations and to investigate whether or not results obtained are repeatable. Other aims were to clarify changes in hormonal profiles of P4 and LH in plasma samples of ewes and to identify related factors that are conducive to pregnancy).

Method

General

Different breeds of multiparous ewes were used in two experiments to investigate the effect of progesterone (P4) administration 24 h prior to intravaginal CIDR-G withdrawal using two protocols conducted in two geographical locations. Ewes were handled humanely in the two experiments and all procedures pertaining to the use of animals complied with the rules approved by the Animal Care committee of each location. Ewes in both experiments had previously lambed during the past lambing season and had their last lambs been weaned about 8-10 weeks before the start of each experiment. During the experimental period, ewes in each experiment were housed together in a single pen and were fed 0.5 kg concentrate mixture (13% CP) per ewe per day, and had *ad libitum* access to alfalfa hay or wheat straw, water, shade and mineral salt blocks.

In experiment 1, 36 thin tail multiparous mixed-breed ewes (Montadale, Southdown, Tunis and Colombia) were assigned randomly to two treatment groups (P4-treated, n= 18 and control n= 18). All ewes were administered with intravaginal CIDR-G Pharmacia and Upjohn Ltd. Co., Mt Wellington, Auckland, New Zealand) devices containing 300 mg P4 for 8 days. Devices were inserted on April 6 (day −8) and were removed 8 days later on April 14 at 08:00 (Day 0 and hour 0). This study was conducted in a farm located in Goodhue County in Minnesota (latitude 44°23'N, longitude 92°26'W) at an altitude of 340 m. Ewes ranged in age from 3 to 6 years and weighed 65.4 ± 1.4 kg (range= 52 to 81) and had a body condition score of 2.5 to 3 (scale= 0 lowest to 5 highest).

In experiment 2, 28 Awassi ewes were assigned at randomly to two treatment groups (P4-treated and control) of 14 ewes each. Ewes were induced to estrus using intravaginal CIDR-G (Pharmacia and Upjohn Ltd. Co., Mt Wellington, Auckland, New Zealand) devices containing 300 mg P4 for 12 days. Devices were inserted on May 27 (day −12) and were removed 12 days later on June 8 at 08:00 (Day 0 and hour 0). This study was conducted in a farm located at the Agricultural Center for Research and Production at Jordan University of Science and Technology (latitude 32º33'N, longitude 35º51'E). Ewes ranged in age from 3 to 7 years old and weighing 51 ± 1.2 kg with a body condition score of 2.5 to 3 (scale= 0 lowest to 5).

One day before CIDR-G removal in both experiments (day −7 in Expt. 1 and day −11 in Expt. 2), half of the ewes (P4-treated) were given an exogenous injection of 25 mg P4 (Intervet UK Ltd., Science Park, Milton Road, Cambridge, UK) supplement each and the second half were given saline solution and served as controls. Fertile rams were allowed with the ewes of both experiments immediately following CIDR-G removal (Day 0 and hour 0) and estrus was monitored at 6-h intervals for 4 days. Blood samples were collected for analysis P4 and LH levels. Jugular venous blood samples were collected every other day starting immediately before CIDR-G insertion until day −2 and once daily from day−1 until day 3 to compare P4 concentrations. Blood samples also were collected at 6-h intervals starting immediately after CIDR-G removal for 72 h for LH analysis and then on alternate days thereafter until day 19 for pregnancy diagnosis.

Plasma P4 concentrations in Expt. 1 were measured using a solid-phase RIA. Sensitivity was 0.1 ng/mL and intraassay coefficient of variation was 2.9%. Plasma P_4 concentrations in Expt. 2 were measured by a solid-phase RIA using a commercial kit [Coat-A-Count procedure, Diagnostic Products Corporation (DPC), Los Angeles, CA]. Sensitivity was 0.1 ng/ml and intraassay coefficient of variation was 5.3%.

Plasma LH concentrations in Expt. 1 were measured in duplicate of 100 µl plasma aliquots using a double-antibody RIA (Husein et al., 1996). Assay components were NIH-LH-S19 for reference, NIADDK-oLH-I-4 (AFP-8614B) for radioiodination, and NIADDK-anti-oLH-1 (AFP-192279) serum. Sensitivity was 0.5 ng/ml and the intraassay CV was 7.1%. Plasma LH levels in Expt. 2 were determined using ovine LH ELISA commercial kit (Endocrine Technologies Inc., Newark, CA). Sensitivity was 0.1 ng/ mL and intraassay coefficient of variation was 7.9%. Pregnancy was diagnosed on day 30 using transrectal ultrasound transducer (Aloka 500V scanner and a 7.5-MHz human prostate transducer; Corometrics Medical Systems, Inc., Wallingford, CT).

Statistical Analysis

Data were analyzed by using SAS/STAT ANOVA procedures (SAS Inst. Inc., Cary, NC., 2006). Data in text, tables and figures are presented as means ± SEM. Onset of estrus was considered to have occurred 6 h before observation of a breeding mark among ewes of both experiments. The effects of P_4 supplementation on incidence of estrus, preovulatory LH surge and pregnancy were analyzed using the *Chi-square* test. The effects of P_4 supplementation on the amplitude of LH surge and various intervals to onset of estrus and LH surge were analyzed using least-square means of the GLM procedures. Plasma P_4 and LH concentrations were analyzed for the effect of treatments and time using the repeated-measures procedure of GLM.

Results and Discussion

Experiment 1

Progesterone Concentration during the 8-Day Period in Which CIDR-G were in Place:

Ewes maintained CIDR-G devices during the period of insertion until they were pulled out. Progesterone concentrations in plasma samples of ewes during the period from day –8 to day 3 are illustrated in Figure 1. Initial P4 concentrations in plasma samples taken prior to CIDR-G insertion were 0.49 ± 0.06 ng/ml among ewes of the two groups, indicating probably seasonal anestrus and/or transitional period from estrus to anestrus in some ewes. Following CIDR-G insertion P_4 concentrations increased sharply and maximum values were reached 2 days post insertion (day –6) and were 5.7 ± 0.8 and 5.9 ± 0.6 ng/ml in the P4-treated and control groups, respectively. Differences in maximum P_4 concentrations on day –6 were not significant ($P > 0.1$). Progesterone gradually decreased by day from day –6 until day –1 and concentrations on day –1 were similar ($P > 0.05$) between P4-treated and control groups 3.1 ± 0.3 and 2.9 ± 0.2 ng/ml, respectively .

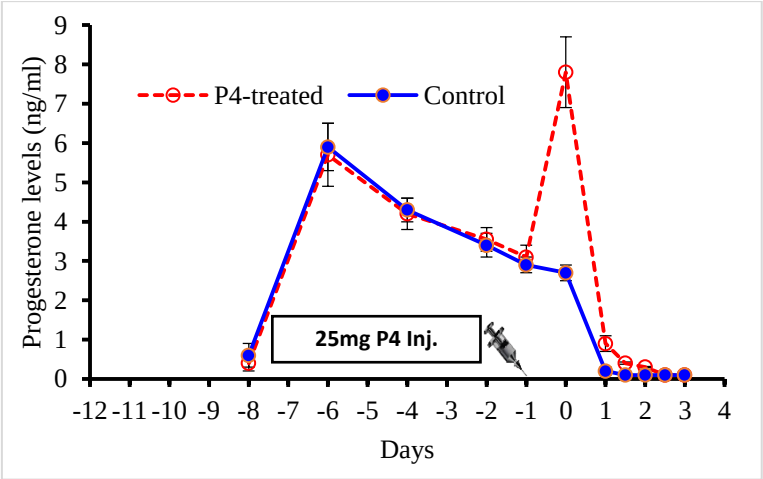

Figure 1. Plasma progesterone concentrations in Expt. 1 during the period in which CIDR-G were in place in P4-treated and Control groups from day –8 to day 3.

In the P_4-treated ewes, P_4 concentrations increased abruptly in response to the P_4 supplementation on day –1 from 3.1 ± 0.3 to 7.8 ± 0.9 ng/ml on day 0, then decreased sharply to 0.9 ± 0.2 ng/ml on day 1. Progesterone concentrations in the control group decreased from day –1 values of 2.9 ± 0.2 to 2.7 ± 0.2 ng/ml on day 0, then gradually declined to a value of 0.2 ± 0.1 ng/ml on day 1. Plasma P_4 concentrations between days –1 and 0 differed ($P < 0.001$) significantly between P_4-treated and control groups (Figure 1).

Estrus Responses and the Preovulatory LH Surge

Reproductive parameters occurring following day 0 among ewes of the two groups are presented in Table 1. The overall estrus expression rate was 72.3% (26/36 ewes). Cyclic activity was induced in 67.7 and 77.8% in P4-treated and control groups, respectively. There was no treatment effect ($P > 0.05$) on the incidence of estrus between P4-treated and control ewes. Intervals from 0 h to onset of estrus differed ($P < 0.05$) significantly between the two groups and were shorter (42.4 ± 2.5 h) in control than P4-treated (51.0 ± 2.6 h) ewes.

Occurrence of the preovulatory LH surge was similar (P > 0.05) between the P4-treated (44.4%) and control (27.8%) ewes.

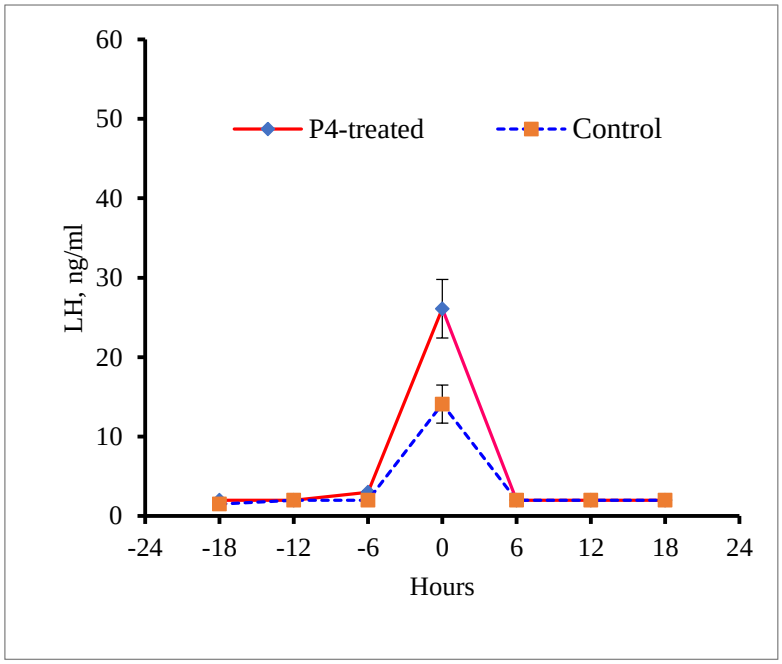

Figure 2. Plasma LH concentrations and magnitude of the preovulatory LH surge in P4-supplement-treated and control ewes in Expt. 1. The time (0 h) represents the aligned peak LH levels in both groups.

Intervals from 0 h to onset and peak LH surge were shorter (P < 0.05) in the control (44.4 ± 4.5 h) group than the P4-treated (51.8 ± 3.4 h) group ewes. Occurrence of estrus and LH surge coincide and intervals from onset of estrus to onset or peak LH surge were similar between the two groups and averaged 1.0 ± 1.1 h in the P4-treated and 2.0 ± 0.9 h in the control. The amplitude of the preovulatory LH surge was greater in the P4-treated (26.1 ± 2.8 ng/ml) than the control (14.1 ± 1.9 ng/ml) group (Figure 2).

Progesterone Profiles after CIDR-G Removal, Pregnancy and Lambing Rates

Progesterone levels in the P4-treated and control groups dropped after day 1 to basal values and remained low until day 5 and then rose gradually until day 15. Differences in P4 rise between days 5 and 15 were not significant (P > 0.1). Progesterone levels remained elevated through day 19 in 8/18 P4-treated and 11/18 control ewes. These ewes were confirmed pregnant by ultrasonography performed on day 30. Differences in pregnancy rates were not significant (P > 0.05) between the two groups. Progesterone levels among ewes that did not become pregnant in groups dropped spontaneously after Day 15 and concentrations were typical of those detected during the process of luteal regression. Of the ewes that became pregnant from mating at induced estrus 7/18 (P4-treated) and 11/18 (control) lambed on day 148.6 ± 0.3. The number of lambs born and the multiple birth rates were similar (P > 0.1) between the P4-treated and control ewes (Table 1).

Experiment 2

Progesterone Concentration during the 12-Day Period in Which CIDR-G were in Place:

Ewes maintained CIDR-G devices during the period of insertion until they were pulled out. Progesterone concentrations in plasma samples of ewes during the period from day –12 through day 3 are illustrated in Figure 3. Initial P4 concentrations in plasma samples taken prior to CIDR-G insertion were <0.2 ng/ml among ewes of the two groups, indicating seasonal anestrus. Differences between the two groups in initial P4 values on day –12 were not significant (P > 0.5). Following CIDR-G insertion P_4 concentrations increased sharply and maximum values were reached 2 days post insertion (day –10) and were 5.7 ± 0.8 and 5.9 ± 0.6 ng/ml in the P4-treated and control groups, respectively. Differences in maximum P_4 concentrations on day –10 were not significant (P > 0.1). Progesterone gradually decreased by day from day –10 until day –1 and concentrations on day –1 were similar (P > 0.05) between P4-treated and control groups 1.9 ± 0.1 and 1.8 ± 0.1 ng/ml, respectively.

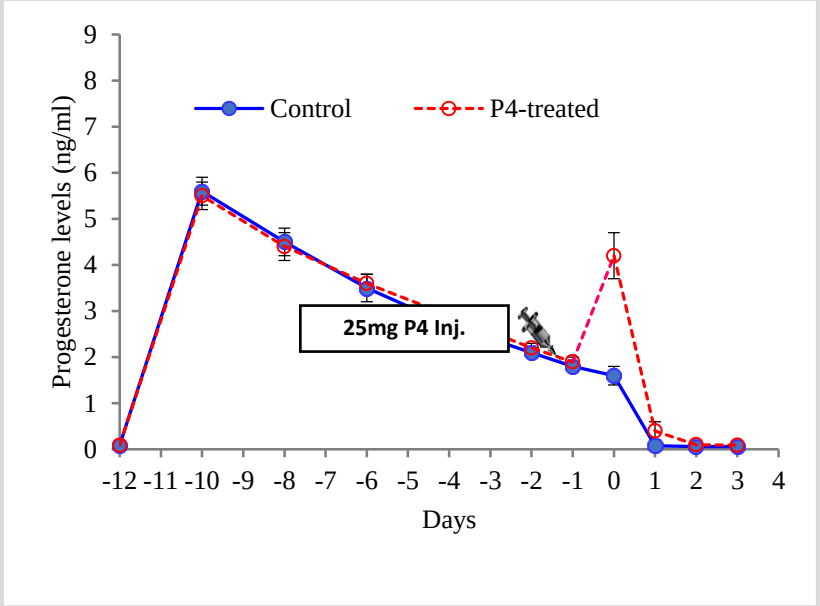

Figure 3. Plasma progesterone concentrations in Expt. 2 during the period in which CIDR-G were in place in P4-treated an d Control groups from day –12 to day 3.

In P4-treated group, plasma P4 concentrations increased sharply from 1.8 ± 0.1 on day –1 to 4.2 ± 0.3 ng/mL on day 0 in response to the 25 mg P4 injection given on day –1. Progesterone concentrations in the control group declined from 1.9 ± 0.1 ng/mL on day –1 to 1.7 ± 0.1 ng/mL on day 0. Plasma P4 concentrations between days –1 and 0 differed ($P < 0.001$) significantly among ewes of the two groups.

Estrus Responses and the Preovulatory LH Surge

Reproductive parameters occurring following day 0 among ewes of the two groups are presented in Table 1. Cyclic activity differed ($P < 0.05$) significantly and was induced in 100 and 64.3% in P4-treated and control groups, respectively. Intervals from 0 h to onset of estrus differed ($P < 0.05$) significantly between the two groups and were shorter (35.3 ± 1.9 h) in the control than in P4-treated (45.4 ± 2.4 h) groups. All ewes in the P4-supplement-treated group and 6/14 control ewes had identifiable surge release of LH. Intervals from 0 h to onset of the preovulatory LH surge were longer in P4-treated (46.6 ± 2.6 h) than control (37.0 ± 2.9 h) ewes, respectively. Differences in occurrence and onset of the preovulatory LH surges were significant ($P < 0.05$) between the P4-treated and control ewes (Table 1). Of the ewes exhibiting a surge release of LH, amplitudes of the preovulatory LH surges were greater ($P < 0.01$) in P4-treated (53.5 ± 4.3 ng/mL; range 33–72) than control (23.7 ± 2.4 ng/mL; range 16–33) ewes (Figure 4).

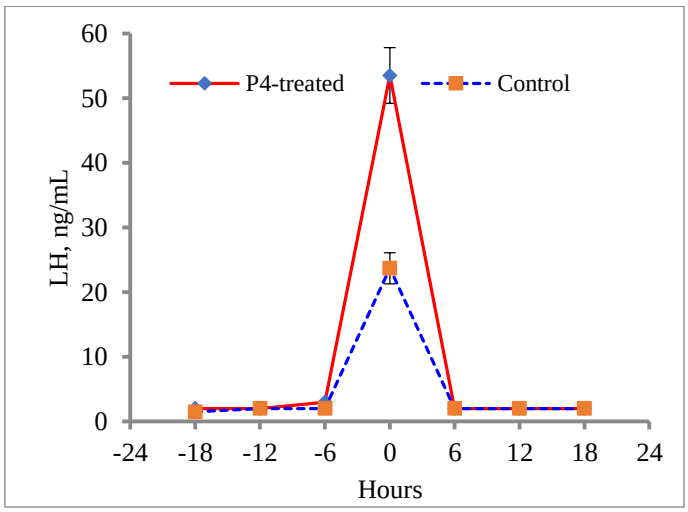

Figure 4. Plasma LH concentrations and magnitude of the preovulatory LH surge in P4-supplement-treated and control ewes in Expt. 2. The time (0 h) represents the aligned peak LH levels in both groups.

Progesterone Profiles after CIDR-G Removal, Pregnancy and Lambing Rates

Progesterone levels in the P4-treated and control groups dropped after day 1 to basal values and remained low until day 5 and then rose gradually until day 15. Differences in P4 rise between days 5 and 15 were not significant ($P > 0.1$). Progesterone levels remained elevated through day 19 in 14/14 P4-treated and 7/14 control ewes. These ewes were confirmed pregnant by ultrasonography performed on day 30. Differences in pregnancy rates were significant ($P < 0.05$) between the two groups. Progesterone levels among ewes that did not become pregnant in groups dropped spontaneously after day 15 and concentrations were typical of those detected during the process of luteal regression. Of the ewes that became pregnant from mating at induced estrus 14/14 P4-treated and 7/17 control lambed on Day 149.3 ± 0.3. Lambing and the multiple birth rates differed significantly ($P < 0.05$) between the P4-treated and control ewes (Table 1).

Table 1. Reproductive parameters following CIDR-G removal in P4-treated and control ewes

Parameter	Treatments[1]			
	Experiment 1 (n= 36 (mixed breed ewes)		Experiment 2 (n=28 Awassi ewes)	
	P4-treated (n = 18)	Control (n = 18)	P4-treated (n = 14)	Control (n = 14)
Body weight (Kg)	64.3 ± 2.5	66.1 ± 2.9	50.8 ± 2.5	49.7 ± 2.7
Ewes displayed estrus*	12 (66.7%)	14 (77.8%)	14 (100%)^{a}	9 (64.3%)^{b}
Intervals to estrus onset (h)*	51.0 ± 2.6^{a}	42.4 ± 2.5^{b}	45.4 ± 2.4^{a}	35.3 ± 1.9^{b}
Ewes displayed LH surge	8/18	5/18	14/14^{a}	6/14^{b}
Intervals to onset of LH surge (h)	51.8 ± 3.4^{a}	44.4 ± 4.5^{b}	46.7 ± 2.6^{a}	37.0 ± 2.9^{b}
Amplitude of LH surge (ng/ml)	26.1 ± 2.8^{a}	51.0 ± 2.6^{a}	53.5 ± 3.7^{a}	23.7 ± 2.4^{b}
Pregnancy rate	8 (44.4%)	11 (61.1%)	14 (100%)^{a}	7 (50%)^{b}
Lambing rate	7 (38.9%)	9 (50%)	14 (100%)^{a}	7 (50%)^{b}
Fecundity	1.4 (144%)	1.35 (135%)	1.29 (129%)^{a}	0.5 (50%)^{b}
Multiple birth (from lambed ewes)	5/7	6/9	4/14	0/7

[ab] means within row with different superscripts for each experiment differed significantly ($P<0.05$).

Discussion

The present study investigated the effectiveness of additional P4 supplement given 24 h before device removal in two estrus synchronization (8-day CIDR-G versus 12-day CIDR-G) protocols in two different geographical locations and to investigate whether or not results obtained are comparable. Reproductive responses resulted in Expt. 2 of the present study using a 12-day CIDR-G-P4 supplement were superior compared to those obtained in Expt. 1 using an 8-day CIDR-G-P4 supplement and were similar to the results reported previously by Husein and Ababneh (2008).

Results obtained from Expt. 2 demonstrate that P4 supplement given one day before device removal was successful in inducing fertile cycles in 100% of the ewes and capable of producing superior reproductive performance compared to those obtained from Expt. 1. Protocols implemented in the present study differed procedurally in the period of CIDR-G insertion (8 vs 12 days). The amounts of circulating P_4 concentrations in Expt. 1 during the 8-day period of pessary insertion protocol are relatively greater than those found Expt. 2 during the 12-day protocol. Progesterone profiles during the 12-day period of pessary insertion determined in Expt. 2 of the present study were typical of those previously reported in the literature (Husein and Kridli, 2002, Husein et al., 1998, Husein et al., 1996). In both experiments, P4 supplement was administered on day –1 at which time concentrations of P_4 increased sharply from about 3 to day 0 values of 7.8 ± 0.9 ng/ml among ewes of Expt. 1. In contrast, P_4 concentrations on day –1 of the 12-day (Expt. 2) protocol were about 1.8 ng/ml to day 0 values to 4.2 ± 0.3 ng/mL (Figure 5).

Therefore, higher and/or sustained P_4 concentrations during the 8-day period are important and maybe associated with down-regulation of LH receptors in a manner affecting dominance of follicles. Thus, follicular dominance is negatively influenced by sustained P_4 levels and therefore, follicular growth is disrupted. In contrast, sensitizing pituitary-ovarian axis with P_4 supplementation at the end of the 12-day protocol would, perhaps, reset recruitment of healthy follicles at the time when gonadotropins and estradiol concentrations are low. However, higher circulating P_4 levels powerfully inhibits LH release, thereby, preventing premature LH surges from occurring and are considered necessary for recruiting a greater number of follicles (Sharma et al., 1993; Caraty &Skinner, 1999).

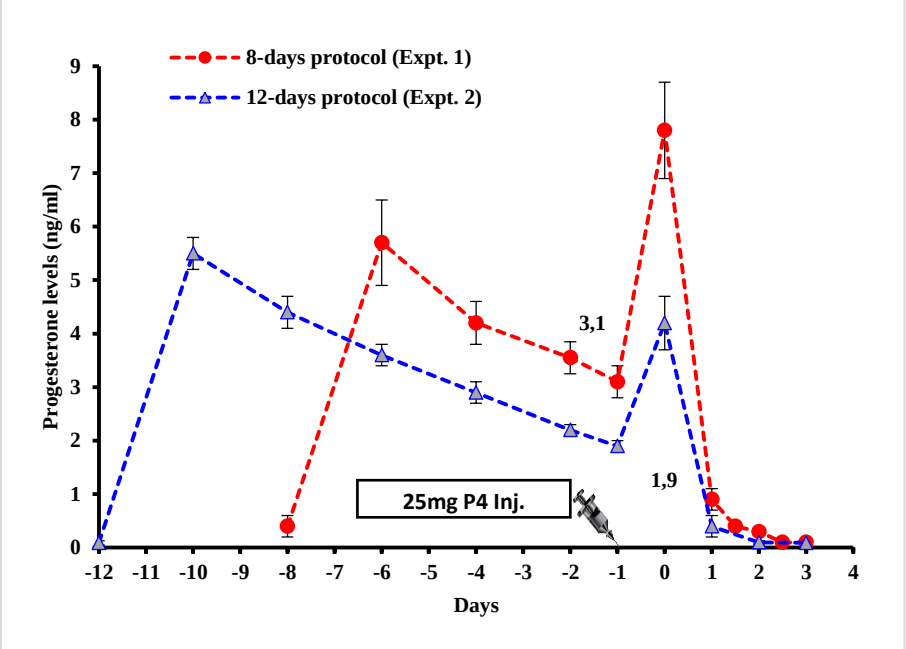

Figure 5. Plasma progesterone concentrations during the period in which CIDR-G were in place in P4-treated ewes in both experiments from day –12 or -8 to day 3.

Similarly, responses of estrus and the preovulatory LH surges were significantly different between the 8-day and the 12-day CIDR-G protocols. More ewes expressed estrus and displayed a surge release of LH in the 12-day CIDR-G than the 8-day CIDR-G protocol. Moreover, the magnitude of the LH surges in the 12-day CIDR-G (53.5 ± 4.3 ng/mL) protocol was greater than those occurring in the 8-day CIDR-G (26.1 ± 2.8 ng/ml) protocol. This evidence may particularly be one of the major contributing factors strengthening follicular dominance and oocyte quality. Likewise, the higher magnitude of LH surges occurring in the 12-day CIDR-G protocol in Expt. 2 may be a reflection of high pro-estrus estradiol levels caused by a sharp decrease in P4 concentrations following CIDR-G removal. Higher amplitudes of LH surges have been reported with high estradiol levels than those with persistently in ewes with persistent follicles (Joseph et al., 1994). In fact, ovulation of mature follicles on the ovary is induced by a large burst of the preovulatory LH surge. Residual cells within ovulated follicles proliferate to form corpora lutea, which secrete progesterone, necessary for the maintenance of pregnancy. In most mammals, LH is required for continued development and functionality of corpora lutea and subsequent embryonic development. However, low LH magnitude occurring in the 8-day CIDR-G protocol may be suboptimal for oocyte quality and subsequent embryonic development competence. It seems that lower magnitude of LH surges was slightly effective because the pituitary gland insufficiently reacted to the positive feedback of rising pro-estrus estradiol levels at the end of the follicular phase. This evidence may inactivate some follicular dominance factors, resulting in premature oocytes at the time of ovulation and may cause supraphysiological thecal androgen production associated with atresia. In addition, the 25 mg P4 injection given one day before device removal caused a significant delay in onset of estrus and the preovulatory LH surge by about 10 h in Expt.2 and 7 h in Expt. 1. The reason for the delay to onset of estrus and LH surge can possibly be attributed to the additional time required for the recruitment and maturation of the ovulatory follicles (Webb et al., 1989) and a slight delay in P4 clearance. These authors indicated that such a delay period is necessary for a large estrogenic follicle (>5 mm in diameter) to develop from a pool of small follicles (<2 mm in diameter) around the time of luteolysis.

The superior pregnancy and lambing rates produced in the 12-day CIDR-G protocol in Expt. 2 of the current study compared to those obtained from the 8-day CIDR-G protocol are believed, perhaps, to be mainly due to eliminating the aged persistent follicles. Thus, our results may provide an indirect evidence for the deleterious effect of aged follicles on fertility in the 8-day CIDR-G protocol due to higher or sustained P4 levels during the 8-day period. In beef cattle, an injection of P4 given 2 days before the end of progestogen treatment induces atresia of persistent follicles and significantly improved pregnancy rates (Anderson a& Day, 1994; McDowell et al., 1998). As a consequence of P4 decline following pessary removal, healthy follicles destined to ovulate, grow and secrete estradiol (Wehrman et al., 1993), which, along with the male effect, induces the preovulatory LH surge leading to ovulation of potential ova. These factors may be considered the key feature for initiating the expression of superior pregnancy and lambing rates in Expt. 2.

In addition, factors including, the sharp P4 decline following pessary removal and the delay in LH surge occurrence and higher amplitude may have participated in resetting the hypothalamic–pituitary–ovarian axis, establishing ovarian-uterine synchrony and thus, superior reproductive performance. It has been indicated that the use of sponges impregnated with high P4 (750 mg) was associated with higher amplitudes of LH surges and higher pregnancy rates in anestrous Finncross ewes (Husein et al., 1996; Husein et al., 1998). In contrast, other studies in sheep associated low P4 concentrations with abnormal follicular development, persistent follicle and reduced fertility (Johnson, 1996; Leyva et al., 1998; Vinoles et al., 1999; Flynn et al., 2000).

Another difference between the two experiments was that ewes were either prolific mixed-breed (Expt. 1) or non-prolific Awassi breed (Expt. 2). Plasma concentrations of P4 play an important role in the control of follicular turnover. This effect is most probably mediated by LH pulsatility, as has been postulated for cattle (Savio et al., 1993). High P4 concentrations lead to lower levels of stimulation of the developing follicles by reducing pulsatile LH secretion (Mann et al., 1992; De Castro et al., 1999). In prolific ewes with multiple ovulations, the ovarian recruitment of follicles is enhanced by the relatively low P4 concentrations. This may limit the subsequent ovulatory outcome and therefore, caliber of oocytes in turn may be influenced by the ewes' exposure to adequate P4 in the few days period prior to ovulation. With higher P4 concentrations being administered to prolific ewes with higher ovulation rate, it is expected that LH receptors will be down-regulated. Thus, the dominance factors responsible for follicles selection are inhibited. Such observation may be beneficial and work better in non-prolific breeds such as Awassi. In this regard the dominance factors are enhanced in selected follicles over the subordinate ones. Thus, a P4 injection given 24-h prior to pessary removal will down-regulate growth of follicles destined to ovulate in prolific ewes, and would rather enhance the dominance of the ovulatory follicles over the subordinate ones in non-prolific ewes (Gonzalez-Bulnes et al., 2004).

The current study also was conducted in two geographical locations. Latitude and day length and breed are not the only factors involved in controlling reproduction in sheep. Some sheep breeds exhibit short seasonal anestrus, particularly when social (ram effect) or nutritional factors are handled appropriately (Quirke & Hanrahan, 1985). Some breeds of sheep in temperate latitude exhibit a seasonal variation in reproductive activity during the year. Despite the fact that P4 profiles in Expt. 1 and Expt. 2 behaved the same, results therefore were not comparable and did not confirm repeatable reproductive performance outcomes. This evidence may eliminate the breed and location effects and establish season of breeding (early April in Expt.1 versus late May in Expt. 2). From this perspective, ewes in Expt. 1 may have been in seasonal transitional period, a crucial factor that may alter reproductive responses compared to ewes in Expt. 2 which were in deep anestrus period.

From another prospective, the amount of P4 rather than the change in magnitude differed between the 8-day and the 12-day protocols and may have played a vital role in producing better reproductive performance. Plasma concentrations of P4 play an important role in controlling follicular turnover. The overall amount of P4 in the 8-day protocol on days –1 and 0 (3.1 ± 0.3 and 7.8 ± 0.9 ng/ml) were greater ($P < 0.05$) than the corresponding values in the 12-day protocol (1.9 ± 0.1 and 5.7 ± 0.8 ng/ml). The higher P4 levels by more than 150% on day –1 in the 8-day protocol in Expt. 1 in comparison to the 12-day protocol in Expt. 2 might have played an important down-regulatory effect on the P4 injection, preventing better suppression of premature LH secretion. Premature LH surge is linked to lower oocyte quality and subsequent lower pregnancy rates. In fact, it is noteworthy that at the time of P4 injection, ewes of Expt. 1 may have had a totally different follicular wave stage than those in Expt. 2. In conjunction with the higher P4 levels arising from CIDR-G in the 8-day protocol, this might have counteracted the desired suppression of endogenous P4 levels to prevent premature LH surge through the expected negative feedback on the hypothalamic-pituitary-ovarian axis. Therefore, the 12-day but not the 8-day protocol with lower P4 levels at day 0 may have permitted better synchrony of estrus occurrence and subsequent LH surge release and, consequently, better overall reproductive performance. To insure effectiveness of the P4 supplement in CIDR-G protocols to produce better LH magnitude and subsequent reproductive responses, mean circulating P4-levels should be < 2 ng/ml, corresponding to normal 12-day protocol P4 profiles. Assuming this is not the case, then failure of the 8-day to produce higher reproductive performance may be attributed to unknown reasons.

Conclusion

In conclusion, there was variability in effectiveness of P4-suppliment to ewes synchronized to estrus with CIDR-G pessaries. Progesterone injection one day before CIDR-G removal is effective in improving LH surge magnitude and superior reproductive responses including pregnancy and lambing rates in 12-day estrus synchronization protocols. However, such improvement is not seen among ewes exposed to the 8-day

progesterone pessary protocol. Such variability in responses between the two protocols might be due to follicular dynamics variations, P4 level at the time of P4 supplement, geographical location and seasonal status.

Recommendations

We recommend that 25 mg progesterone injection can be given to ewes synchronized to estrus for 12 days using CIDR-G protocols 1 day before CIDR-G removal, and would produce superior pregnancy and lambing rates. Nonetheless, the same is not true for ewes synchronized using the same protocols but for only 8 days. Further research is encouraged to incorporate ultrasonographic study of ovarian follicular dynamics concurrent with hormonal profiles in both protocols to ascertain possible follicular dynamic variations between the two protocols.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

* This article was presented as a poster presentation at International Conference on Veterinary, Agriculture and Life Sciences (www.icvals.net) held in Antalya/Turkey on November 16-19, 2023.

References

Anderson, L.H., & Day, M.L. (1994). Acute progesterone administration regresses persistent dominant follicles and improves fertility of cattle in which estrus was synchronized with melenogestrol acetate. *Journal of Animal Sciences, 72,* 2955–2961.

Caraty, A., & Skinner, D.C. (1999). Progesterone priming is essential for the full expression of the positive feedback effect of estradiol in inducing the preovulatory gonadotropin-releasing hormone surge in the ewe. *Endocrinology, 140,* 165–170.

De Castro, T., Rubianes, E., Menchaca, A., & Rivero, A. (1999). Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. *Theriogenology, 52,* 399–411.

Flynn, J.D., Duffy, P., Boland, M.P., & Evans, A.C.O. (2000). Progestagen synchronisation in the absence of a corpus luteum results in the ovulation of a persistent follicle in cyclic ewe lambs. *Animal Reproduction. Sciences ,62,* 285-296.

Gonzalez-Bulnes, A., Souza, C.J.H., Campbell, B.K., & Baird, D.T. (2004). Systemic and intraovarian effects of dominant follicles on ovine follicular growth. *Anim. Rep. Science, 84,*117-119.

Husein, M.Q., & Ababneh, M.M. (2008). A new strategy for superior reproductive performance of ewes bred out-of-season utilizing progestagen supplement prior to withdrawal of intravaginal pessaries. *Theriogenology, 69,* 376-383.

Husein, M.Q., Ababneh, M.M., Crabo, B.G., & Wheaton, J.E. (1996). Out-of-season breeding of ewes using transcervical artificial insemination. *Sheep and Goat Research Journal, 12*(1), 39–45.

Husein, M.Q., Bailey, M.T., Ababneh, M.M., Romano, J.E., Crabo, B.G., &Wheaton, J.E. (1998). Transcervical artificial insemination of ewes out-ofseason using frozen-thawed semen: effect of equine chorionic gonadotropin on pregnancy rate. *Theriogenology, 49,* 997–1005.

Husein, M.Q., & Kridli, RT. (2002). Reproductive responses of Awassi ewestreated with either naturally occurring progesterone or synthetic progestagen. *Asian-Aust. Journal of Animal Science, 5*(9),1257–162.

Johnson, S.K., Dailey, R.A., Inskeep, E.K., & Lewis, P.E. (1996). Effect of peripheral concentrations of progesterone on follicular growth and fertility in ewes. *Dom. Anim. Endocrinol*ogy, *13,* 69–79.

Joseph, B.J.K., Currie, W.D., Ravindra, JP, Cook SJ, Rawlings NC. (1994). Oestradiol and the surge release of gonadotropins in the ewe. *Animal Reproduction Science, 34*(3-4), 217–30.

Kuru, M., Kuru, B.B., Kacar, C., Can Demir M.C., & Cetin, N. (2022). Effect of oestrus synchronization with different lengths of progesterone-impregnated sponges and equine chorionic gonadotropin on reproductive efficiency in Romanov ewes during the non-breeding season. *Acta Veterinaria Brno,* (91), 243-250.

Leyva, V., Buckrell, B.C., & Walton, J.S. (1998). Regulation of follicular activity and ovulation in ewes by exogenous progesterone. *Theriogenology, 50,* 395-416.

Mann, G.E., McNeilly, A.S, & Baird, D.T. (1992). Hormone production in vivo and in vitro from follicles at different stages of the estrous cycle in sheep. *Journal of Endocrinology, 132,* 225–234.

McDowell, C.M., Anderson, L.H., Kinder, J.E., &Day, M.L. (1998). Duration of treatment with progesterone and regression of persistent ovarian follicles in cattle. *Journal of Animal Science, 76*,850–855.

Quirke, J.F., & Hanrahan, J.P. (1985). Breed differences in the breeding season in sheep. In F. Ellendorff & F. Elsaesser (Eds.), *Endocrine causes of seasonal and lactational anestrus in farm animals* (pp.29-43). Dordrecht: Martinus Nijhoff

SAS/STAT.(2006). *SAS/STAT guide to personal computers*. Cary, NC. USA: SAS Instituiton Inc.

Savio, J.D., Thatcher, W.W., Morris, G.R., Entwistle, K., Drost, M., &Mattiacci, M.R. (1993). ffects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. *Journal of Reproduction and Fertility, 98,* 77–84.

Sharma, V.K., Gupta, R.C., Khar, S.K., & Khurana, N.K. (1993). Plasma progesterone profiles, ovarian response and embryo recovery in crossbred ewes superovulated during breeding and non-breeding seasons. *Animal Reproductio Science, 34,* 119–126.

Webb, R., Gauld, I.K., & Driancourt, M.A. (1989). Morphological and functional characterization of large antral follicles in three breeds of sheep with different ovulation rates. *Journal of Reproduction and Fertility, 87*,243–55.

Wehrman, M.E., Roberson, M.S., Cupp, A.S., Kojima, F.N., Stumpf, T.T., Werth, L.A., Wollfe, M.W., Kittok, R.J., & Kinder, J.E. (1993). Increasing exogenous progesterone during synchronization of estrus decreases endogenous 17 betaestradiol and increases conception in cows. *Biology Reproduction, 49,* 214–220.

Vinoles, C., Meikle, A., Forsberg, M., & Rubianes, E. (1999). The effect of subluteal levels of exogenous progesterone on follicular dynamics and endocrine patterns during the early luteal phase of the ewe. *Theriogenology, 51*,1351-1361.

Author Information

Mustafa Q. Husein
Department of Animal Production, Jordan University of Science and Technology, P. O. Box 3030, Irbid 22110, Jordan
Contact e-mail: *huseinmq@just.edu.jo*

Hassan A. Ghozlan
Department of Animal Production, Jordan University of Science and Technology, P. O. Box 3030, Irbid 22110, Jordan

To cite this article:

Husein, M.Q., & Ghozlan, H.A. (2023). A comparison of two estrus synchronization protocols utilizing progesterone supplement 24 hours before CIDR-G removal in sheep. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 11,* 30-39.