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Progesterone concentration and lambing rate of Karakul ewes treated with prostaglandin and GnRH combined with the ram effect during breeding and non-breeding seasons

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ABSTRACT

The combination of ram effect with two injections of $PGF_{2\alpha}$ 10-days apart and the same protocol plus an additional injection of GnRH prior to the first injection of $PGF_{2\alpha}$ were examined in Karakul ewes during breeding and non-breeding seasons, respectively. Plasma progesterone (P_4) concentrations (to detect the presence of active corpus luteum), twin lambing, litter size and synchronization of lambing were evaluated. In each study 70 ewes (2–4 years old) were divided to a treatment (n = 40) and a control (n = 30) group. During the breeding season, on days −10 and 0 before ram release, the treatment group was injected intramuscularly with $PGF_{2\alpha}$ (D-Cloprostenol; 0.15 mg). During the non-breeding season, on day −15 before ram release the treatment group was injected with GnRH (buserelin; 4.2 μg) intramuscularly followed by two injections of $PGF_{2\alpha}$ on days −10 and 0. In both studies, the rams were released into the ewe flock after the second prostaglandin injection (day 0). Blood samples of ewes were collected on days −10, 0, 20 and 70 of the study in breeding season and on days −15, −10, 0, 20 and 70 during non-breeding season. The treatment group had higher P_4 concentrations compared to the control ewes on day 0 in the breeding season (5.80 ± 0.61 vs. 5.0 ± 0.93 ng/mL) and day −10 in the non-breeding season (3.50 ± 0.33 vs. 2.70 ± 0.35 ng/mL) though the differences were not significant ($P > 0.05$). Based on plasma P_4 concentrations (>1 ng/mL) on day 70, in the breeding season all control ewes (100%) and 91.9% of the treatment ewes were detected to have active corpus luteum ($P = 0.09$). An almost inverse result (90% vs. 97.5%; $P = 0.2$) was detected in the non-breeding season. The lambing rate was higher ($P = 0.03$) in the treatment group compared to the control ewes during the non-breeding season (90% vs. 70%), but tended to be lower ($P = 0.07$) in the breeding season (73% vs. 90%). Twin lambing rate was higher in the treatment group compared to the control ewes in the breeding (40.7% vs. 0.0%; $P < 0.05$) and non-breeding (22.2% vs. 0.0%; $P < 0.05$) seasons. The litter size of the control and treated ewes were 1.0 ± 0.0 vs. 1.40 ± 0.10 in the breeding and 1.0 ± 0.0 vs. 1.22 ± 0.10 in the non-breeding season ($P < 0.05$). No effect was observed regard to synchronization of the treated ewes. In the breeding season two injections of $PGF_{2\alpha}$ ten days apart combined with ram effect, may lower the lambing rate, but may enhance twin pregnancies and litter size in Karakul ewes. In the non-breeding season, however, the GnRH-$PGF_{2\alpha}$ treatment plus ram effect may enhance the lambing rate, twin pregnancies and litter size.

1. Introduction

Ewes exhibit seasonal reproduction in response to the shortening the day-length during summer or early autumn. The non-breeding season begins in late winter and ends in early- or mid-

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summer [1]. The increasing need for conserving land resources necessitates some methods to increase the reproductive functions during the breeding season or to induce them during the non-breeding season. The ram effect increases LH secretion in cycling ewes and is used for induction of ovulation in breeding season [1,2]. Induced estrous and ovulation could also be achieved by injection of progestagen [3] and prostaglandin [4]. Numerous studies have investigated the administration of hormones such as progesterone (P4) or its analogues, prostaglandin (PG) and gonadotropin releasing hormone (GnRH) to modify the luteal phase of the estrous cycle with considerable effects on reproductive performance of ewes [5–10].

Controlled internal drug release (CIDR) device and intravaginal sponges are two main intravaginal devices that mimick the activity of corpus luteum and are used for ewes' estrous synchronization [1,11]. Progestagen-based protocols, however, have environmental contamination and are not clean, green and ethical [6,12,13]. Induction of luteolysis by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is an alternative method to manage flock reproduction especially in the breeding season [14] and a possible tool for reproductive management [15]. Two doses of $PGF_{2\alpha}$ injected to cycling ewes at a 9-day interval induced estrus in 95% of treated animals within 72 h after the second injection [16]. Almost all ewes respond to the second injection of $PGF_{2\alpha}$ because they are most probably in mid-luteal phase at that time [1].

The injection of GnRH can result in ovulation by stimulating LH release [17]. Beck et al. [18] reported a high fertility rate in ewes during breeding season following GnRH injection five days prior to $PGF_{2\alpha}$ treatment. A high pregnancy rate was obtained in anoestrous ewes treated with progesterone-GnRH-$PGF_{2\alpha}$ [19]. Exogenous GnRH immediately prior to a short-term (7d) progesterone treatment at the onset of breeding season increased fertility rate, multiple births and litter size [8]. The use of GnRH in combination with other hormones including P4 has been reported in several studies with increased prolificacy of reproductive performance of ewes [8,9,20]. Mirzaei et al. [21], however, reported that the treatment with GnRH analogue (buserelin) combined with the male effect negatively affected the plasma P4 concentration during the transition period from anoestrus to the natural breeding season. Jordan et al. [20] reported that formation of corpus luteum, estrous response and pregnancy rate tended to be greater in the ewes treated with GnRH than those exposed to rams only during seasonal anestrus.

In the present study we examined 1) combination of ram effect with two injections of $PGF_{2\alpha}$ 10-days apart in breeding season and 2) the same protocol plus an additional injection of GnRH prior to the first injection of $PGF_{2\alpha}$ during non-breeding season in Karakul ewes under the field condition.

2. Materials and methods

2.1. Animals

Two sets of study were conducted during breeding (October to February) and non-breeding (May to September) seasons in a Karakul flock consisting of 350 ewes in Saadat Shahr, Fars province, Iran (latitude 30° 3′ N; longitude 53° 7′ E; height 1892 m). In each study 70 ewes were randomly divided to a treatment (n = 40) and a control (n = 30) group. Body condition score (BCS) of the ewes was evaluated by palpation of their back (1–5 points, 0.5-point intervals) [22]. The age of the ewes were obtained from the farm's records and examining the teeth formula of the animals [22]. The ewes were stained on their back for easy recognition among the rest of the flock.

The ewes grazed on the existing pastures and stubbles during the corresponding season: medium-to low-quality forages with slightly higher quality forages during the non-breeding season (late spring). The animals were nutritionally flushed for about 3 weeks before breeding. The flushing ration (in addition to grazed feeds) was a mixture of alfalfa hay (23%), corn silage (67%) and barley grain (10%) ad libitum and was extended to the first month of the breeding period. White salt and a trace mineralized supplement lick containing 19.6% calcium and 9.6% phosphorus were also available free choice. The ewe flock was kept away from the rams for about 2 months before the breeding period. One week prior to the introduction of the rams, the ewes and rams were kept in close vicinity of one another at afternoons and nights, separated by fences.

2.2. Treatments

During the breeding season, on days −10 and 0 before ram introduction, the treatment group (n = 40) was injected intramuscularly with 0.15 mg of prostaglandin (D-Cloprostenol) and the control group (n = 30) was injected with distilled water as placebo (injections on October 8 and October 18). Three ewes of treatment group were lost during the study and the final number of the ewes in this group was 37. During the non-breeding season, on day −15 before ram introduction the treatment group (n = 40) was injected with 4.2 μg of GnRH analogue (buserelin) intramuscularly followed by two injections of 0.15 mg of prostaglandin (D-Cloprostenol) on days −10 and 0 before ram introduction. The control group (n = 30) received distilled water as placebo on the same days (injections on May23, May 28 and June 6). In both studies, the rams were released into the ewe flock after the second prostaglandin injection (day 0). Twenty fertile rams, 4–5 years old, selected based on their scrotal girth were released into the whole ewe flock (n = 350) for 50 days.

2.3. Samplings and experiments

The ewes were sampled for blood on days −10, 0, 20 and 70 before and after ram introduction in breeding season and on days −15, −10, 0, 20 and 70 during non-breeding season (Fig. 1). Blood samples were collected by jugular venipuncture into citrate containing tubes. Plasma was separated by centrifugation at 750×g for 15 min and was stored for further analyses at −20 °C. Plasma P4 concentrations were determined using an ELISA kit (DRG Instruments GmbH, Germany), which detected concentrations as low as 0.045 ng/ml, recording intra- and inter-assay coefficients of variation of 6.86 and 5.59%, respectively.

The ewes with active corpus luteum (CL) were detected based on P4 concentrations>1 ng/mL [23,24] on days 20 and 70 after ram release. The lambing performance in all groups was determined using the lambing data recorded by the farmer. Litter size (number of lambs born per ewe lambed) were calculated using the farm's data to evaluate twin lambing.

2.4. Statistical analysis

Data were analyzed at $P \leq 0.05$ using the SPSS statistical software (Version 15.0, SPSS Inc, Chicago, Illinois). The rates of lambing and single and twin births of the control and treatment groups during the breeding and non-breeding seasons were analyzed using Chi-square test; Fisher correction was used in cases that the counts were <5. The percentages of the ewes with active CL (P4>1 ng/mL) on days 20 and 70 after ram release were compared by the same tests. The means (±SEM) of plasma P4 at different times of sampling and the litter size were compared between studied groups with one-way analysis of variance (ANOVA).