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Research Article

Effects of Double Injections of $PGF_{2\alpha}$ at Different Intervals on Some Reproductive Traits in Tuj Ewes*

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Abstract: The present study investigated the effects of double injections of $PGF_{2\alpha}$ at different intervals (10 vs. 14 days) on plasma progesterone levels, oestrus and lambing rates in Tuj ewes during the breeding season. Thirty clinically healthy mature ewes were assigned randomly into 2 experimental groups: 2 injections of 125 µg of Cloprostenol (a $PGF_{2\alpha}$ analogue) at either 10 (Group I, n = 15) or 14 day (Group II, n = 15) intervals. Blood samples were collected from the jugular vein on the day of the second injection of $PGF_{2\alpha}$, 4 days post-injection and 16 days after insemination to determine plasma progesterone levels. The oestrus and lambing rates were also determined. Findings showed that double injections of $PGF_{2\alpha}$ at 10 or 14 day intervals did not significantly affect either the concentrations of plasma progesterone or the rates of oestrus and lambing. Plasma progesterone concentrations on the day of the second injection, 4 days post-injection and 16 days after insemination were 4.29 ± 1.96, 0.27 ± 0.19 and 7.39 ± 3.5 ng/ml in group I and 3.45 ± 1.45, 0.10 ± 0.02 and 9.21 ± 4.29 ng/ml in group II, respectively. The oestrus and lambing rates were 86.7% and 60.2% in group I, and 100% and 73.3% in group II, respectively. In conclusion, it appears that for oestrus synchronisation, shortening the interval of double injections of $PGF_{2\alpha}$ by 4 days has little effect on plasma progesterone concentrations, oestrus or lambing rates in Tuj ewes during the breeding season.

Key Words: $PGF_{2\alpha}$, synchronisation, progesterone, oestrus, lambing, Tuj ewe

Tuj Irkı Koyunlarda Farklı Aralıklarla Çift Doz $PGF_{2\alpha}$ Enjeksiyonunun Bazı Üreme Özellikleri Üzerine Etkileri

Özet: Sunulan çalışmada, üreme sezonundaki Tuj ırkı koyunlarda farklı aralıklarla (10 veya 14 gün) çift doz $PGF_{2\alpha}$ enjeksiyonunun plazma progesteron düzeyleri, östrus ve kuzulama oranları üzerine etkileri araştırılmıştır. Klinik açıdan sağlıklı otuz adet yetişkin koyun rasgele seçilerek iki deneme grubuna ayrıldı; ilk gruptaki hayvanlara 10 gün (Grup I, n = 15), ikinci gruba ise 14 gün (Grup II, n = 15) ara ile 125 µg Cloprostenol (bir $PGF_{2\alpha}$ analoğu) enjeksiyonu uygulandı. Plazma progesteron düzeylerini belirlemek amacıyla, ikinci $PGF_{2\alpha}$ enjeksiyonu günü, enjeksiyon sonrası 4. gün ve tohumlama sonrası 16. günde V. jugularis'ten kan örnekleri alındı. Ayrıca, östrus ve kuzulama oranları belirlendi. Elde edilen bulgulara göre, farklı aralıklarla çift doz $PGF_{2\alpha}$ enjeksiyonunun plazma progesteron konsantrasyonları veya östrus ve kuzulama oranları üzerine belirgin bir olumsuz etkisi bulunamadı. Plazma progesteron konsantrasyonları, ikinci $PGF_{2\alpha}$ enjeksiyonu günü, enjeksiyon sonrası 4. gün ve tohumlama sonrası 16. günde grup I'de sırasıyla 4,29 ± 1,96, 0,27 ± 0,19 ve 7,39 ± 3,5 ng/ml iken, grup II'de ise sırasıyla 3,45 ± 1,45, 0,10 ± 0,02 ve 9,21 ± 4,29 ng/ml idi. Östrus ve kuzulama oranları grup I'de sırasıyla % 86,7 ve 60,2 iken, grup II'de ise sırasıyla % 100 ve 73,3 idi. Sonuç olarak, üreme sezonundaki Tuj ırkı koyunlarda östrus senkronizasyonu amacıyla çift doz $PGF_{2\alpha}$ enjeksiyonu aralığınının 4 gün kısaltılmasının plazma progesteron konsantrasyonları, östrus veya kuzulama oranları üzerine etkisinin önemsiz düzeyde olabileceği anlaşılmıştır.

Anahtar Sözcükler: $PGF_{2\alpha}$, senkronizasyon, progesteron, östrus, kuzulama, Tuj koyun

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Introduction

Tuj is a local sheep breed that is low yielding compared to Morkaraman (1), and is well adapted to the Caucasian region. Due to the high altitude (around 1500-2000 m) and harsh climate (namely the long-lasting winter lasting about 6 months) the grazing period on green/growing pasture is usually relatively short (3-4 months) in this region.

As with many other animal species, oestrus synchronisation techniques have been widely used for improving the reproductive performance of sheep (2,3). For this aim, various hormones (e.g., progesterone, $PGF_{2\alpha}$ and melatonin) and hormone analogues can be used (2,4,5). Among them, $PGF_{2\alpha}$ (single or double injection) can be administered either alone (2,5) or following progesterone sponge treatment (3,6-8) in ewes.

Henderson et al. (6), studying the effect of double injections of different doses of $PGF_{2\alpha}$ at 9 day intervals along with the administration of progesterone-impregnated vaginal sponges, reported that there were no differences in the lambing rates of ewes treated with different doses of $PGF_{2\alpha}$. Beck et al. (7) reported that the oestrus rates during 2 consecutive breeding seasons were greater in ewes treated with double injections of $PGF_{2\alpha}$ at 11 day intervals than in those treated with a single injection only. In a subsequent study, they reported that there was no difference between the lambing rates of ewes injected with 125 µg of Cloprostenol (a $PGF_{2\alpha}$ analogue) at 11 day intervals and in the lambing rates of those injected with a combination of $PGF_{2\alpha}$ and GnRH (8). A 2-step injection of $PGF_{2\alpha}$ may thus improve the rates of oestrus synchrony in low-yielding ewes. However, the reproductive performance of Tuj ewes subjected to a 2-step injection of $PGF_{2\alpha}$ at different intervals is largely unknown.

The objective of this study was therefore to determine the effects of double injections of 125 µg of Cloprostenol at 10 or 14 day intervals on plasma progesterone concentrations and the rates of oestrus and lambing in Tuj ewes during the breeding season.

Materials and Methods

Animals and Experimental Design

Thirty clinically healthy mature (3-4 years old) Tuj ewes kept at the Research and Experimental Station at the Kafkas University farm (43° E, 40.5° N), were used in this study. The animals were fed daily (at 8.00 a.m. and 4.00 p.m.) with a diet containing standard concentrated meal (150-200 g/day per ewe) and medium quality grass-hay (about 1500 g/day per ewe) during the breeding season and then grass-hay only afterwards. Grass-hay constituted mainly Gramineae (64.2%) along with Leguminoseae (22.8%) and other plant families (13.0%) (9). Drinking water in easily accessible metal containers was provided ad libitum. The experiment was conducted in November (when the routine mating season starts; it continues until the end of December). The ewes, similar in terms of body weight etc., were assigned randomly to receive double intramuscular injections of 125 µg/ewe L-Cloprostenol, a $PGF_{2\alpha}$ analogue (Estrumate®, Sanofi Doğu İlaç AŞ, İstanbul, Turkey) at either 10 (Group I, n = 15) or 14 day (Group II, n = 15) intervals. Oestrus was characterised mainly by the 'standing reflex', i.e. the female stands and allows the male to mount (10,11). The rates of oestrus were observed 3 times in total by introducing teaser rams into each group at 24, 48 and 72 h after the second injection of $PGF_{2\alpha}$. The lambing rates were recorded 150 ± 5 days after hand mating with Tuj rams (2 rams per group) previously known to be fertile (over 90%, according to farm records).

Blood Collection and Analytical Procedures

Blood samples were collected from the jugular vein on the day of (just prior to) the second injection of $PGF_{2\alpha}$, day 4 post-injection and 16 days after the inseminations. Plasma was obtained after centrifugation of blood at 2000 *g* for 15 min at room temperature and stored at –20 °C until laboratory analysis. Plasma progesterone concentrations were determined by using double antibody enzyme immunoassay (EIA) procedures, as described by Prakash et al. (12). Anti-progesterone-7α-carboxyethylthioether-bovine thyroglobulin, produced in rabbits, was kindly supplied by D.F.M. van de Wiel (ID-DLO, Lelystadt, The Netherlands). For the preparation of enzyme labelled progesterone, 6β-OH-progesterone-hemisuccinate was labelled with horseradish peroxidase by the mixed anhydride method (13). The affinity-purified goat IgG-anti rabbit IgG was produced as described by Meyer and Guven (14). The microtitration plates were coated with 1 µg/well affinity-purified goat IgG produced against rabbit IgG. The immune reaction

was performed by incubating a mixture of 10 µl of plasma, 100 µl of enzyme label and 100 µl of antiserum. After the plates were washed, 150 µl of substrate solution (0.01% 3,3`5,5` tetramethylbenzidine, 0.004% H_2O_2 in 100 mM sodium acetate, pH 5.5, adjusted with citric acid) was added. The plates were incubated in the dark for 40 min, and the substrate reaction was ceased by adding 50 µl of 4 N H_2SO_4. The optical density was measured at 450 nm with a microtitration plate reader. The results were determined by using Easy WIN fitting program E 5.0a. The standard curve was sensitive at 0.25 to 16 ng/ml (2.5 to 160 pg/well). Intra- and interassay coefficients of variations were less than 14%.

Statistical analysis

Data from the concentrations of plasma progesterone were analysed as repeated measures, with the time being the sub-plot, by using a linear model computed in Minitab (Release 13.0, Minitab Inc., USA, 2000), as follows:

$$Y_{ijk} = \mu + G_i + t_j + (G*t)_{ij} + e_{ijk}$$

Y_{ijk} = response variable, μ = population mean, G_i = experimental group (I = short or II = long intervals), t_j = time relative to the second injection of $PGF_{2\alpha}$ (j = 0, 4, 16), $(G*t)_{ij}$ = treatment by time interaction, and e_{ijk} = residual error.

The effects of $PGF_{2\alpha}$ treatments on the rates of oestrus and lambing were analysed by chi-square test. Differences in the least square means between the experimental groups were considered significant when $P < 0.05$. Data were presented as mean ± SE (for progesterone).

Results

Oestrus signs (of 13 and 15 ewes in groups I and II, respectively) were detected at 48 h in the vast majority (92.3%) of ewes in group I, whereas they were observed within 48 h (33.3% at 24 h and 60% at 48 h) in group II following the second injection of $PGF_{2\alpha}$ (Table 1). At 72 h, only a small number of ewes showed oestrus (7.6% in group I and 6.6% in group II).

However, there were no significant effects of treatments or of treatments by time interaction on the concentrations of plasma progesterone (Figure). The concentrations on the day of the second injection, day 4 post-injection and 16 days after inseminations were 4.29 ± 1.96, 0.27 ± 0.19 and 7.39 ± 3.5 ng/ml in group I and 3.45 ± 1.45, 0.10 ± 0.02 and 9.21 ± 4.29 ng/ml in group II, respectively. The average progesterone concentrations decreased dramatically on day 4 after the second injection of $PGF_{2\alpha}$ as compared to just prior to the second injection. These lower values of progesterone along with behavioural signs of ewes were considered 'typical oestrus'. Progesterone concentration was then increased dramatically on day 16 after inseminations in both groups (time effect, $P < 0.001$).

Table 1. Oestrus detection (%) at different times relative to the second injection of $PGF_{2\alpha}$ at 10 or 14 days apart in Tuj ewes (of 13 and 15 animals in Groups I and II, respectively). ND = not detected.

	Time relative to the second injection of $PGF_{2\alpha}$		
Distribution of Oestrus in Groups	24 h	48 h	72 h
%Oestrus in Group I	ND	92.3	7.6
%Oestrus in Group II	33.3	60	6.6

Neither the oestrus rates nor the lambing rates were significantly affected by double injections of $PGF_{2\alpha}$ at either short (10 days) or long (14 days) intervals (Table 2). The oestrus and lambing rates were 86.7% and 60.2% in group I and 100% and 73.3% in group II, respectively. Despite the lack of statistical difference between the groups, the values given were numerically greater in ewes in the latter group.

Discussion

Overall, the signs of oestrus were detected within 72 h following the second injection of $PGF_{2\alpha}$ in the vast majority (28 out of 30) of ewes in both groups (with 10 vs. 14 day intervals). During this period, however, the first appearance of oestrus signs was more common among ewes in group II.

Öztürkler et al. (3) reported a 100% oestrus rate, 80% conception rate and 53.3% lambing rate in Tuj ewes injected with 0.075 mg of D-Cloprostenol at 11 day intervals. Likewise, İleri (15), using Tiaprost (İliren®, a $PGF_{2\alpha}$ analogue) with the same intervals, reported 100% oestrus and 52.2% fertility in ewes. The present results were consistent with these previous reports.

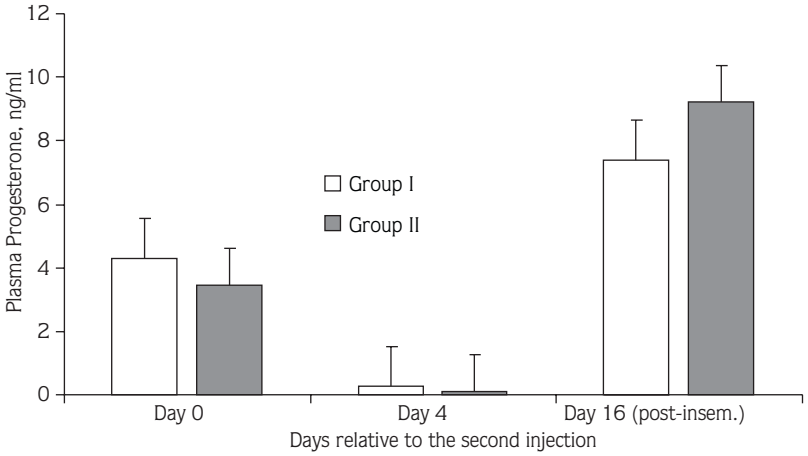

Figure. Plasma progesterone concentrations of Tuj ewes double-injected with $PGF_{2\alpha}$ at 10 (Group I) and 14 day (Group II) intervals for oestrus synchronisation.

Table 2. Oestrus and lambing rates in ewes synchronised by double injections of $PGF_{2\alpha}$ at 10 (Group I) or 14 day (Group II) intervals. The values within each row are not significantly different from each other.

Parameters	Group I (n = 15)	Group II (n = 15)
Oestrus rate, %	86.7	100
Lambing rate, %	60.2	73.3

Furthermore, the fertility of induced oestrus is generally poor, due likely to the reduction in exposure of the reproductive tract to progesterone (11). Indeed, all the parameters studied herein were numerically (but not statistically) higher in ewes double-injected with $PGF_{2\alpha}$ at 14 day intervals than in those receiving the same treatment but for a shorter (10 days) interval (oestrus rates: 100% vs. 86.7%, lambing rates: 73.3% vs. 60.2%, progesterone concentrations: 9.21 ± 4.29 vs. 7.39 ± 3.5 ng/ml, respectively). In comparison with the literature, differences in the rates of non-return (herein as 'high' progesterone levels on day 16 post-insemination) and of lambing could be considered 'normal' since one-third (approximately 25%-40%) of embryos are lost between the time of sperm penetration of the ovum and the end of implantation in sheep, cattle and swine (16).

Double injections of $PGF_{2\alpha}$ at 10 or 14 day intervals did not significantly impair either the concentrations of plasma progesterone or the rates of oestrus or lambing. Reduced (around minimum) plasma progesterone concentrations on day 4 after the second injection of $PGF_{2\alpha}$ indicate the regression of corpus luteum. Although there were no statistical differences, the rates of oestrus and lambing in group II (with longer injection interval) were numerically greater than those in group I (short interval). In a similar study by Tekeli et al. (17) using Konya Merino ewes inseminated artificially following a double injection of $PGF_{2\alpha}$ at 8, 11 and 14 day intervals, the greatest rates of oestrus (83.9%) and lambing (80.6%) were observed when ewes received double injections of $PGF_{2\alpha}$ at the longest (14 days) intervals. Additionally, Horoz et al. (18) reported that in ewes inseminated artificially following double injections of 100 µg of Cloprostenol at 9 day intervals, the rates of oestrus and pregnancy were 47.6% and 70.0%, respectively. Although our findings indicate that there was no significant reduction in plasma progesterone concentrations, oestrus or lambing rates with a shorter injection interval (10 days) of $PGF_{2\alpha}$, this requires confirmation with further investigations by using a higher number of animals of different breeds. On the other hand, the present results might have been confounded by the way of insemination, as the pregnancy rates of animals inseminated naturally or artificially may vary according to the methodology of oestrus synchronisation (19). However, oestrus synchronisation could be successful when 2-step injections of $PGF_{2\alpha}$ administrated at 11, 12 or 13 day intervals are used (2). In the present

study, the lack of significant treatment effect on the rates of oestrus and lambing might be related to the marginal range (10 vs. 14 days) of injection intervals. Despite the conceptual consistency as compared with the literature, dissimilarities in the present data could be related to (i) the potency of hormone, (ii) doses of $PGF_{2\alpha}$ analogues (20), and (iii) the breed of ewes (11,21) used.

In conclusion, the double injection of $PGF_{2\alpha}$ at 10 or 14 day intervals did not markedly alter the concentrations of plasma progesterone or the rates of oestrus synchrony or lambing in Tuj ewes. This might be related to the marginal range of the injection intervals tested (10 vs. 14 days). Future studies with a higher number of animals of different breeds may thus verify whether there is an interaction between the method of insemination (natural or artificial) and the method of administration of $PGF_{2\alpha}$ at larger time intervals for oestrus synchronisation in ewes during the breeding season.

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