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The Effect of PMSG on Follicular Development and Ovulation Time of $PGF_{2\alpha}$ Treated Awassi Ewes

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Abstract: The objective was to examine the effects of PMSG administration before or at the time of $PGF_{2\alpha}$ injection on reproductive responses of ewes out-of-season. In late June and early July, 57 anestrus ewes were induced to estrus using CIDR-G devices for 12 days. Six days following device removal ewes were randomly allocated into three treatment groups and each received an i.m injection of 20mg $PGF_{2\alpha}$ (day 0, 0 h). PMSG (500 IU) was administered either 24 h before (group A, n=20) or at (group B, n=19) the time of $PGF_{2\alpha}$ injection. Group C ewes (n=18) served as control. Half of the ewes in each group were exposed to three intact rams at 0 h to be naturally mated and the other half were exposed to three aproned rams and inseminated 50-56 h following 0 h. Ewes were checked for breeding marks at 6-h intervals for 4 days. Progesterone levels from day –1 until day 20 were monitored. Occurrence of estrus was similar among ewes of the three treatment groups and averaged 79%. PMSG-treated ewes had shorter ($P<0.01$) intervals to estrus and ovulation, and a higher ovulation rate than non-PMSG treated ewes. Pregnancy and lambing rates were similar among PMSG- and non-PMSG-treated ewes. Although reproductive responses were similar among artificially-inseminated and naturally-mated ewes, the latter had a higher ($P<0.05$) lambing rate (14.3% vs 41.4%, respectively). Ewes inseminated close to the time of ovulation (7.7 h earlier) produced higher ($P<0.05$) pregnancy rate than those inseminated at a wider interval from ovulation (16.7 h earlier). In conclusion, the $PGF_{2\alpha}$ treatment given during luteal phase was effective in resetting subsequent cyclic activity of ewes. Although it did not increase the number of ewes detected in estrus, PMSG shortened intervals to estrus and ovulation, increased ovulation and induced–estrus pregnancy rates.

Keywords: Awassi, PMSG, $PGF_{2\alpha}$, Follicle, Ovulation, ewes

Introduction

Induced-estrus pregnancy rates of < 40% have been reported out-of season for Awassi ewes using different estrus synchronization protocols (Husein & Kridli, 2002a). Several studies have been conducted to determine reasons responsible for low induced-estrus pregnancy rates (Husein & Kridli, 2002b; Abdullah et al., 2002). These authors reported some contributing factors, which include drought, environmental stress and ram inexperience. Other factors may have been season, temperature, disease, nutrition, management, semen quality, artificial insemination method, insemination time, synchronization protocols and the reproductive condition of the ewe (Husein et al., 1996; Husein

et al., 1998). Although the use of various methods of estrus synchronization utilizing progesterone and PMSG produced a little improvement, the overall fertility rate was still reduced (Husein & Kridli, 2002a).

In sheep, administration of the exogenous $PGF_{2\alpha}$ in cyclic ewes between days 4 and 14 of the estrous cycle causes corpus luteum regression and allows a new follicular phase to start and subsequent return to estrus within 2-3 days (Chamley et al., 1972). The use of $PGF_{2\alpha}$ is limited in anestrous ewes because they do not possess active corpora lutea. The intention of this study, therefore, was to use animals of similar stage of the estrous cycle for an attempt to minimize variation in reproductive responses among ewes. Variable fertility has been reported among ewes induced to estrus using $PGF_{2\alpha}$ alone or any of its analogues (Gordon, 1997). However, when PMSG was incorporated into a $PGF_{2\alpha}$ protocol, estrus exhibition was improved and intervals to onset of estrus were shortened (Trounson et al., 1976) and pregnancy rate and incidence of multiple births were increased (Madani et al., 1984). The objective was to examine the effects of PMSG administration before or at the time of $PGF_{2\alpha}$ injection on reproductive responses of ewes out-of-season.

Materials and Methods

Animals

In late June and Early July, 57 anestrus Awassi ewes weighing 49.0 ± 1.0 kg were used in an experiment conducted at the Agricultural Center for Research and Production at Jordan University of Science and Technology (32°33'N, 35°51'E). All ewes had previously lambed at least once and their last lambing dates ranged from November 6 to January 23. Ewes were offered a diet of 1.2 kg wheat straw and 0.5 kg concentrate mixture (soybean, corn, bran and barley) per ewe per day. Mineral blocks and water were available on an ad libtium basis.

Experimental Design

Ewes were induced to estrus using a 12-day CIDR-G protocol and randomly allocated into three treatment groups in a completely randomized design 6 days after CIDR-G removal. Each was given a 20 mg i.m injection of $PGF_{2\alpha}$ (lutalyse, Pharmacia and Upjohn n.v./s.a. Puurs, Belgium) on July 2 (day 0, 0 h). Ewes in groups A (n=20) and B (n=19) received a single i.m injection of 500 IU PMSG (Sanofi Animal Health, Libourne Cedex, France) 24 h before and at the time of $PGF_{2\alpha}$ injection, respectively. Ewes in group C (n=18) did not receive PMSG and served as control. Ewes in each group were divided into two subgroups and were either naturally-mated (n=29) or artificially-inseminated (n=28). The naturally-mated or artificially-inseminated ewes were isolated into two separate adjacent pens. Immediately after $PGF_{2\alpha}$ injection, three intact and three aproned Awassi rams were turned-in with naturally-mated and artificially-inseminated (AI) ewes, respectively. Ewes were checked for breeding marks at 6-h intervals for 4 days (Figure 1). The AI group ewes were inseminated 50-56 h following 0 h using the Guelph (T-AI) equipment. Average number of spermatozoa per straw was approximately 500 x 10^6.

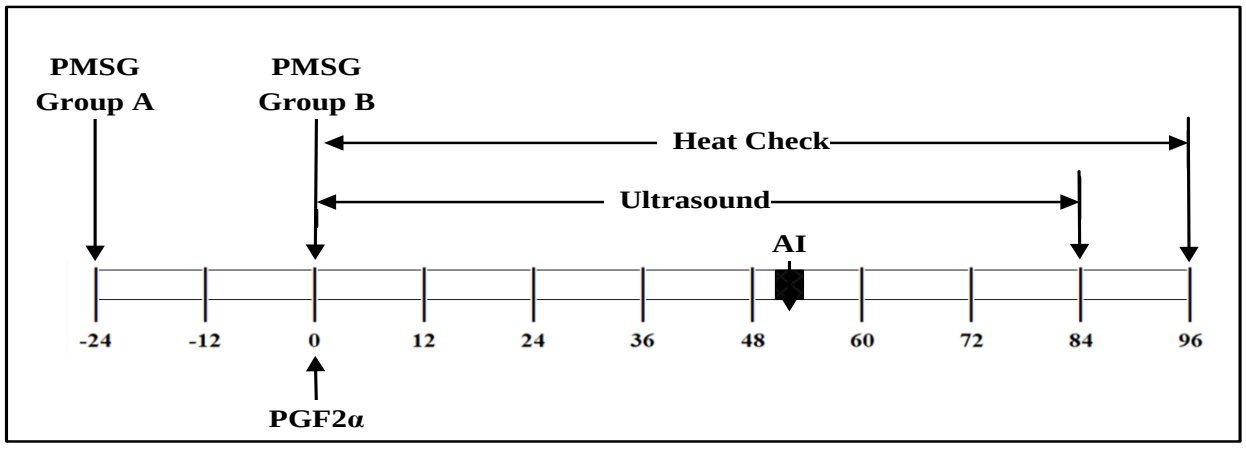

Figure 1. Time line for the treatment groups

Blood Sampling and Progesterone Assay

Blood samples were collected via jugular vein puncture once daily from day −1 until day 6 and on alternate days thereafter until day 20 to verify comparison in progesterone concentrations among treatments and for pregnancy diagnosis. Blood samples (5 ml each) were collected in heparinized tube (5 IU/ml blood) and centrifuged soon after at 3000 rpm for 15 min. Plasma was pipetted and stored at −20 °C until assayed by RIA using Coat-A-Count kit (Diagnostic Products Corporation, DPC, Los Angeles, CA). Sensitivity was 0.1 ng/ml and intraassay CV was 3.4%.

Statistical Analysis

Data were analyzed using SAS/STAT ANOVA procedure (SAS, 2006). Means ± SE are presented in text and tables unless otherwise noted. Effect of PMSG of treatments on incidence of estrus, ovulation, pregnancy, and lambing were analyzed using "Chi square" test. Onset of estrus was considered to have occurred 3 h before observation of the breeding mark. Time to ovulation was considered to have occurred 6 h before disappearance of the large follicles. Effect of PMSG treatments on various intervals were analyzed using least square means (LSM) procedure of the general linear model (GLM). Progesterone levels were analyzed for the effect of treatment and time using the repeated measure procedure of the GLM.

Results

Estrus occurrence did not differ ($P > 0.1$) among groups and was detected in 16/20 (80%), 16/19 (84.2%) and 13/18 (72.2%) ewes in groups A, B and C, respectively. Intervals from $PGF_{2\alpha}$ injection to onset of estrus were shorter ($P < 0.01$) in ewes of groups A (32.4±1.3 h), and B (32.8±1.3 h) than ewes in group C (47.1±1.5 h) (Table 1). Ovulation occurred earlier ($P < 0.01$) in groups A (58.8±2.9 h) and B (61.2±2.9 h) than that (75.0±2.3 h) of group C ewes, with no difference among ewes of groups A and B. Intervals from onset of estrus to ovulation were similar ($P > 0.1$) and averaged 27.9±1.4 h among ewes of the three treatment groups (Table 2).

Table 1. Reproductive responses following $PGF_{2\alpha}$ injection in Awassi ewes treated with PMSG 24 h before (group A), at the time of $PGF_{2\alpha}$ injection (group B) and control (group C)

Parameter	Treatment		
	Group A	Group B	Group C
Number of ewes	20	19	18
Ewes detected in estrus	16 (80%)	16 (84.2%)	13 (72%)
Interval to onset of estrus (h)	32.4 ± 1.3^{a}	32.8 ± 1.3^{a}	47.1 ± 1.5^{b}
Ewes pregnant	11 (55%)	10 (52.6%)	6 (33.3%)
Ewes lambed	4 (20%)	9 (47.4)%	3 (16.7%)

[a, b] Means within row with different superscripts differ ($P < 0.01$)

Table 2. Ovulatory responses following $PGF_{2\alpha}$ injection in naturally-mated Awassi ewes treated with PMSG 24 h before (group A), at the time of $PGF_{2\alpha}$ injection (group B) and control (group C)

	Treatment		
Parameter	Group A	Group B	Group C
Number of ewes	5	5	4
Ewes ovulated	5/5	5/5	4/4
Interval to onset of estrus (h)	31.8 ± 2.4^{a}	32.4 ± 2.4^{a}	47.2 ± 2.7^{b}
Ovulation (h)	58.8 ± 2.9^{a}	61.2 ± 2.9^{a}	75.0 ± 3.2^{b}
Interval from onset of estrus to ovulation (h)	27.0 ± 2.7	28.8 ± 2.7	27.8 ± 3.0
Ovulation rate	1.6 ± 0.2	1.6 ± 0.2	1.0 ± 0.2

[a, b] Means within row with different superscripts differ ($P < 0.01$)
[c, d] Means within row with different superscripts differ ($P < 0.08$)

Number of follicles greater than 5 mm in diameter at 0, 12, and 24 h was similar ($P > 0.1$) among ewes of the three treatment groups. However, number of large follicles at 36 and 48 h was greater ($P < 0.01$) in groups A and B than

ewes in group C. Fewer large follicles were observed at 60 h in groups A and B and no follicles were seen by 72 h. In group C, the number of large follicles dropped after 84 h. Based upon ultrasonic examination, ovulation rate tended (P = 0.08) to be lower in ewes of group C (1.0 ± 0.2) than those of groups A and B (1.6 ± 0.2 and 1.6 ± 0.2, respectively) (Figure 2).

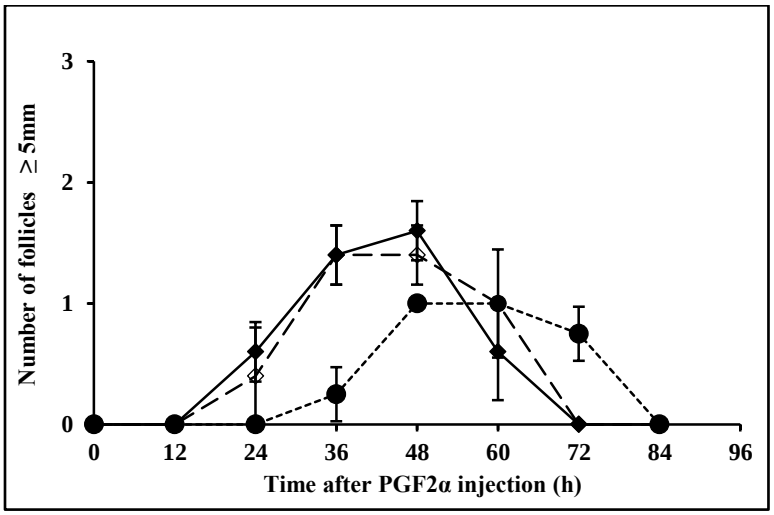

Figure 2. Number of large follicles in groups A (PMSG 24 h before) (♦), B (PMSG at the time) (◊), and C (Control) (●) after PGF2α injection.

Mean plasma progesterone concentrations at the time of $PGF_{2\alpha}$ injection (0 h) were similar (P > 0.5) and averaged 4.2 ± 2.0 ng/ml among ewes of the three treatment groups. Following 0 h, progesterone concentrations rapidly fell in all ewes to ≤ 0.5 ng/ml within 24 h and remained low until day 4. Progesterone concentrations increased gradually after day 4 in all ewes. Apparently, ovulation occurred in all ewes during this period based upon the subsequent rise in progesterone concentrations.

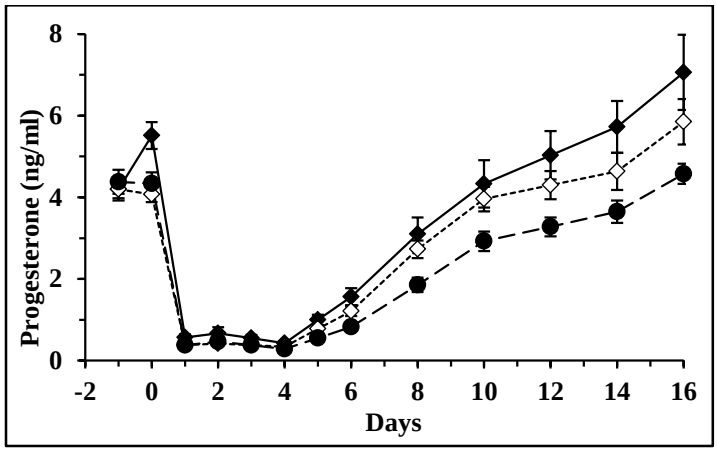

Figure 3. Plasma progesterone concentrations in groups A (PMSG 24 h before, n=20) (♦), B (PMSG at the time, n=19) (◊), and C (Control, n=18) (●) following PGF2α injection until day 16.

Maximum progesterone concentrations were reached on day 16 and averaged 7.1 ± 0.9, 6.0 ± 0.5, and 4.2 ± 0.2 ng/ml for ewes of the three treatment groups (A, B and C, respectively). The PMSG-treated (groups A and B) ewes had higher (P < 0.05) progesterone concentrations between days 4 and 16. Progesterone concentrations remained elevated through day 20 in 11/20 (55%), 10/19 (53 %) and 6/18 (33%) ewes of groups A, B and C, respectively (Table 1). Progesterone concentrations after day 16 dropped spontaneously in 9/20 (45%), 9/19 (47%) and 12/18 (67%) ewes of groups A, B and C, respectively, (Figure 4). After 151 days had elapsed, 4/20 (20%), 9/19 (47%) and 3/18 (17%) of ewes had lambed in groups A, B and C, respectively (Table 1).

Discussion

In the present study, both PMSG treatment 24 h before or at the time of $PGF_{2\alpha}$ injection produced similar estrus responses. Shorter intervals to onset of estrus in these ewes are attributed to faster endocrine responses following PMSG treatment. Earlier endocrine responses in PMSG-treated ewes are the outcomes of earlier estrogen production from the growing follicles (McNatty et al., 1982; Baby et al., 2011; Bartlewski et al., 2011). It is believed that PMSG can markedly increase aromatase activity (Brandt et al., 1988). Similarly, treatment with PMSG was effective in shortening the interval from $PGF_{2\alpha}$ injection to ovulation by about 15 h. These results resembled those reported by Trounson et al. (1976). Quinlivan. (1980) reported that PMSG treatment at the time of progesterone withdrawal resulted in shorter interval to ovulation. Reduced intervals to ovulation have been attributed to the rapid maturation of follicles induced by PMSG prior to the regression of the corpus luteum (Trounson et al., 1976).

Examination of data (Table 2) showed that ovulation occurred in a constant interval from the onset of estrus. Consistency in this interval is due to the fact that LH surge occurs at the onset of estrus (Husein et al., 1997; Shackell et al., 1991; Quirke et al., 1981) as a result of estradiol positive feedback (Khalid et al., 1991) and to the constant interval (21-26) between the preovulatory LH surge and ovulation (Cumming et al., 1973; Baby et al., 2011; Bartlewski et al., 2011).

Treatment with PMSG resulted in earlier appearance of large size (≥5mm) follicles in PMSG- than in non-PMSG-treated ewes. Similar advancement in the time of emergence of large follicles in response to PMSG treatment has been reported (McNatty et al., 1982; Husein et al., 1998a). Advancement in the time of appearance of large follicles has been attributed to the antiatretic effect of PMSG, which results in recruitment of more than one follicle and development of many preovulatory estrogenic follicles from the extent of large follicles pool (McNatty et al., 1982; Junqueira et al., 2019).

Treatment with PMSG at or 24 h before the time of $PGF_{2\alpha}$ injection tended to increase ovulation rates compared to treatment with $PGF_{2\alpha}$ alone. Higher ovulation rates with PMSG treatment have been reported (Henricks and Hill, 1978; Noël et al., 1994; Husein et al., 1998a). This has been attributed to the fact that PMSG has an FSH like activity and results in more follicles to grow and develop (Gordon, 1997a; Baby et al., 2011; Bartlewski et al., 2011).

Mean plasma progesterone concentrations prior to $PGF_{2\alpha}$ injection were similar among ewes of the three treatment groups. Elevated concentrations of progesterone in ewes are a reflection of their cyclicity. After $PGF_{2\alpha}$ injection, concentrations of progesterone fell to ≤ 0.5 ng/ml within 24 h. Thereafter, progesterone concentrations started to increase gradually after day 4. The increase in plasma progesterone concentrations at that time indicates that all ewes had ovulated irrespective to whether or not they exhibited estrus.

Progesterone concentrations in PMSG-treated ewes (Group A and B) were similar during the entire period of the estrous cycle. Progesterone concentrations declined to ≤ 0.5 ng/ml and remained low until day 4. This indicates that $PGF_{2\alpha}$ was effective in inducing luteolysis of corpora lutea, which allowed all ewes to ovulate. After day 4, progesterone concentration started to increase in both groups A and B indicating that new corpora lutea formed and started to secrete progesterone. Progesterone concentrations among ewes of the three treatment groups continued to rise until those reached maximum concentrations on day 16. Higher progesterone concentrations in PMSG-treated ewes (Groups A and B) during the luteal phase has been related to the action of PMSG in advancing the time of ovulation (Jabbour & Evans, 1991) and in producing multiple corpora lutea through increasing ovulation rate (Henricks and Hill, 1978). The overall pregnancy rate was 47.4% for ewes of the three treatment groups and was lower than that reported by Trounson et al. (1976).

Pregnancy rates in this study were higher than those reported earlier in the JUST sheep flock (Husein & Kridli, 2002a, 2002b). Haynes and Haresign (1987) reported that running rams with ewes prior to ram introduction in a breeding program increased the fertility of the ewes. Breeding ewes at the second cycle improved pregnancy rates since the reproductive condition of the ewe at that time may be set better. In addition, ewes treated were of similar stage of the estrous cycle which allows greater proportion of ewes to be in approximately similar or uniform stages of follicular development (Beck et al., 1996).

Uniformity in follicular growth and development enhances pregnancy rate. Proportions of ewes lambed were 20%, 47%, and 17% in groups A, B, and C, respectively. Reasons for overall low lambing rate are due to the fact that half of the ewes were artificially inseminated. Other contributing factors will be discussed in the following part of discussion (subheading 5.5.). Lambing rate in artificially-inseminated ewes was 14.3% compared to 41.4% for the naturally-mated ewes. This could be attributed to the fact that some ewes may have been traumatized due to the T-AI technique.

Conclusion

Results indicate that $PGF_{2\alpha}$ injection during luteal phase (2 to 5 day old corpus luteum) can be effectively used to induce estrus irrespective to whether or not eCG treatment is given. However, eCG administration 24 h before or at the time of $PGF_{2\alpha}$ injection shortened the intervals to onset of estrus and ovulation. eCG treatment resulted in higher progesterone concentrations during luteal phase. Although pregnancy and lambing rate were not influenced by eCG treatment, ewes inseminated near to the time of ovulation had higher pregnancy and lambing rates.

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

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