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Effect of Ram Introduction in Combination with Progesterone or Cloprostenol on Estrus Induction Rates of Konya Merino Ewes in the Anestrous Season

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Contents

The effect of ram introduction, either alone or in combination with progesterone or cloprostenol applications, on estrus occurrence and pregnancy rates of Konya Merino ewes in anestrous season was investigated. The study was carried out on a total of 100 ewes and 12 rams. Ewes were divided into four groups in June, near the end of the anestrous season. Weekly blood samples were collected to detect the proportion of ewes undergoing cycling activity in group I during the study. Ewes in group II, III and IV were exposed to rams for 4 days; four rams for each group were used. In addition to this, ewes in group III were injected with 20 mg of progesterone 5 days before the ram introduction and ewes in group IV were injected with 125 µg of cloprostenol 13 days after ram introduction. Cloprostenol injections were repeated on day 21 for ewes which did not display estrus in group IV. In all groups, estrous ewes were detected by teaser rams and allowed to mate. Although the proportion of ewes undergoing cycling activity during the study was around 15.38-23.07%, estrus and pregnancy rates in groups II, III and IV were 44%, 87.5% and 56% for estrus and 32%, 66.7% and 44% for pregnancy, respectively. As a result, it was concluded that the combination of ram introduction and progesterone application is an effective method for the induction of estrus in Konya Merino ewes in the non-breeding season.

Inhalt

Brunstinduktion durch Anwesenheit eines Bockes in Kombination mit Progesteron oder Cloprostenol in der anöstrischen Saison bei Konya-Merinoschafen. Es wurde bei anöstrischen Konya-Merinoschafen eine Brunstinduktion durch Stimulierung mit einem Schafbock in Kombination mit Progesteron oder Cloprostenol versucht. Für die Untersuchungen standen 100 Schafe und 12 Schafböcke zur Verfügung. Am Ende der Anöstrusphase (Juni) wurden die Schafe in vier Gruppen aufgeteilt. Zur Feststellung der Zyklusaktivität wurde bei Tieren der Gruppe I einmal pro Woche Blut entnommen. Bei den übrigen drei Gruppen wurden jeweils vier Schafböcke für die Dauer von 4 Tagen bei den Schafen zugelassen. In Gruppe III wurden 5 Tage zuvor 20 mg Progesteron je Schaf verabreicht. An 13 Tagen nach Trennung der männlichen und weiblichen Tiere

erhielten die Schafe der Gruppe 4 125 µg Cloprostenol. Bei Tieren, die nicht in Brunst kamen, wurde die Cloprostenolgabe 9 Tage nach der Erstinjektion wiederholt. Zur Brunstfeststellung wurden Suchböcke verwendet. Brünstige Schafe wurden besamt. Die Zyklusaktivität der Kontrollgruppe lag bei 15.4%–23.1%, in Gruppe II bei 44%, in Gruppe III bei 87.5% und in Gruppe IV bei 56%. Die entsprechenden Trächtigkeitsraten betragen 32% (Gruppe II), 66% (Gruppe III) und 44% (Gruppe IV). Die Untersuchungen zeigen, daß die besten Ergebnisse zur Brunstinduktion durch Progesterongaben mit anschließender Stimulation durch einen Schafbock erreicht werden.

Introduction

The introduction of rams to seasonally anovulatory ewes, isolated from rams, stimulates ovulation and synchronizes estrus in a high proportion of ewes (Fulkerson et al. 1981; McDonald 1986). This response is known as 'ram effect'. Ovulations induced by the ram introduction are unaccompanied by behavioural estrus but are followed by *corpora lutea* with either a normal or short life span (Pearce et al. 1985). Ewes with *corpora lutea* of normal life span first display estrus at their second ovulation, about 18 days after ram introduction. But the *corpora lutea* with a short life span persist for 5–7 days and are followed by a second ovulation with a normal life span. Thus, ewes come into estrus for the first time at their third ovulation, approximately 24 days after ram exposure. The distribution of estrus in a flock is, therefore, spread over 9–10 days (Oldham and Martin 1979; Pearce and Oldham 1984). Estrous cycles induced by ram introduction can be synchronized if early regression of *corpora lutea* can be eliminated. Oldham et al. (1985) and Pearce et al. (1987) reported that progesterone applications on the day of ram introduction or earlier can eliminate the early regression process and concentrate the estrous periods of ewes into a shorter period. An alternate method of concentrating the estrous periods is the injection of prostaglandin F_{2α} analogues after ram introduction (Smith et al. 1986). Lopez-Sebastian and Inskoop (1988) reported that 14 and 16-day intervals from ram exposure to prostaglandin injection give better conception and pregnancy rates compared to an interval of 12 days for Rasa Aragonesa ewes in first service.

The aim of this study was to evaluate the effect of ram introduction alone, or in combination with progesterone or cloprostenol injections, on estrus occurrence and pregnancy rates of Konya Merino ewes in anestrus season.

Materials and Methods

Animals: A total of 100 Konya Merino ewes (80% German Mutton Merino × 20% Native Akkaraman), 2–7 years of age, from one flock, and 12 intact rams of the same breed were used. The experiment was carried out in June and July, near the end of anestrus season for the ewes in Konya province. After an isolation period of 2 months from rams, ewes were randomly divided into four groups.

Treatments: Ewes in group I (n = 26) were isolated from rams and used as control for monitoring the ovarian function during the study. In group II (n = 25) ewes were exposed to rams only. Ewes in group III (n = 24) were intramuscularly injected with 20 mg of progesterone (Corluton, I.E., Turkey) 5 days before ram introduction. In group IV (n = 25) ewes were injected with 125 µg of cloprostenol

Table 1. Effect of ram introduction alone or in combination with progesterone or prostaglandin $F_{2\alpha}$ on estrus and pregnancy rates

Variable	Group II	Group III	Group IV
No. of ewes	25	24	25
Ewes exhibiting estrus (%)	44.0 ^b	87.5 ^a	56.0 ^b
Pregnancy rate after first service (%)	32.0 ^c	66.7 ^d	44.0 ^{cd}

^{ab}Percentages within the same column having different superscripts are statistically different ($p < 0.05$); ^{cd}($p < 0.01$)

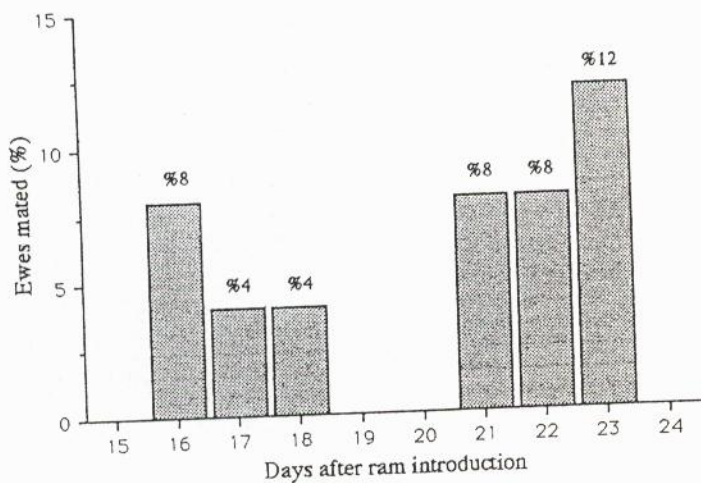


Fig. 1. The distribution of estrous periods of ewes after introduction of rams

(Estrumate, D.I.F., Turkey) 13 days after the introduction of rams. At intervals of 8 days, following the first treatment, cloprostenol treatment was repeated at the same dosage for the ewes which did not display estrus in group IV. Each group of ewes, II, III and IV, were introduced to four rams and allowed to stay with them for 4 days. Ram introduction and withdrawal was carried out on the same days in all groups. After withdrawal of rams, estrous ewes were detected by teaser rams twice daily and permitted to mate. Pregnancy rates following first service were calculated on the basis of lambing records.

Blood sampling: Jugular blood samples in group I were collected at weekly intervals, transferred into heparinized polyethylene tubes, and centrifuged for 5 min at $4000 \times g$. Then plasma was removed and stored at -22°C until assayed for progesterone.

Hormone assays: Plasma progesterone concentration was determined using the double-antibody enzymeimmunoassay procedure described by Prakash et al. (1987). The anti-progesterone- 7α -BSA-serum was obtained from Dr. D.F.M. van de Wiel (IVO-Zeist-The Netherlands). Coupling of enzyme (Horse radish

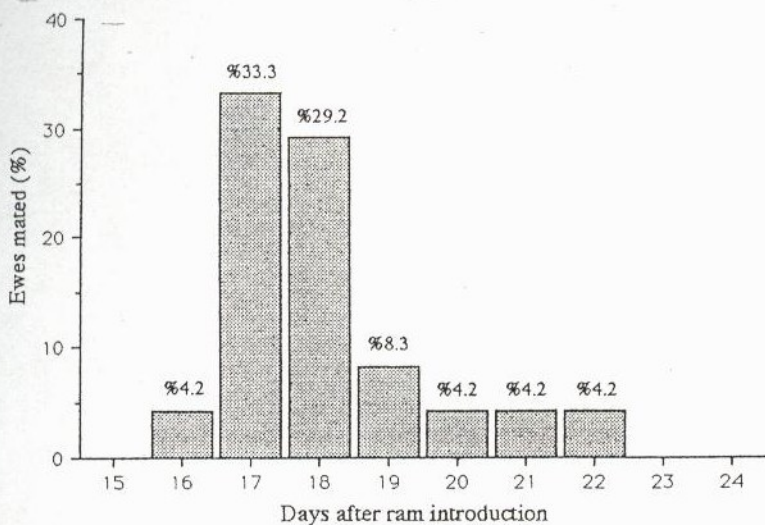


Fig. 2. The distribution of estrous periods of ewes treated with progesterone 5 days before introduction of rams

peroxidase) to progesterone-6 β -hydroxyhemissuccinate was carried out according to the procedure described by Meyer et al. (1986). Double antibody against rabbit IgG was produced in goats and the IgG fraction of goat antiserum was purified through the affinity column (Meyer et al. 1986). A single ml of ovine plasma was analysed for progesterone. The sensitivity of progesterone measurements was 0.5 pg/well and inter- and intra-assay coefficients of variation were 11.2% and 6.5% N, respectively. In group I, ewes with a progesterone concentration higher than 1 ng/ml on any sampling day and on consecutive sampling days were considered as spontaneously cycling and potentially estrous.

Statistical analyses: Statistical analyses were carried out using the χ^2 test.

Results

The proportion of ewes spontaneously cycling at the time of ram introduction in group I (control) was 15.38% and increased up to 23.07% towards the end of the study. The majority of ewes were, therefore, anestrous during the experiment. Following a 4-day ram exposure and progesterone or cloprostenol treatment, the proportion of ewes displaying estrous activity in group II, III and IV were significantly higher than the rate of ewes which had already been cycling in group I ($p < 0.01$; Table 1).

Estrous activity of ewes was spread over 8 and 7 days in group II and III, respectively. Ewes in group II displayed estrus with two peaks which occurred 16–18 and 21–23 days after ram introduction (Fig. 1). In group III, estrous periods were concentrated around day 18 with only one peak (Fig. 2). Following the cloprostenol injection on days 13 and 21, 16% and 40%, respectively, of the ewes in group IV exhibited estrus. Estrous activities were concentrated within 48–72 hours after both injections (Fig. 3).

Discussion

The effect of ram introduction can be measured by the proportion of ewes

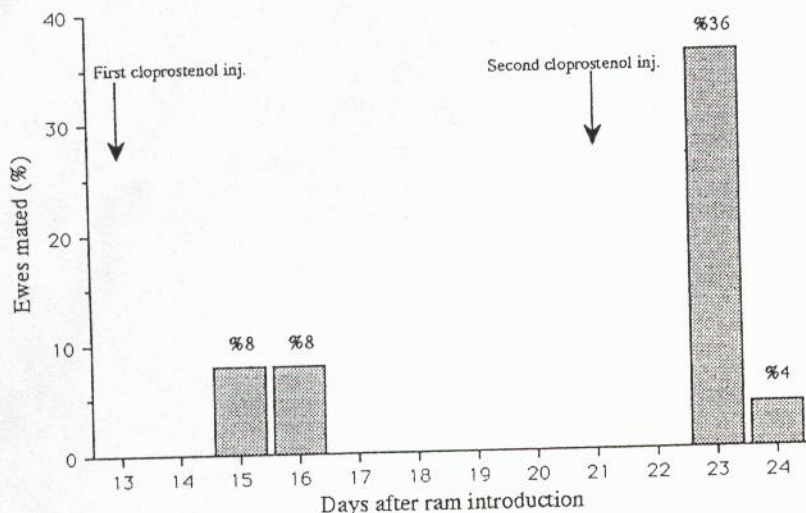


Fig. 3. The distribution of estrous periods of ewes treated with cloprostenol on day 13 and 21 after introduction of rams.

displaying estrus within 16–24 days after introduction (Murtagh et al. 1984). In group II and III, the rate of ewes which displayed estrus within 16–24 days after ram exposure were 44% and 87.5%, respectively. Estrus rates obtained in this study are in agreement with reported values by Oldham et al. (1985) and Pearce et al. (1987). However, in group I the proportion of ewes which were spontaneously cycling during the experiment fluctuated between 15.38% and 23.07%. Similar findings in Merino ewes in anestrus season were also reported by Pearce and Oldham (1988). Initiation and cessation of anestrus period may differ between the breeds (Quirke et al. 1988). The duration and intensity of seasonal anestrus are not well documented in either Konya Merino or Native Akkaraman ewes. The rate of ewes undergoing cycling activity between the breeds in the non-breeding season could not be compared here. In this study, estrous activities in group I could not be detected by teaser rams because the LH surge, which is regarded as a major factor in the commencement of ovarian activity in ram-exposed anestrus ewes, can be stimulated as early as 10 min after contact with rams (Martin et al. 1980).

Estrous activity with two peaks, which was observed in group II, has previously been reported (Knight et al. 1981). Estrous activities of ewes in group III were concentrated around day 17–19 with only one peak. This indicates that progesterone injection at a dose level of 20 mg per animal 5 days before ram introduction effectively blocks early regression of the *corpora lutea* (Pearce et al. 1987). In addition to the effect on the life span of *corpora lutea*, progesterone pretreatment also facilitates behavioural estrus after ram introduction (Oldham and Martin 1979) and increases estrus occurrence following a normal luteal phase regardless of the day of injection, whether on the day of ram introduction or 5 days earlier (Pearce et al. 1987). Likewise, the proportion of ewes displaying estrus in group III was significantly higher than in group II ($p < 0.01$) and group IV ($p < 0.01$).

Lopez-Sebastian and Inskip (1988) suggested that estrus occurrence was highest when prostaglandin injection was applied on day 12 or 14 following ram introduction. In this experiment, only 16% of ewes in group IV displayed estrus after the first cloprostenol injection. The poor estrus rate possibly resulted from the

high rate of ewes with *corpora lutea* of short life span, which caused the ewes to respond in the unsensitive period to the effect of cloprostenol on day 13. Ewes detected as being in estrus 16–20 days after ram introduction can be deemed to have a normal luteal life span (Pearce et al. 1987). Thus, it can be concluded that 7 out of 11 (63.6%) of the estrous ewes had *corpora lutea* of a short life span in group II. The detected high rate for ewes with a short luteal life span also supports this hypothesis for group IV. Murtagh et al. (1984) reported that some ewes return to anestrus before becoming pregnant, following one or two estrous cycles induced by the introduction of rams. After the second cloprostenol injection on day 21 in group IV, 10 out of 21 (47.6%) of the ewes displayed estrus and the recorded poor estrus rate was possibly due to the reversion of a considerable proportion of the ewes to anestrus period.

In addition to the classic treatments with hormone combinations and ram introduction, melatonin preparations are also used to induce ovarian activity in ewes in the anestrus season. English et al. (1986) reported that ovarian activity can commence 5–10 weeks earlier as a result of melatonin feeding or melatonin implants when compared to control ewes maintained in natural photoperiod where the treatment is begun in June. Williams et al. (1992) pointed out that a single treatment with a subcutan melatonin implant, commencing 30–40 days prior to ram introduction in spring or early summer, maximizes the potential advantages of ram introduction.

In conclusion, introduction of ram alone, or in combination with progesterone pretreatment or cloprostenol injections, increased the proportion of ewes displaying estrus relative to the control group. The combination of ram effect and progesterone pretreatment was more effective than ram effect and prostaglandin $F_{2\alpha}$ combinations in producing estrus induction and synchronization of Konya Merino ewes in anestrus season.

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Submitted: 1.11.1993

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