A new strategy for superior reproductive performance of ewes bred out-of-season utilizing progestagen supplement prior to withdrawal of intravaginal pessaries

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Abstract:

Two experiments were conducted to examine the effect of progestagen supplement 24 h prior to intravaginal pessary withdrawal on reproductive performance of seasonal anestrous ewes. Ewes in each experiment were allocated to treatment and control and all were induced to estrus using either intravaginal MAP (Exp. 1; n = 24) or CIDR-G (Exp. 2; n = 28) pessaries for 12 days. Half of the ewes in each experiment were supplemented 24 h before withdrawal of pessaries with either 10 mg oral MAP tablets (Exp.1) or 25 mg i.m. progesterone (P4) administration (Exp.2; P4-supplement-treated group). Fertile rams were allowed with the ewes at sponge removal (Day 0, 0 h) and estrus was monitored at 6-h intervals for 3 days. Blood samples were collected for measurements of P4 (Exp.1 and Exp.2) and LH (Exp. 2). In both experiments, the percent of ewes in estrus was greater ($P<0.05$) and intervals to estrus were longer ($P<0.05$) in progestagen-supplement-treated than control ewes. In Exp. 2, the occurrence and magnitude of LH surges were greater ($P<0.01$) and intervals to onset of LH surge were longer ($P<0.01$) in P4-supplement-treated than control ewes. In Exp. 2, P4 supplement elevated P4 levels from 1.8 _ 0.1 ng/mL on Day _1 to 4.2 _ 0.3 on Day 0 ($P<0.001$). Following pessaries

removal, P4 concentrations fell to basal values on Day 1 in both groups and remained low until Day 5. Then, P4 concentrations increased and remained elevated through Day 19 in all (100%) progestagen-supplement-treated in Exp. 1 (12/12) and Exp. 2 (14/14) and in only 5/12 (41.7%) and 6/14 (42.9%) control ewes, respectively. These ewes were confirmed pregnant by ultrasonography and lambed on Day 149.2 _ 0.2 following Day 0. In conclusion, progestagen supplement 24 h prior to removal of pessary can be used successfully to improve reproductive performance of ewes bred out-of-season.

Keywords: Progesterone; Estrus synchronization; Sheep; LH surge.

1. Introduction:

The use of estrus synchronization in the sheep industry has been practiced for over 50 years [1–3].

Synchrony of estrus following the most traditional methods is not precisely enough to enhance first-cycle pregnancy and lambing rates [4,5]. The most widely used protocols are based on the use of intravaginalprogestagen-releasing pessaries and eCG at pessary withdrawal [6,7]. Although about 90% of ewes express estrus, approximately 50% become pregnant and lamb from mating at induced estrus using such protocols [5]. The remaining percentage of ewes do not become pregnant even though they display estrus, ovulate and are mated.

We have previously indicated that the possibility to maximize fertility rates in ewes reside in improving the process of follicular growth and development [8]. Driancourt [9] stated that ''synchronization treatment protocols involving the use of progesterone/progestagen will only be successful if they prevent the development of a persistent dominant follicle''. Prolonged P4 exposure using an intravaginal device induces subluteal serum P4 concentrations in sheep toward the end of the treatment [10]. It has been previously shown that low P4

concentrations are associated with abnormal follicular development and presence of persistent follicle, leading to low fertility [10–16]. In cattle, altered follicular wave pattern and development of persistent follicle have been proposed to be one of themajor causes of infertility in P4-based synchronization programs [17–19]. In sheep, the effects of aged follicles on pregnancy rate are controversial. It has been reported that aged follicles do reduce [11,20] or do not have any effect on pregnancy rates [16]. Progestagen-based protocols practiced over the years should take into account the deleterious effect of prolonged progestagen treatment on fertility and therefore ovulation of persistent dominant follicles must be avoided [14,19]. In cattle, an approach to overcome such a problem is by acute P4 supplement [19].Administration of P4 reduces the frequency of LH pulses and therefore, induces regression of the persistent ovarian follicle [19,21]. The objectives of this study were to examine the effects of progestagen supplement 1 day before intravaginal pessary removal on pregnancy and lambing rates of ewes bred out-of-season.

2. Materials and methods:

2.1. General:

Anestrous pluriparous Awassi ewes were used in two experiments conducted at the sheep unit at the Agricultural Center for Research and Production at Jordan University of Science and Technology (328330N,358510E) located in the northern part of Jordan at an altitude of 520 m. Ewes ranged in age from 3 to 7 years and had a body condition score of 2.5–3 (scale = 0 lowest to 5 highest) and weighed between 43 and 65 kg (mean _ S.E.M. of both experiments = 49.3 _ 1.1). Cyclicity in Awassi ewes ceases during the months of May and June and part of July in our region [4,5,22]. All ewes had previously lambed during the past lambing season and had their last lambs been weaned about 6 weeks before the start of each experiment. Ewes in each experiment were housed together in a single pen (10 m _ 6 m). Ewes were fed 1.2 kg

wheat straw and 0.5 kg concentrate mixture per ewe per day, and had ad libitum access to water, shade and mineral salt blocks.

2.2. Experimental design:

2.2.1. Exp. 1:

In June 2005, 24 Awassi ewes were administered intravaginally withmedroxyprogesterone acetate (MAP) pessaries (Synchron sponges, Farvet, Bladel, Holland) for 12 days and were randomly allocated 1 day before pessary removal to two treatment groups of 12 ewes each. Ewes in the first group (MAP-supplement-treated) were orally administered with 10 mg MAP tablets (Provera, Pharmacia & Upjohn n.v/s.a. Puurs, Belgium) 24 h prior to MAP pessaries removal and those in the second group were orally administered with 1 mL saline solution and served as controls (control group). Ewes were exposed to two fertile Awassi rams, fitted with crayon-marking harnesses, immediately following pessary removal (Day 0, 0 h) and estrus was monitored at 6-h intervals for 3 days. Blood samples were collected once daily on Day _12 and from Day 0 until Day 5, and once on alternate days thereafter untilDay 19 to compare P4 concentrations between the two groups. Pregnancy diagnosis was determined based upon sustained high P4 concentrations between Days 15 and 19 and later confirmed by ultrasonography on Day 30 using a modified 5-MHz transrectal ultrasound transducer (485 Anser Vet, Pie Medical Equipment B.V., Philipsweg, AJ Maastricht, The Netherlands). Plasma P4 concentrations were measured by a solid-phase radioimmunoassay (RIA) using a commercial kit (Coat-A-Count Progesterone1;Diagnostic Products Corporation, DPC, Los Angeles, CA). Sensitivity was 0.1 ng/mL and intraassay coefficient of variation was 4.3%.

2.2.2. Exp. 2:

In May 2006, 28 Awassi ewes were assigned at randomto treatment (n=14) and control (n=14) groups. Ewes were administered on May 27 with intravaginal P4-releasing

pessaries (CIDR-G, Pharmacia & Upjohn n.v/ s.a. Puurs, Belgium) containing 300 mg P4 which were withdrawn 12 days later on June 8 at 08:00 (Day 0 and hour 0). Ewes in the treatment group received i.m. injections of 25 mg P4 (Intervet UK Ltd., Science Park, Milton Road, Cambridge, UK) given 24 h prior to CIDRG removal and those in the control group were administered with saline solution. Ewes were exposed to three fertile Awassi rams, fitted with crayon-marking harnesses immediately following CIDR-G removal and estrus was monitored at 6-h intervals for 3 days. Jugular venous blood samples were collected every other day starting immediately before CIDR-G insertion from Day _12 until Day_2 and once daily from Day_1 until Day 5 to compare P4 concentrations between the two groups. Additional blood samples were obtained on alternate days after Day 5 until Day 19 for comparison of P4 levels and for pregnancy diagnosis. Pregnancy diagnosis was performed also on Day 30 using ultrasonography. For measurement of LH concentrations, blood samples were also collected at 6-h intervals starting immediately after CIDR-G removal (Day 0, hour 0) for 72 h. Plasma P4 concentrations were measured using a solid-phase RIA. Sensitivity was 0.1 ng/mL and intraassay coefficient of variation was 2.9%. Plasma LH levels 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%.

2.3. Statistical analysis:

Data on reproductive responses of ewes in both experiments were analyzed using SAS/STAT ANOVA procedures [23]. Onset of estrus was considered to have occurred 3 h before observation of a breeding mark. Onset of the preovulatory LH surge was considered to have occurred 6 h before the first plasma sample having an LH concentration of _10 ng/mL [8]. Effects of progestagen supplement, prior to pessary removal, on incidence of estrus, preovulatory LH surge, pregnancy, lambing and multiple birth rates were analyzed using Fisher's exact test. Effects of progestagen supplement on amplitude of

the LH surge and on various intervals to onset of estrus and LH surge and peak LH surge were tested using Student's t-tests. Plasma P4 and LH concentrations were analyzed for the effect of treatments and time using the repeated-measures procedure of GLM. First-cycle pregnancy rate was defined as the number of ewes bred by rams within 3 days following Day 0 and became pregnant based upon sustained P4 concentrations of _3 ng/mL from Day 15 through Day 19 and confirmed by ultrasonography on Day 30 [5,24]. Lambing rate was defined as the number of ewes that became pregnant from mating at induced estrus and lambed 145–155 days following Day 0.

Table 1: Reproductive responses following MAP sponge removal in MAP-supplement-treated and control ewes bred out-of-season

Parameter	Control (n = 12)	MAP-supplement-treated (n = 12)
Ewes displayed estrus[a]	7/12 a	12/12 b
Intervals to onset of estrus (h)[a]	43.7 _ 2.8 a	54.5 _ 2.9 b
First-cycle pregnancy rate[b]	5/12 c	12/12 d
First-cycle lambing rate[c]	5/12 c	12/12 d
Fecundity (%)[d]	0.4 (41.6) c	1.3 (133) d
Prolificacy[e]	1.0 _ 0.0 a	1.3 _ 0.1 a
Multiple birth rates	0/5 a	4/12 a

Numbers within row with different letters (a and b) differ (P < 0.05). Numbers within row with different letters (c and d) differ (P < 0.01).
[a] Occurring within 72 h following MAP sponge removal.
[b] Occurring based upon P4 concentrations on Day 19 and ultrasonography on Day 30.
[c] Ewes lambing from mating at induced estrus.
[d] Number of lambs born per ewe exposed to rams.
[e] Number of lambs born per ewe lambing.

3. Results :

3.1. Exp. 1:

Reproductive parameters are illustrated in Table 1. Estrus was observed in 12/12 MAP-supplement-treated and in 7/12 control ewes. Intervals to onset of estrus were longer by 10.8 h in MAP-supplement-treated (54.5_2.9h) than control (43.7_2.8h) ewes. Differences in estrus expression and intervals to onset of estrus were significant (P<0.05). Initial plasma P4

concentrations in samples taken prior to MAP sponge insertion on Day_12 were less than 0.3 ng/mL among ewes of both groups (P>0.2) indicating seasonal anestrus. Progesterone concentrations at the time of MAP sponge removal (Day 0) were basal (<0.2 ng/mL) and remained low until Day 5. Concentrations of P4 increased gradually after Day 5 among ewes of both groups until Day 15 and remained elevated through Day 19 in 12/12 MAP-supplement-treated and in only 5/12 control ewes (P<0.01). These ewes were diagnosed pregnant based upon sustained P4 concentrations between Days 15 and 19 and confirmed by ultrasonography performed on Day 30 and lambed 149.1_0.3 days following Day 0. In the remaining 7/12 control ewes, P4 concentrations dropped after Day 15 and concentrations were typical of those seen during the process of spontaneous luteal regression. MAP supplement significantly increased the number of lambs born per ewe exposed (P<0.01) but not the multiple birth rates (P=0.14) compared to the control.

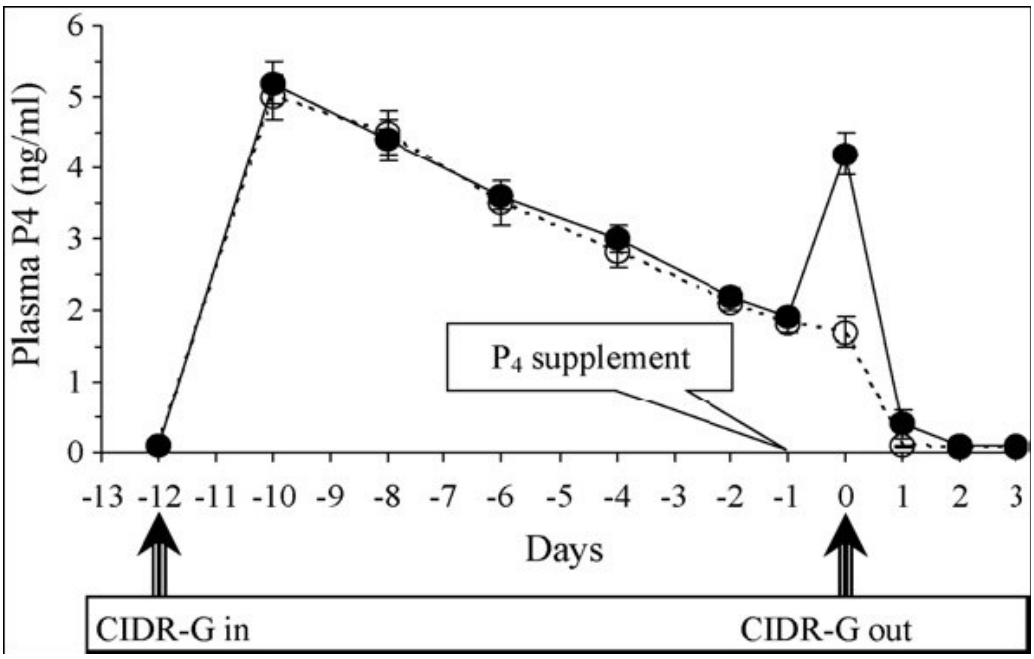

Fig. 1. Plasma P4 concentrations during the 12-day period in which CIDR-G were in place in P4-supplement-treated (*) and control (*) ewes bred out-of-season.

3.2. Exp. 2:

3.2.1. Progesterone concentrations during the 12-day period in which CIDR-G were in place:

Ewes maintained CIDR-G until they were pulled out. Progesterone concentrations in plasma during the 12-day period of CIDR-G insertion are illustrated in Fig. 1. Progesterone concentrations in plasma samples taken prior to CIDR-G insertion were less than 0.2 ng/Ml among ewes of both groups (P>0.2) indicating seasonal anestrus. Following CIDR-G insertion, P4 concentrations rapidly increased and reached maximum mean values 2 days post-insertion (Day_10) and were 5.2_0.3 and 5.0_0.3 ng/mL for the P4-supplement-treated and control ewes, respectively. Differences between the two groups in maximum mean P4 values on Day_10 were not significant (P>0.5). Progesterone gradually decreased from Day_10 by day until Day_1 and concentrations on this day were similar (P>0.5) between P4-supplement-treated (1.8_0.1 ng/mL) and control (1.9_0.1 ng/mL) ewes. In P4-supplementtreated group, plasma P4 concentrations increased sharply from 1.8_0.1 ng/mL on Day_1 to 4.2_0.3 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.

Table 2: Reproductive parameters following CIDR-G removal in P_4-supplement-treated and control ewes bred out-of-season

Parameter	Control (n = 14)	P_4-supplement-treated (n = 14)
Estrus[a]		
Ewes displayed estrus	9/14 a	14/14 b
Intervals to onset of estrus (h)	35.3 _ 1.9 c	45.4 _ 2.4 d
Interval from estrus to onset of LH surge (h)	1.0 _ 1.8 a	2.8 _ 0.9 a
Plasma LH[a]		
Ewes had LH surge	6/14 c	13/14 d
Intervals to onset of LH surge (h)	37.0 _ 2.9 a	46.6 _ 2.6 b
Intervals to peak LH surge (h)	43.0 _ 2.9 a	52.6 _ 2.6 b
Amplitude of LH surge (ng/mL)	23.9 _ 3.9 c	51.1 _ 7.1 d
Pregnancy and lambing		
First-cycle pregnancy rate[b]	6/14 c	14/14 d
First-cycle lambing rate[c]	6/14 c	14/14 d
Fecundity (%)[d]	0.4 _ 0.1 (42.8) e	1.4 _ 0.1 b (142.8) f
Prolificacy[e]	1.0 _ 0.0 a	1.4 _ 0.1 b
Multiple birth rates	0/6 a	6/14 a

Numbers within row with different letters (a and b) differ (P<0.05). Numbers within row with different letters (c and d) differ (P<0.01). Numbers within row with different letters (e and f) differ (P<0.001).

[a] Occurring within 72 h following CIDR-G removal.
[b] Occurring based upon P4 concentrations on Day 19 and ultrasonography on Day 30.
[c] Ewes lambing from mating at induced estrus.
[d] Number of lambs born per ewe exposed to rams.
[e] Number of lambs born per ewe lambing.

3.2.2. Estrus responses and the preovulatory LH surge:

The incidence of estrus and the preovulatory surges of LH were detected in more P4-supplement-treated than control ewes (Table 2). One ewe from the P4-supplement-treated group and eight ewes from the control group did not have an identifiable surge release of LH. Intervals from 0 h to onset of estrus (45.4_2.4 versus 35.3_1.9 h; P<0.01) and to onset of the preovulatory LH surge (46.6_2.6 versus 37.0_2.9 h; P<0.05) were longer in P4-supplement-treated than control ewes,

respectively. Onset of the preovulatory LH surge occurred after onset of estrus by 2.8_0.9 h in P4-supplement-treated and 1.0_1.8 h in control ewes with no difference (P=0.4) between two groups. Of the ewes exhibiting a surge release of LH, amplitudes of the preovulatory LH surges were greater (P<0.01) in P4-supplement-treated (51.1_7.1 ng/mL; range 21.4–100) than control (23.9_2.9 ng/mL; range 14.9–41.6) ewes (Fig. 2).

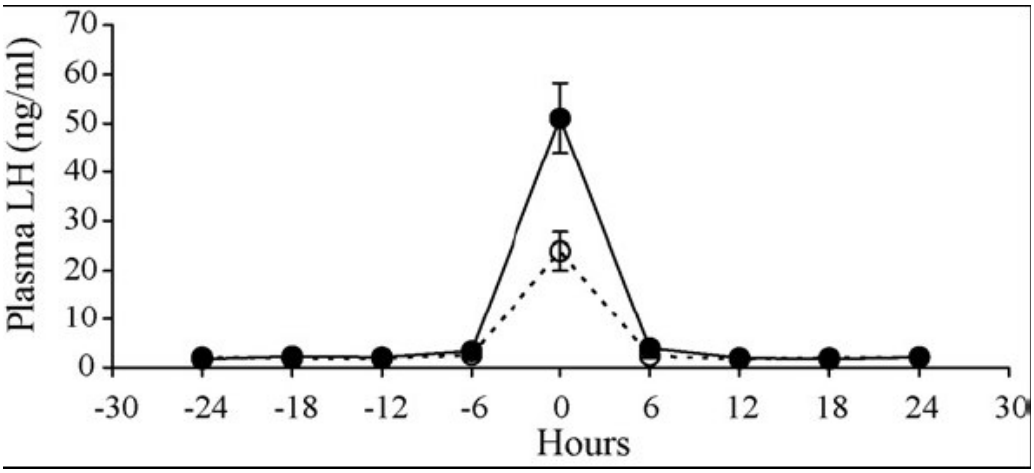

Fig. 2. Plasma LH concentrations and magnitude of the preovulatory LH surge in P4-supplement-treated (*) and control (*) ewes. The time (0 h) represents the aligned peak LH levels in both groups.

3.2.3. Progesterone profiles following CIDR-G removal, pregnancy and lambing rates:

Plasma P4 concentrations at the time of CIDR-G removal on day 0 differed significantly (P<0.001) between the P4-supplement-treated (4.2_0.3 ng/mL) and the control (1.7_0.1 ng/mL) groups. Following Day 0, P4 concentrations fell within 24 h from 4.2 to <0.5 ng/mL in P4-supplement-treated and from 1.7 to <0.1 ng/mL in the control group. Progesterone 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-supplement-treated and 6/14 control ewes. These ewes were confirmed pregnant by ultrasonography performed on Day 30. Differences in pregnancy rates were significant (P<0.01) between the two groups. In the remaining 8/14 control ewes P4 concentrations dropped spontaneously after Day 15 and concentrations were typical of those detected during the process of luteal regression. Ewes that became pregnant from mating at induced estrus in both groups lambed on Day 149.3_0.3 and the number of lambs born was greater (P<0.01) in P4-supplement-treated than control ewes (Table 2). The multiple birth rate between the two groups tended to be different (P=0.09).

4. Discussion:

The present study provides a new insight into the estrus synchronization schemes and to our knowledge this is the first study to test the effect of administration of progestagen 1 day before pessary removal on fertility rates in sheep. In the present study, Exp. 2 utilized natural P4 (CIDR-G) and confirmed repeatability of the results in Exp. 1 which utilized synthetic progestagen (MAP). Results obtained from both experiments in the present study demonstrate that the progestagen supplement strategy was successful in inducing fertile cycles in 100% of the ewes and capable of producing full reproductive performance out-of-season. Such successful outcome illustrates the important requirement of progestagen supplement prior to pessary removal and the ram effect in eliminating/substituting the need for gonadotropins in out-of-season breeding programs. Progesterone profiles during the 12-day period of pessary insertion and the preovulatory LH surges were determined in Exp. 2 and were typical of those previously reported in the literature [5,8,24]. An exception was the abrupt rise in P4 concentrations from 1.8 to 4.2 ng/mL following the P4 supplement on Day _1. From our prospective, the abrupt rise in P4 levels followed by sharp decline to basal values after withdrawal of pessaries resets the hypothalamic–

pituitary–ovarian axis to regress persistent follicles and recruit new healthy ones in the milieu of low gonadotropin (especially LH) and estradiol 17-b (E2) concentrations. Progestagen-based synchronization protocols in sheep cause the development of persistent follicle and maintenance of high E2 concentrations over longer period of time [11]. In cattle, the acute injection of P4 induces atresia of persistent follicles [19,25]. As a consequence of P4 decline following pessary removal, healthy follicles destined to ovulate, grow and secrete E2 [26], which along with the male effect induce the preovulatory LH surge leading to ovulation of potential ova [27]. These factors maybe considered the key feature for initiating the expression of superior reproductive performance. The amount rather than the change in magnitude of P4 concentrations has been suggested to be more important in regulation of LH pulses [27]. In the present study, we have used 10 mg MAP and 25 mg P4 which are well above the minimum suggested doses of 2.5 mg MAP [28,29] and 20 mg P4 [27,30]. In fact, P4 supplement in Exp. 2 of the present study resulted in a significantly higher amplitude of LH surges than the control. Husein et al. [24] indicated that the use of sponges impregnated with 750 mg P4 were associated with higher amplitudes of LH surges compared with those containing 500 mg in anestrus Finncross ewes. Pearce et al. [27] suggested that P4 injection at ram introduction delays the LH surge, increases the duration of gonadotropin priming of follicles and causes a dosedependent increase in peak E2 levels. Increasing E2 levels have been shown to be positively associated with pulse frequency, amplitude and mean concentrations of LH [25]. Furthermore, LH surges of higher amplitudes have been reported with rising high E2 levels than those with persistently high E2 levels in ewes with persistent follicles [31]. In both experiments of the present study estrus was detected in 100% of ewes that had MAP and P4 supplement compared with 61.5% in the control groups. Synthetic analogues of progesterone such asMAP are at least 20 times more potent than natural P4 [32]. It is well known that MAP has a longer half-life than P4 [33] and therefore, ewes that received MAP

(Exp. 1) exhibited estrus 8–9 h later than ewes treaded with CIDR-G (Exp. 2). Intervals to onset of estrus in the present study were in agreement with those previously reported [5,7,34]. The intervals from pessary removal to the onset of estrus (Exp. 1 and Exp. 2) and to the LH surge (Exp. 2) were delayed in the progestagen-supplement-treated groups compared with the control groups. Results of the present study are in agreement with those of Wheaton et al. [7] who reported that administration of P4 at the time of sponge removal caused 9 h delay in the onset of estrus from 41 _ 2 to 50 _ 2. In addition, a single P4 injection at the time of ram introduction caused a significant delay in the preovulatory LH surge from 11.5 _ 2.6 to 71.8 _ 6.2 [27], and from 20.5 _ 10.7 to 58.8 _ 10.1 h [35]. The reason for this 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. Interestingly the 10.8 and 10.1 h delay in the onset of estrus in Exp. 1 and Exp. 2, respectively, is equal to the 10-h delay reported by Webb et al. [36]. 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. Rodriguez Iglesias et al. [29] reported that a single i.m. injection of 2.5 mg MAP at the time of ram introduction caused a 3-day delay in peak estrus occurrence when compared with the 20 mg i.m. P4 injection. In contrast, our study demonstrated that MAP and P4 supplements were similar and did not show any differences in reproductive patterns between Exp. 1 and Exp. 2. The contradiction between our results and those of Rodriguez Iglesias et al. [29] maybe attributed to the timing and the route of MAP supplement. MAP, due to its long half-life, may act for more than 1 day [28]. In the present study, the 1 day earlier administration of MAP before ram introduction could have negated the delaying effect expected when MAP was administered at ram introduction. Ungerfeld et al. [28], using a single 2.5 mg i.m. MAP injection 1 day before ram introduction, showed a 4.5-day advancement in peak estrus occurrence when compared with

MAP injection on the day of ram introduction. In addition, elimination of orally administered MAP is reported to be shortened by about 6–10 times compared to intramuscularly administered MAP [37]. Moreover, Ungerfeld et al. [28] and Rodriguez Iglesias et al. [29] did not use any progestagen-based synchronization protocols. Higher pregnancy rates in P4-supplement-treated groups in the current study are believed to be mainly due to eliminating the aged persistent follicles. Thus, our results provide an indirect evidence for the deleterious effect of aged follicles on fertility. In beef cattle, P4 administrated i.m. 2 days before the end of progestagen treatment regressed persistent follicles and significantly improved pregnancy rates [19,25]. 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. Husein et al. [8,24] indicated that the use of sponges impregnated with high P4 (750 mg) were associated with higher amplitudes of LH surges and higher pregnancy rates in anestrous Finncross ewes [24]. In contrast, other studies in sheep associated low P4 concentrations with abnormal follicular development, persistent follicle and reduced fertility [10–16]. It is generally accepted that progestagen-eCG treatment is necessary for out-of-season breeding in small ruminants. In Awassi ewes, low but acceptable (between 40 and 50%) fertility rates have been reported out-of-season using a 12-day FGA-eCG or MAP-eCG [4,5]. In the present study, the improved pregnancy and lambing rates and the increase in the number of lambs born in progestagen-supplement-treated groups may in part be due to the exclusion of the eCG from the synchronization scheme when compared with those previously reported in literature using progestagen-eCG in Awassi ewes [4,5]. Due to its long half-life, administration of eCG may lead to the development of large anovulatory estrogenic follicle that may, in turn, negatively influence early embryonic development and oviductal transport. Under Jordanian conditions, the prolificacy

rate averages about 0.9 lambs per ewe bred naturally and a little more than one lamb per ewe treated with progestagen-eCG. The strategy of progestagen supplement implemented in the present study has the potential to improve such low reproductive performance without using eCG.

In conclusion, the present study demonstrates for the first time that progestagen supplement 24 h prior to pessary removal can be used successfully for expression of superior reproductive performance of ewes bred outof-season. The actions of progestagen supplement reside, perhaps, in enhancing the exponential follicular growth and development, preventing the development of large persistent follicle and expression of estrus and LH surges. However, progestagen supplement may have additional ameliorative effects on the hypothalamus–pituitary–ovarian axis. Acknowledgements Authors are grateful to Dr. Ali Debiri and Dr. Nabeel Salameh (Pharmacia and Upjohn) for providing CIDRG. Authors express their appreciation to Mr. H.A. Ghozlan and Dr. A. Altilawi for data collection and lab analysis and staff led by I.M. Tahat for technical assistance and animal management and care at the sheep unit at the Center of Agricultural Research and Production at JUST.

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ملخص البحث

لقد خلق هذا البحث بشقيه (التجربة الأولى والتجربة الثانية) بعداً تطبيقياً جديداً للأبحاث المستقبلية في إنتاج الأغنام باستخدام البروجسترون الإضافي قبل نزع اسفنجات البروجسترون. كما حدد عاملاً جديداً مهماً في تحديد نسبة الحمل الناتج عن التلقيح خارج موسم التزاوج.

أجريت **التجربة الأولى** في يناير 2004، وكان الهدف منها هو فحص أثر إعطاء حبوب تحتوي على 10 ملغم من مادة أسيتات ميدروكسي بروجسترون (MAP) قبل 24 ساعة من سحب الاسفنجات. وقد استخدمت في التجربة 24 نعجة غير شائعة تم توزيعها عشوائياً على مجموعتين. وقد أعطيت كل النعاج في المجموعتين اسفنجات بروجسترون نوع MAP لمدة 12 يوماً. وأعطيت النعاج في المجموعة الأولى حبوب بروجسترون عن طريق الفم تحتوي كل حبة على 10 ملغم من أسيتات ميدروكسي بروجسترون (MAP) قبل 24 ساعة من سحب الاسفنجات، بينما كانت المجموعة الثانية مجموعة الشاهد. بعد سحب الاسفنجات مباشرة أدخل على النعاج جميعاً كبشين لكشف الشياع والتلقيح. وأخذت عينات دم من جميع النعاج لفحص مستوى هرمون البروجسترون والكشف عن الحمل كما تم الكشف عن الحمل أيضاً في اليوم 30 باستخدام الأمواج فوق الصوتية. كانت نسبة حصول الشياع والإباضة في المجموعة المعاملة بالبروجسترون الإضافي بناءاً على مستويات البروجسترون أعلى والفترة من سحب الاسفنجة حتى حصول الشياع أطول منها في المجموعة الشاهد. وكانت مستويات البروجسترون في المجموعتين مشابهة لما هو في الواقع. كما بقيت مستويات البروجسترون مرتفعة في 12/12 (100%) من مجموعة المعاملة وفقط في 12/5 (41.7%) من مجموعة الشاهد. وقد حددت هذه النعاج على أنها حوامل في اليوم 30 عند الفحص بالأشعة فوق الصوتية وقد ولدت جميعاً خلال 150.2 يوماً من اليوم 0. كانت هذه النتائج مشجعة، وأصبح الاهتمام هو فحص موثوقية النتائج، وتحديد العوامل التي أدت إلى نسبة الحمل الرائعة هذه.

أجريت **التجربة الثانية** في مايو 2005، لفحص أثر البروجسترون الإضافي قبل نزع أداة البروجسترون

المهبلية (CIDR-G) في تحسين الأداء التناسلي لأغنام العواسي خارج موسم التلقيح. شملت التجربة 28 نعجة تم توزيعها بشكل عشوائي إلى مجموعتين: مجموعة المعاملة ومجموعة الشاهد بعدد 14 نعجة لكل منهما. تم وضع أداة بروجسترون المهبل لجميع النعاج لمدة 12 يوماً، وحقنت نعاج مجموعة المعاملة بـ 25 ملغم بروجسترون

للنعجة قبل 24 ساعة من سحب الأداة، بينما حقنت مجموعة الشاهد بمحلول ملحي. تم تعريض النعاج إلى 3 كباش فور سحب أداة البروجسترون للتلقيح وكشف الشياع. وقد أخذت عينات الدم من النعاج لفحص مستويات هرمونات البروجسترون والهرمون المنشط للجسم الأصفر (LH). تم تحديد الحمل عن طريق مستوى البروجسترون على يوم 19 وبالموجات فوق لصوتية على يوم 30. وكانت نسبة الشياع، وجود ارتفاع في مستوى LH وذروته أكبر في مجموعة التجربة منها في مجموعة الشاهد. وكانت الفترات حتى هذه الاستجابات أطول في المجموعة المعاملة بالبروجسترون منها في مجموعة الشاهد. كما كانت مستويات البروجسترون عادية في جميع النعاج ومطابقة لمثيلاتها في بروتوكولات تنظيم الشياع إلا في حالة واحدة في مجموعة المعاملة إذ ارتفعت من حوالي 1.8±0.1 في اليوم -1 إلى 4.2±0.3 في اليوم 0 بقيت مستويات البروجسترون عالية حتى اليوم 19 في 14/14 (100%) من مجموعة المعاملة، وفي 14/6 (42.9%) في مجموعة الشاهد. وقد حدت هذه النعاج حوامل في اليوم 30 باستخدام الموجات فوق الصوتية. كما ولدت بعد 149.4 يوم بعد سحب الأداة.

وكنتيجة، فان استخدام البروجسترون الإضافي قبل سحب الأداة قد أنتج أداءً تناسلياً عالياً في النعاج خارج موسم التلقيح.

A new strategy for superior reproductive performance of ewes bred out-of-season utilizing progestagen supplement prior to withdrawal of intravaginal pessaries

Theriogenology, *Volume 69, Issue 3*, *February 2008*, *Pages 376-383*

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Two experiments were conducted to examine the effect of progestagen supplement 24 h prior to intravaginal pessary withdrawal on reproductive performance of seasonal anestrous ewes. Ewes in each experiment were allocated to treatment and control and all were induced to estrus using either intravaginal MAP (Exp. 1; *n* = 24) or CIDR-G (Exp. 2; *n* = 28) pessaries for 12 days. Half of the ewes in each experiment were supplemented 24 h before withdrawal of pessaries with either 10 mg oral MAP tablets (Exp. 1) or 25 mg i.m. progesterone (P_4) administration (Exp. 2; P_4-supplement-treated group). Fertile rams were allowed with the ewes at sponge removal (Day 0, 0 h) and estrus was monitored at 6-h intervals for 3 days. Blood samples were collected for measurements of P_4 (Exp. 1 and Exp. 2) and LH (Exp. 2). In both experiments, the percent of ewes in estrus was greater ($P<0.05$) and intervals to estrus were longer ($P<0.05$) in progestagen-supplement-treated than control ewes. In Exp. 2, the occurrence and magnitude of LH surges were greater ($P<0.01$) and intervals to onset of LH surge were longer ($P<0.01$) in P_4-supplement-treated than control ewes. In Exp. 2, P_4 supplement elevated P_4 levels from 1.8 ± 0.1 ng/mL on Day −1 to 4.2 ± 0.3 on Day 0 ($P<0.001$). Following pessaries removal, P_4 concentrations fell to basal values on Day 1 in both groups and remained low until Day 5. Then, P_4 concentrations increased and remained elevated through Day 19 in all (100%) progestagen-supplement-treated in Exp. 1 (12/12) and Exp. 2

(14/14) and in only 5/12 (41.7%) and 6/14 (42.9%) control ewes, respectively. These ewes were confirmed pregnant by ultrasonography and lambed on Day 149.2 ± 0.2 following Day 0. In conclusion, progestagen supplement 24 h prior to removal of pessary can be used successfully to improve reproductive performance of ewes bred out-of-season.