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The Effect of Post-Mating Progesterone Supplement on Pregnancy and Lambing Rates of Ewes Bred Out-of-season

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Abstract: The objective of this experiment was to determine whether post-mating progesterone (P_4) supplement improves pregnancy and lambing rates in ewes bred during the seasonal anestrous period. In June, 39 synchronized to estrus Awassi ewes were allowed with four harnessed fertile rams immediately following CIDR-G device removal (day 0 and 0 hour). Five days following ram introduction, ewes were randomly assigned to four groups to be treated post-mating with intramuscular injections of 20 mg P_4 supplement once daily from day 5 to day 9 (P_4-D5-9), days 10 to 14 (P_4-D10-14), days 5 to 14 (P_4-D5-14) or did not receive P_4 supplement (control). Blood samples were collected from all ewes for P_4 analysis. Progesterone concentrations prior to CIDR-G insertion were basal and no differences in P_4 concentrations were found on days –12, –10 and between days 0 and 5 among groups. Progesterone concentrations between days 5 and 15 differed ($p<0.001$) significantly due to treatment effect. Maximum P_4 concentrations were reached on day 9 in group P_4-D5-9 and between days 11 and 15 in groups P_4-D10-14, P_4-D5-14, and control. Pregnancy was diagnosed based on day 19 P_4 levels and day 30 ultrasonic examination in 5/10, 6/10, 5/10, and 5/9 ewes in groups P_4-D5-9, P_4-D10-14, P_4-D5-14 and control, respectively. Pregnancy loss was detected by ultrasonography on day 45 in 3/6 and 3/5 ewes in P_4-D10-14, P_4-D5-14 groups only. Overall pregnancy (53.8%) and lambing (41%) rates were similar among groups and were not influenced by P_4 supplement. In conclusion, P_4 supplement administered intramuscularly between days 5 and 14 post-mating is not effective in improving pregnancy, embryonic survival and lambing rates in Awassi ewes pretreated out-of-season. Pregnancy loss which occurred only in P_4-D10-14 and P_4-D5-14 groups maybe attributed to factors including the sharp decrease in P_4 concentrations on day 15 and stress experienced during the period of maternal recognition of pregnancy process.

Key words: post-mating, progesterone supplement, Awassi ewes, out-of-season

INTRODUCTION

Reproductive performance of Awassi ewes has been low under semi arid conditions[1]. The fertility rate has been estimated to be 0.9 lambs per ewe mated and the majority of lambs born are singles, with some twins and rare triplets. Of approximately the 90% ewes that exhibit estrus, between 25 and 55% conceive and lamb from mating at first service[1,2,3]. The remaining ewes that do not become pregnant to first service remain prone to be bred during the second or third service or even some are not bred at all during a given breeding season. The low fertility rate is primarily attributed to factors including breed, heredity, environment, management and the reproductive soundness of the ewes[1,4]. Ovulation, fertilization and early embryonic mortality rates are also among factors influencing litter size[2]. Of these factors embryonic mortality has been considered to be the greatest limitation to reproductive efficiency across mammalian species and has been estimated to between 25 and 60%[5]. Early embryonic mortality usually occurs during the first 3 weeks of gestation which results in pregnancy rates ranging from 16 to 76%[2,3,6,7].

Although factors causing early embryonic mortality in sheep are not well established, there is evidence suggesting the involvement of luteal inadequacy[8]. Luteal inadequacy, resulting from environmental factors such as heat stress or nutrition, has been shown to be a major cause of embryonic loss in sheep[8]. Progesterone is the principal hormone maintaining pregnancy and controlling embryo

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development. It is well known that P_4 levels during the early-mid luteal phase increase rapidly from day 4 to day 10 after estrus in ewes[9]. Pregnancy maintenance is largely dependent on P_4 of luteal source for approximately the first 50 days of gestation[10]. Therefore, it would be advantageous to note that high plasma P_4 concentrations during the luteal phase are important for maintaining pregnancy[11]. Moreover, a positive association exists between additional corpora lutea and the maintenance of pregnancy[12]. Low P_4 concentrations can lead to poor pregnancy and fertility rates[13]. Progesterone concentrations in ewes with luteal inadequacy remain below the level required to provide suitable uterine environment for normal embryo growth and development[9]. In addition, ewes with lower concentration of P_4 are more prone to embryonic loss, perhaps, as a result of insufficient maternal recognition of pregnancy signals[12]. It is during the second week of pregnancy that the changes in the uterine secretions are critical for embryo survival[11]. Since P_4 is essential for maintenance of pregnancy there would appear to be a rationale for its use to improve conception rates and to minimize early embryonic mortality[14]. Therefore, we hypothesize that post-mating P_4 supplement is important for improving pregnancy and lambing rates in anestrous ewes pretreated with intravaginal P_4 inserts for 12 days. The objective of this study was to determine whether or not post-mating P_4 supplement improves pregnancy and lambing rates of ewes bred out-of-season.

MATERIALS AND METHODS

Animals: The experiment was conducted during the months of May and June at the sheep unit at the Agricultural Center for Research and Production at Jordan University of Science and Technology (32°33'N, 35°51'E) located in the northern part of Jordan at an altitude of 510 m above sea level. Ewes age ranged from 3 to 5 years and had a body condition score of 2.5 to 3 (scale= 0 lowest to 5 highest, Russel[15] and weighed between 40 and 59 kg (Mean ± SEM = 47.3 ± 1.1). All ewes had previously lambed during the past lambing season and their lambs weaned at least 6 weeks before the start of the experiment. Ewes were housed in a single pen (18 x 6 m), one-third of which is sheltered and the south wall is open. During the experimental period, ewes were fed 1.2 kg wheat straw and 0.5 kg concentrate mixture per ewe per day and had free access to water, shade and mineral salt blocks.

Experimental design: Thirty-nine Awassi ewes were synchronized to estrus using intravaginal P_4 inserts (CIDR-G, Pharmacia & Upjohn n.v./s.a. Puurs, Belgium) containing 300 mg P_4. Inserts were inserted on May 27 and were removed 12 days later on June 8 at 0600. At the time of CIDR-G removal (day 0 and 0 hour) four Awassi rams fitted with marking harnesses were turned in with the ewes which were checked for breeding marks at 6-h intervals for 5 days. After five days of ram exposure, ewes were randomly assigned to four groups to be treated with intramuscular (i.m.) injections of 20 mg P_4 (Acros Organics, New Jersey, USA) supplement given once daily from day 5 to day 9 (P_4-D5-9), from day 10 to day 14 (P_4-D10-14), and from day 5 to day 14 (P_4-D5-14) or did not receive P_4 supplement (control group). Ewes from the four groups were all run together in one mob in a single pen with the four rams and no ram rotation was used. Transrectal ultrasonography was performed on day 30 for pregnancy diagnosis and confirmed on day 45 using transabdominal ultrasonography.

Blood sampling and hormone assay: Blood samples were collected via jugular venipuncture once on days-12 and-10 and once daily from day 0 until day 5 and then on alternate days thereafter until day 19 to compare P_4 concentrations among groups and for pregnancy diagnosis. All blood samples (5 ml each) were drawn into heparinized tubes (5 IU/ml blood) and centrifuged within 30 min of collection at 1500 g for 15 min. Plasma was pipetted and stored at –20ºC until assayed. Plasma P_4 concentrations were measured using a solid phase RIA kit containing antibody coated tubes and ^{125}I-labeled P_4 (Coat-A-Count kit, Diagnostic Products Corporation, DPC, Los Angeles, CA, USA). Sensitivity of the assay was 0.1 ng mL^{-1}. Intra-assay coefficient of variation (CV%) was 7.2%. The experimental protocols were performed according to the guidelines made by the Animal Use and Care committee at Jordan University of Science and Technology.

Statistical analysis: Data were analyzed using SAS/STAT ANOVA procedures[16]. Data in text, tables and figures are presented as means ± SEM. The effects of P_4 supplement following CIDR-G removal on pregnancy and lambing rate were analyzed using Chi-square test. Plasma P_4 concentrations were analyzed for the effect of post-mating P_4 supplement and time using the repeated-measures procedure of the GLM. Pregnancy rate was defined as the number of ewes bred

by the rams within 5 days following day 0 and became pregnant based upon sustained P_4 concentrations of ≥ 3 ng mL between days 15 and 19[17]. Lambing rate was defined as the proportion of ewes that became pregnant from mating at first service and lambed between 145 and 155 days following day 0.

RESULTS

Progesterone levels before CIDR-G removal and estrus responses: Mean initial plasma P_4 concentrations on day –12 were basal and averaged 0.2 ± 0.04, 0.3 ± 0.1, 0.4 ± 0.1 and 0.3 ± 0.1 ng mL^{-1} for the P_4-D5-9, P_4-D10-14, P_4-D5-14 and control groups, respectively. Following CIDR-G insertion plasma P_4 concentrations increased rapidly in all ewes and values on day –10 were 5.2 ± 0.2, 5.4 ± 0.1, 5.3 ± 0.2 and 5.5 ± 0.1 ng mL^{-1} for the P_4-D5-9, P_4-D10-14, P_4-D5-14 and control groups, respectively. Progesterone concentrations declined to day 0 values of 2.4 ±0.1, 2.4 ± 0.1, 2.3 ± 0.1 and 2.5 ± 0.2 ng mL^{-1} for the P_4-D5-9, P_4-D10-14, P_4-D5-14 and control groups, respectively. Plasma P_4 concentrations on days –12, –10 and 0 were not significantly different among groups ($p>0.05$). All ewes expressed estrus at similar intervals following CIDR-G removal among groups (Table 1).

Progesterone profiles following CIDR-G removal, pregnancy and lambing rates: Following day 0, plasma P_4 concentrations rapidly fell to ≤ 0.3 ng mL^{-1} within 24 h of CIDR-G withdrawal and remained basal through day 4. All ewes ovulated during this period based upon the subsequent rise in P_4 concentrations following CIDR-G removal. On day 5 (at the beginning of P_4 treatment), P_4 concentrations began to increase and did not differ ($p>0.05$) significantly among ewes of the four treatment groups and averaged 0.9 ± 0.1 ng mL^{-1}. Progesterone concentrations increased gradually thereafter until day 15 and differed significantly ($p<0.001$) by day among groups. Mean P_4 concentrations between days 5 and 15 were 4.94 ± 0.17, 4.78 ± 0.26, 6.70 ± 0.22 and 3.99 ± 0.17 ng mL^{-1} for groups P_4-D5-9, P_4-D10-14, P_4-D5-14 and control, respectively.

Concentrations of P_4 in the control group increased gradually until day 15 as those typically seen during the process of luteal development. Likewise P_4 concentrations in group P_4-D10-14 increased gradually until day 10 and then increased sharply until day 15.

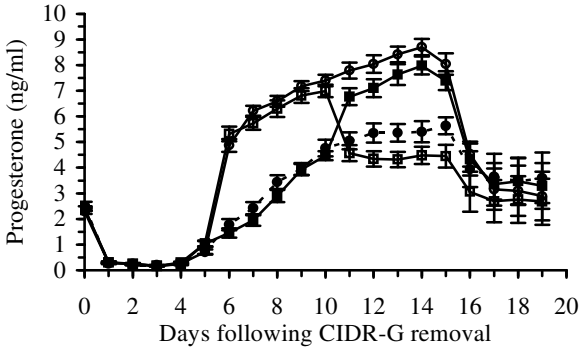

Fig. 1. Plasma P_4 profiles following CIDR-G removal in Awassi ewes treated with P_4 supplement from days 5 to 9 (P_4-D5-9, □), days 10 to 14 (P_4-D10-14, ■), days 5 to 14 (P_4-D5-14, ○), and control (●)

Table 1: Reproductive responses following CIDR-G removal in Awassi ewes treated during the seasonal anestrous period with 20 mg P_4 supplement between days 5 and 9 (P_4-D5-9 group), 10 and 14 (P_4-D10-14 group), 5 and 14 (P_4-D5-14 group) and control group

	Treatments			
Parameter	P_4-D5-9 (n=10)	P_4-D10-14 (n=10)	P_4-D5-14 (n=10)	Control (n=9)
Ewes expressing estrus	10/10	10/10	10/10	9/9
Interval to onset of estrus (h)	37.3 ± 2.5	36 ± 2.5	34.2 ± 2.5	35.3±2.7
Ewes pregnant (%)[1]	5 (50%)	6 (60%)	5 (60%)	5 (55.6%)
Ewes pregnant (%)[2]	5 (50%)	3 (30%)	3 (30%)	5 (55.6%)
Pregnancy loss (%)[2]	0/5 (0%)	3/6 (50%)	2/5 (40%)	0/5 (0%)
Embryonic/fetal survival (%)[3]	5/5 (100%)	3/6 (50%)	3/5 (60%)	5/5 (100%)
Ewes lambed /ewes exposed (%)[3]	5 (50%)	3 (30%)	3 (30%)	5 (55.6%)
Prolificacy[a]	1.0	1.0	1.0	1.0

[a] No. of lambs born live per ewes lambing
[1]Occuring based upon P_4 concentration between days 15-19 and transrectal ultrasonic examination on day 30
[2]Occuring based upon transabdominal ultrasonic examination on day 45
[3]Ewes lambing from mating at first service (145-155 days following day 0)

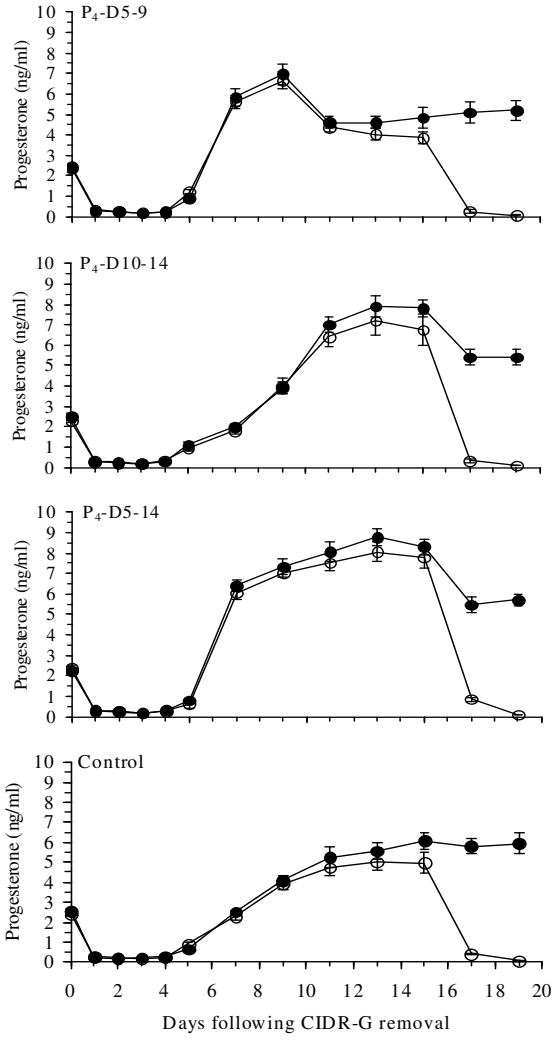

Fig. 2. Plasma P_4 profiles in P_4-D5-9, P_4-D10-14, P_4-D5-14 and control groups following CIDR-G removal in pregnant (●) and non-pregnant (○) ewes

Progesterone concentrations in groups P_4-D5-9 and P_4-D5-14 rose in a similar manner between days 5 and 10 due to treatment effect (Fig. 1). Maximum P_4 concentrations were reached on day 9 in group P_4-D5-9 and between days 11 and 15 in group P_4-D5-14. Concentrations of P_4 decreased after day 9 only in group P_4-D5-9 ewes and then were maintained from day 11 to day 15 at levels typical of those usually detected during normal luteal phase. Plasma concentrations of P_4 were greater ($p<0.001$) in P_4-D5-9 and P_4-D5-14 groups than P_4-D10-14 and control from day 5 to day 9. Between days 11 to 14, P_4 concentrations were significantly higher ($p<0.001$) in P_4-D10-14 and P_4-D5-14 groups than P_4-D5-9 and control (Fig. 1).

Progesterone concentrations remained elevated from day 15 through day 19 in 5/10, 6/10, 5/10, and 5/9 ewes for groups P_4-D5-9, P_4-D10-14, P_4-D5-14 and control, respectively, and these ewes were confirmed pregnant based upon ultrasonography performed on day 30. However, early signs of embryonic demise were evident in 3/6 and 2/5 ewes in groups P_4-D10-14 and P_4-D5-14, respectively. Pregnancy loss was confirmed to have occurred in these ewes later on day 45 by ultrasonography. Progesterone concentrations dropped spontaneously after day 15 in the remaining 5/10, 4/10, 5/10, and 4/9 ($p>0.2$) ewes of groups P_4-D5-9, P_4-D10-14, P_4-D5-14, and control, respectively (Fig. 2). These ewes were confirmed non-pregnant based upon ultrasonography on days 30 and 45. Of the 21 ewes that became pregnant from mating at first service, 16 lambed 149.4 ± 0.3 days following day 0 and were 5/10, 3/10, 3/10 and 5/9 ewes of groups P_4-D5-9, P_4-D10-14, P_4-D5-14 and control, respectively (Table 1). Embryonic survival rates were 100, 50, 60 and 100% for groups P_4-D5-9, P_4-D10-14, P_4-D5-14 and control, respectively. There was no significant difference ($p>0.05$) among ewes of the four treatment groups in the number of ewes that became pregnant or lambed. The number of lambs born live per ewes lambed was similar among groups and was not influenced by P_4 supplement (Table 1). The overall pregnancy and lambing rates were 53.8 and 41%, respectively.

DISCUSSION

As expected, post-mating plasma P_4 concentrations increased during the periods of treatment between days 5 and 9, 10 and 14 and 5 and 14 for groups P_4-D5-9, P_4-D10-14 and P_4-D5-14, respectively. The number of ewes failing to establish pregnancy was similar among P_4 groups. The overall pregnancy (53.8%) and lambing (41%) rates obtained in this study, do not suggest direct involvement of P_4 supplement, rather it may be considered as a normal outcome of the synchronization protocol (CIDR-G for 12 days) used since no gonadotropin treatments were incorporated. The acceptable pregnancy rates obtained in the present

study using CIDR-G were better than those previously reported using 500 mg P_4 sponges[18]. The use of CIDR-G in estrus synchronization in sheep has been shown to improve estrus responses and pregnancy rates compared with progestagen sponges[19,20].

The experimental hypothesis was that P_4 supplement post-mating between days 5 and 14 is crucial for improving embryonic survival in ewes pretreated out-of-season with exogenous P_4 for 12 days. Results of the present study did not support the experimental hypothesis in that the P_4 supplement did not improve pregnancy and lambing rates. No differences were found between groups supplemented with P_4 and the control and all groups showed similar reproductive performance pre- and post-mating. Notably, P_4 supplement in P_4-D10-14 and P_4-D5-14 groups, although not significant, numerically decreased the number of ewes lambing compared with the two other groups. On the other hand, the P_4 supplement from day 5 to day 9 post-mating (group P_4-D5-9) did not affect lambing rate and was similar to the control.

Results of the present are in agreement with those reported in sheep[3,21,22], cattle[23] and women[24], which showed no benefit of using post-mating P_4 supplement in improving or minimizing early embryonic losses. According to McMillan[22], P_4 supplement using intravaginal inserts from day 7 to day 14 post-mating did not improve pregnancy rate in mature ewes but increased litter size by 23%. In contrast, other researchers indicated that the use of intravaginal P_4 inserts has a potential of reducing the incidence of pregnancy loss during the early fetal period in dairy cattle[24]. Villarroel and coauthors[26] showed that intravaginal P_4 supplement administered from day 5 to day 9 post insemination prevented pregnancy losses in dairy cows. In hoggets, P_4 supplement using CIDR-G from day 7 to day 14 post-mating positively affected both pregnancy rate and litter size which increased by 17 and 40%, respectively[22].

Based on the results obtained in the present study and those reported in the literature, it would be advantageous to note that P_4 supplement has been administered by means of injections, implants, oral or intravaginal inserts. In this regard, fertility rates were improved when P_4 supplement was administered intravaginally rather that orally, intramuscularly or by implants. Therefore, it seems likely that vaginal deposition of P_4 was superior to other routes[26]. These authors indicated that vaginal administration of P_4 could have acted to increase P_4 levels in uterine arteries by means of a counter-current transfer from the vaginal and cervical venous drainage into the corresponding arterial blood. Such application results in avoidance of first pass metabolism in the liver and in preventing sustained high plasma concentration of P_4[28]. In the present study, P_4 was administered intramuscularly and its influence may have been predominantly to raise P_4 concentrations in the systemic circulation. Approximately 90% of P_4 in hepatic portal blood is metabolized during the first pass through the liver[29]. Moreover, P_4 administration by mean of injections results in its accumulation in the fat tissues within the muscles, resulting in more sustained serum P_4 concentrations after injection[26]. However, vaginal administration of P_4 seems to disappear more rapidly from the circulation than intramuscular administration[27].

Various other reproductive parameters were examined for differences in the overall ewes that did (groups P_4-D5-9, P_4-D10-14 and P_4-D5-14; n=30) or did not (control; n=9) receive P_4 supplement. Results of the present study indicated similar overall pregnancy (16/30 {53.3%} versus 5/9 {55.6%}) and numerically different but not significant lambing rates (11/30 {36.7%} versus 5/9 {55.6%}) among groups receiving P_4 supplement versus the control group, respectively. Progesterone supplement in groups P_4-D10-14 and P_4-D5-14 tended (p=0.1) to negatively affect lambing rates. In fact, of the ewes that became pregnant in groups P_4-D10-14 and P_4-D5-14, 3/6 (50%) and 2/5 (40%), respectively, lost their embryos and did not lamb. Notably, the reduction in pregnancy occurred only in groups P_4-D10-14 and P_4-D5-14 (shared P_4 supplement during the overlap period between days 10 and 14) but not in groups P_4-D5-9 and control. In this regard, P_4 concentrations in group P_4-D5-9 declined after day 10 and then approached their corresponding typical (sustained) luteal phase values between days 11 and 15. On the other hand, for groups P_4-D10-14 and P_4-D5-14 which had P_4 supplement through day 14, P_4 levels sharply declined after day 15 and may have been responsible for initiating embryonic loss process in some ewes (Fig. 1). Interestingly, the overlap period of P_4 supplement in groups P_4-D10-14 and P_4-D5-14 corresponds well with period of maternal recognition, which takes place about days 12-13 of pregnancy[30]. Maintaining high P_4 levels during this period of time is critical for establishment of pregnancy by the maternal

recognition process. More specifically, the concentration of P_4 in maternal blood must be sustained at a high level in order that the endometrium is maintained in a state conducive to embryonic survival[11].

Thus, pregnancy diagnosis based on P_4 levels and ultrasonography revealed that the process of pregnancy loss started before day 30 since ultrasonic examination performed on this day demonstrated early signs of embryonic death. Authors suggest two factors attributing to pregnancy loss; the sharp decline in P_4 after the end of P_4 supplement and stress imposed on ewes due to handling and treatment in groups P_4-D10-14 and P_4-D5-14 between days 10 and 14. Stressful conditions result in elevated levels of cortisol[31,32] and have been associated with decreased reproductive responses. Doney et al.[33] provided evidence of decreased pregnancy rates due to stress through exposing ewes to stressful conditions.

In conclusion, P_4 supplement administered intramuscularly between days 5 and 14 post-mating is not effective in improving pregnancy, embryonic survival and lambing rates in Awassi ewes pretreated out-of-season with CIDR-G for 12 days. Factors attributing to pregnancy loss may include the sharp decrease in P_4 concentrations on day 15 and stress experienced during the period of maternal recognition. The overall acceptable pregnancy and lambing rates obtained are normal outcome of using the 12-day CIDR-G estrus synchronization protocol out-of-season. Further studies are needed to ascertain the negative impact of the sharp decline in P_4 and stress during the period of maternal recognition and their interaction on pregnancy and lambing rates.

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