Determination of reproductive response to different synchronization methods and profitability during the transition to anoestrus in multiparous Kangal sheep

Abdurrahman Takci[1], Mehmet Buğra Kivrak[1], Hakan Murat[2], Murat Yuksel[1]

Sivas Cumhuriyet University, Faculty of Veterinary Medicine, [1]Department of Obstetrics and Gynecology, [2]Department of Livestock Economics and Management, Sivas, Türkiye

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Abstract

The aim of the study was to determine a reproductively and economically successful synchronization method for Kangal sheep during the transition period from the breeding season to anoestrus. A total of 212 Kangal sheep were divided into three groups. On day 0, a sponge containing progesterone (P4) hormone was inserted intravaginally into the animals of Group 1 (n = 75). The sponges were removed 9 days later, and equine chorionic gonadotropin (eCG) and prostaglandin F2 alpha (PGF2α) hormones were injected. Animals in Group 2 (n = 65) were given PGF2α hormone at 9-day intervals. In addition to the second PGF2α injection, eCG was also administered. A double dose of PGF2α was administered to animals in Group 3 (n = 72) at 9-day intervals. The animals in this group were administered eCG along with the first PGF2α injection. As a result of the applications, Group 1 showed a higher rate of oestrous than the other two groups ($P < 0.05$). There was no difference in pregnancy rates between Group 1 and the other groups. The pregnancy rate of Group 2 was significantly lower than that of Group 3. While there was no difference between the groups regarding multiple pregnancies, the fertility rate of Group 3 was higher than in the other two groups. Cost evaluations showed that the synchronization technique used in Group 3 was the most cost-effective. It was determined that the synchronization method used in Group 3 is preferred for Kangal sheep during the transition period.

Ovine, progesterone, fertility, oestrus induction, pregnancy

The world's rapidly increasing population is putting a tremendous strain on animal production, especially animal protein (Hill and Wall 2017; Van Zanten et al. 2018). Climate and environmental changes, especially due to the countries that account for the majority of the world's meat supply, are posing challenges to cattle breeding worldwide (Renaudeau et al. 2012; Rust 2019; El-Sayed and Kamel 2020). In this context, modern sheep breeding aims to effectively improve reproductive management to increase yield (Lindsay 1991). Because sheep have a long anoestrus, only one brood can be produced per year, and births occur at a single time of the year. However, there is a year-round demand for lamb meat (Goulet and Castonguay 2002). To implement production programs aimed at increasing lamb breeding to meet consumption, sheep must be impregnated as soon as possible while considering the physiological limits after birth (O'Shea and Wright 1984; Goulet and Castonguay 2002).

To implement increased production systems, such as obtaining three lambs in 2 years, the sheep should be bred up to 95 days postpartum. Weaning should be stopped between the puerperal days 60 and 70 to achieve this goal. Concurrently, uterine involution and luteal function normalization in sheep determine the time between birth and conception. This physiological process typically takes two months to complete. It is essential to wait for the specified process to complete before performing manipulations to increase reproduction (O'Shea and Wright 1984; Goulet and Castonguay 2002).

Regarding follicular dynamism, it is known that the ovaries of sheep are somewhat dynamic and not completely stagnant during the transition phase from the breeding

Address for correspondence:
Abdurrahman Takci
Department of Obstetrics and Gynecology
Faculty of Veterinary Medicine
Sivas Cumhuriyet University, Sivas, Türkiye

Phone: +905317077488
E-mail: abdurrahmantakci@cumhuriyet.edu.tr
http://actavet.vfu.cz/

season to anoestrus. During this period, follicle-stimulating hormone (FSH) synthesis continues, follicular development fluctuations occur in parallel with these FSH fluctuations (Bartlewski et al. 1999), and reproduction can be induced through ovulation interventions based on the continuation of follicular activity even during the anoestrus period (Simões 2015).

Although ovulation rates are lower in the transitional phases from the breeding season to anoestrus than in the breeding season, the corpus luteum is larger (Bartlewski et al. 1999). However, luteal progesterone (P4) secretion is suppressed due to decreased ovulation and total luteal volume, and the decrease in gonadotropic support further exacerbates this reduction (Bartlewski et al. 1999). To stimulate fertile oestrus during this period, intravaginal placement of P4-containing devices, followed by equine chorionic gonadotropin (eCG) injection when these devices are removed, should be performed (Abecia et al. 2012). Vaginitis with abnormal (purulent or haemorrhagic) and foul-smelling vaginal secretion may occur after the use of P4-containing intravaginal devices (sponge). Alternative applications are needed because of complications affecting fertility, cost concerns, environmental contamination risk, and workload (Gonzalez-Bulnes et al. 2005; Viñoles et al. 2011; Martinez-Ros et al. 2018).

Although too many special programs have been implemented in different seasons on different breeds of sheep and herds in terms of ideal oestrus synchronization for breeding, programs suitable for specific periods in which reproductive efficiency is different have not been established (Yu et al. 2018). Some studies found that even if it was done during the breeding season, prostaglandin F2 alpha (PGF2α) applications with 11-day intervals performed better than standard P4 applications for inducing pregnancy (Rekik et al. 2016). During the transition period, luteal deficiencies occur because of the decrease in gonadotropic support, and the desired responses to prostaglandins are not obtained (Bartlewski et al. 1999). Based on these findings, adding gonadotropin to double-dose PGF2α applications may be an alternative to P4 applications.

Kangal sheep are a valuable genetic source in Türkiye. This breed originated in Sivas Province and is now grown throughout Central Anatolia and the neighbouring provinces. While the Kangal sheep breed was previously considered a variety of the Akkaraman sheep breed, which made up more than half of Türkiye's sheep population, it was accepted as a breed in 2012 owing to its superior milk and meat yield and differences in body shape, head structure, and wool. Pure-bred Kangal sheep are now raised in many parts of Central Anatolia, especially in Sivas Province and its districts. In recent years, there has been a surge in interest in intensified lambing methods among Kangal sheep breeders who have abandoned wool and milk production for various reasons, and whose sole source of income is now lamb production. Furthermore, Kangal sheep is non-prolific and has a short mating period. Therefore, there is a need for effective and breed-specific sexual stimulation programs or synchronization programs that are suitable for all seasons. The present study aimed to identify a reproductively and economically suitable protocol for Kangal sheep by performing different applications during the transition period from the breeding season to anoestrus.

Materials and Methods

Location

The study was conducted on a sheep farm with coordinates of 39.83371433796894 and 36.34688098838113 and an altitude of 1,290 metres in Ortaklar Village, Yıldızeli District, Sivas Province, Türkiye. Its pasture is located in a geography dominated by steppes between high mountains.

Animals and treatment schedule

This study was approved by the Animal Research Ethics Committee of Cumhuriyet University on 11.11.2020 (decision no. 361).

The study sample consisted of 21 brood stock rams and 212 multiparous sheep that mated out of season (in the spring), gave birth in September–October, and nursed their lambs for 60–75 days. At the start of the application, ewes' and rams' average body weights were 53 ± 5 kg and 102 ± 5 kg, respectively, and their body condition scores were 2.5–3.25 and 3–3.5, respectively. The body condition scoring method explained by Ferguson et al. (1994) was used, and the body condition values of these ewes were scored from 1 to 5 (Ferguson et al. 1994). All applications were made during the transition period between late February and mid-March (starting on February 23). When the business records were examined, it was determined that pregnancy was not achieved in the same period in previous years, although the ewes were kept together with the rams during the anoestrus period.

Approximately two months before the applications, the rams were separated from the sheep and placed in wooden partitions in the same barn. From the start of the applications until the first pregnancy examination, the animals in all groups spent 6 h in the pasture after waking up and the rest of the time on the farm; they were given a ration of 750 g of meadow grass + 750 g of wheat straw + 500 g of alfalfa grass and 250 g of barley paste per animal per day. The pasture was used the whole time after the first pregnancy examination.

A P4-containing vaginal sponge (a sponge containing 20 mg of flugestone acetate [Chronogest® CR, MSD, Ankara, Türkiye]) was vaginally inserted into the animals in Group 1 (n = 75) on day 0 and removed 9 days after the application (day 9). On day 9, the animals were given 500 IU of eCG (Chronogest/PMSG®, MSD, Ankara, Türkiye) and 250 µg of PGF2α (PGS, Alke, Tokat, Türkiye) hormones. Animals in Group 2 (n = 65) received 250 µg of PGF2α hormone on day 0. On day 9, the animals were given 500 IU of eCG and 250 µg of PGF2α hormones. Animals in Group 3 (n = 72) received 500 IU of eCG and 250 µg of PGF2α hormones on day 0. Only PGF2α hormone was administered to this group on day 9. Rams were kept with all groups one day after the last application (day 10) and with ewes for 5 days (until day 15). Subsequent oestrus cycles were followed to determine whether the animals had become cyclic. Inseminations were observed and recorded during these five days. Pregnancy was determined via ultrasonography twice (at 28–35 days [38–45 days] and 60 days [70 days]) after the rams were introduced to all ewes. The effect of different synchronization applications on reproductive indices such as oestrus, pregnancy, multiple pregnancies, and litter size was compared statistically. The economic outcomes of the specified reproductive indices were also evaluated.

Pregnancy examination

Pregnancy was examined twice. The first examination was performed using the transrectal ultrasonographic method 1 month (day 38) after the rams were introduced; the second examination was performed using the transabdominal ultrasonographic method 2 months (day 68) after the rams were introduced. Pregnancy examinations were performed using a B-mode, linear-array ultrasonography device (Mindray DP50/Vet/US) and a 5.0–7.5 MHz rectal probe rectally in the supine position to determine pregnancies and offspring counts in the early period or transabdominally to determine embryonic and foetal losses that may occur in the following days of pregnancy. During the transabdominal examination, the hairless area above the breast ventral to the right fasting pit was preferred for probe placement. Based on the presence or absence of pregnancy-related findings in this region, the breast was completely scanned in the dorso–caudal direction. Pregnancy was confirmed based on the gestational period by observing the gestational sac, followed by detecting the embryo/foetus, brood membranes, fluids, heartbeat, and placentomas. Farm visits were made at certain intervals (monthly) to follow up on the induced pregnancies. One week before the expected date of birth, daily visits were made to the farm, and birth records were maintained.

Economic analysis

The findings obtained in the present study were analysed using the following formula variations. The economic efficiency of different synchronization methods used in the three groups was evaluated using these formulas:

Group 1 Total Income: $\sum M_1 = (\sum Kg_1 - \sum P_1)$
Group 2 Total Income: $\sum M_2 = (\sum Kg_2 - \sum P_2)$
Group 3 Total Income: $\sum M_3 = (\sum Kg_3 - \sum P_3)$

M: Total monetary income obtained as a result of the method applied in that group

∑Kg: (Total lambs obtained × market value*)/current Dollar exchange rate**

∑P: (Cost of applied treatment*** + travel fee + veterinarian practice fee + labour cost****)/current Dollar exchange rate

*The current market price is included in the calculation separately for male and female lambs.

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*** P_1: Cost of one P4 sponge + 500 IU of eCG hormone cost + 263 µg of PGF2α hormone cost + consumables required for the unit animal (injector, cotton, alcohol, etc.)

P_2: Cost of one 500 IU of eCG hormone + 2 × cost of 263 µg of PGF2α hormone + consumables required for the unit animal (injector, cotton, alcohol, etc.)

P_3: Cost of one 500 IU of eCG hormone + 2 × the cost of 263 µg of PGF2α hormone + consumables required for the unit animal (injector, cotton, alcohol, etc.)

****Calculation was made based on the total working hours over the minimum wage for 2020 (Türkiye Ministry of Family and Labour 2020).

Statistical analysis

The data obtained in the study were statistically analysed using the SPPS 25 (IBM Corp. Released 2017, IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical package program. The Shapiro-Wilk and Levene tests were used to determine whether the variables met the assumptions of normality and homogeneity, respectively. Chi-square test was used to examine the single effects of the treatments on oestrus, pregnancy, conception, and multiple pregnancy rates. To determine the significance between groups, litter size data were log-transformed to achieve normal distribution and analysed with one-way ANOVA, followed by Bonferroni adjustment for *post hoc* testing. $P < 0.05$ was considered significant in all analyses.

Results

After the applications, reproductive indices such as the oestrus rate, pregnancy rate, multiple pregnancy rate, embryonic death rate, number of births, litter size, and productivity were compared between the groups to determine the reproductive response to the applications.

The indices used in the interpretation of reproductive results were established as follows:

Oestrus rate: Number of animals showing oestrus in the group/Total number of animals in the group
Pregnancy rate: Number of pregnant animals in the group/Total number of animals in the group
Multiple pregnancy rates: Number of multiple pregnancies in the group/Total number of pregnant animals in the group
Embryonic death rate: Number of embryonic deaths in the group/Total number of pregnant animals in the group
Number of births: The number of pregnant animals who gave birth after completing their pregnancy
Number of litters: The number of litters born from the number of pregnant animals
Productivity: Total number of litters/Total number of pregnant animals

In addition, on the day the sponge was removed, eight animals in the first group that received P4-supported stimulation developed a clinical picture of vaginitis with bloody purulent discharge. Although all of these animals showed oestrus and mated, only one pregnancy was achieved.

Economic findings

When the number of pregnancies, multiple pregnancies, and litters were analysed in relation to the number of animals in each group, it was found that Group 3 was more successful compared to Groups 1 and 2 (Table 1). These simple ratios also provide information about the study's direction. The most economical and productive group was Group 3 because it yielded better results than other groups. To further support this with concrete results, an economic analysis was conducted for each group based on the findings. The results of the analysis are as follows:

Group 1 Total Income: $\sum M_1 = 7{,}770.50$ USD
Group 2 Total Income: $\sum M_2 = 4{,}958.90$ USD
Group 3 Total Income: $\sum M_3 = 9{,}214.30$ USD

Based on these results, profitability was ranked as follows: $\sum M_3 > \sum M_1 > \sum M_2$. Group 3 yielded higher profits than Groups 1 and 2. Earnings of Group 3 were higher than those of Groups 1 and 2 by 1.443.80 USD and 4,255.40 USD, respectively.

Table 1. The effect of the synchronization protocol on oestrus, pregnancy, conception rate, and multiple pregnancies following different synchronization protocol the transition during period in Kangal sheep.

		Groups			Total
		1	2	3	
Oestrus Rate	Positive	74[a] (98.7%)	55[b] (84.6%)	53[b] (73.6%)	182 (85.8%)
	Negative	1[a] (1.3%)	10[b] (15.4%)	19[b] (26.4%)	30 (14.2%)
	Total	75	65	72	212
Pregnancy rate	Positive	42[ab] (56.0%)	26[b] (40.0%)	47[a] (65.3%)	115 (54.2%)
(pregnancies/all)	Negative	33[ab] (44.0%)	39[b] (60.0%)	25[a] (34.7%)	97 (45.8%)
	Total	75	65	72	212
Fertility rate	Positive	42[a] (56.8%)	26[a] (47.3%)	47[b] (88.7%)	115
(pregnancy/ mated)	Negative	32[a] (43.2%)	29[a] (52.7%)	6[b] (11.3%)	67
	Total	74	55	53	182
Multiple pregnancies	Twin and Triplet	23[a] (54.8%)	15[a] (57.7%)	24[a] (51.1%)	62 (53.9%)
	Total	42	26	47	115

[ab] Percentages that do not share a common superscript letter in the same row are significantly different from each other ($P < 0.05$)

Assuming all other conditions were the same between the groups (same field/ environment), Group 3 was 42.0% more successful, Group 1 was 35.4% more successful, and Group 2 was 22.6% more successful than standard methods.

These results indicate that the treatment method used in Group 3 was the most cost-effective and profitable. The synchronization method for Group 3 was more economical compared to the other two groups.

Reproductive findings

Table 1 shows the effect of the synchronization protocol on oestrus rates, pregnancy rates, and multiple pregnancies. Table 1 shows singleton, twin, and triplet lamb-bearing ewes according to groups. Almost all of the sheep in Group 1 were in oestrus, and the oestrus rate in Group 1 was significantly higher than in other groups. There was no significant difference in pregnancy rates between Group 1 and the other groups. Group 2 had a significantly lower pregnancy rate than Group 3 ($P < 0.05$). Group 3 had a significantly higher fertility rate than the other groups ($P < 0.001$). Multiple pregnancy rates were similar between all groups. Litter sizes (number of lambs/number of ewes lambing) were 1.5952 ± 0.09055, 1.6800 ± 0.12543, and 1.6383 ± 0.10281 in Group 1, Group 2, and Group 3, respectively, and differences between groups were not significant.

Discussion

During the transition period from the breeding season to anoestrus in Kangal sheep, intermittent double-dose PGF2α combined with gonadotropin (eCG) supplementation during the first PGF2α application increased both reproductive efficiency and economic gain of synchronization.

The lower level of ovulation and total luteal volume in the transition from the breeding period to the anoestrus in sheep suppresses luteal P4 secretion, resulting in lower pregnancy rates in this period than in the breeding season (Bartlewski et al. 1999).

Outside the breeding season, groups treated with different doses of eCG had similar reproductive efficiency. Regardless of the dose, groups that received eCG had significantly

higher oestrous response and pregnancy rates than groups that did not receive any eCG (Atalla and Abu Gazal 2018).

In a study in which two different P4 forms (medroxyprogesterone acetate and flugestone acetate) were used to stimulate Dorper sheep, a prolific breed, during the transition to anoestrus, pregnancy rates of 70.6% and 74%, respectively, were obtained (Zeleke et al. 2005). In contrast, lower pregnancy rates were obtained in all groups in the present study. Similar to the results of Zeleke et al. (2005), although Group 1 was a P4-supplemented group, the 56% pregnancy rate in this group could be attributed to the non-prolific sheep used in this study. In addition, in another study investigating the effect of exposure to P4 on reproductive performance during the anoestrus period, pregnancy rates of 43.5% and 37.8% were obtained with short-term (6 days) and traditional long-term (14 days) vaginal applications of a P4-containing device (Menchaca et al. 2017). In the present study, the higher pregnancy rate (56%) in Group 1, in which 9-day P4 administration was carried out, was associated with different mating patterns (natural insemination vs. fixed-time insemination) and application periods (transition to anoestrus vs. anoestrus) between studies (Menchaca et al. 2017).

During the breeding season, similar net benefits were obtained after synchronization with PGF2α alone or with P4-assisted synchronization in sheep (Rosasco et al. 2019). Another breeding study found that intermittent double-dose PGF2α administration improved pregnancy rates than P4 (Rekik et al. 2016). However, because the present study included an economic evaluation and was conducted outside of the breeding season and because double-dose PGF2α applications result in low pregnancy rates (Almadaly et al. 2016; Hasani et al. 2018) such a group was not included in the present study.

Hasani et al. (2018) found that P4-supported stimulation was 33.43% more expensive than eCG + PGF2α stimulation. Similarly, in the present study, the P4-assisted stimulation method was more expensive.

Hasani et al. (2018) also found that eCG + PGF2α was more productive compared to P4 supplementation in terms of reproduction (Hasani et al. 2018). In the present study, productivity was highest in Group 3, which received eCG and PGF2α in various ways. We hope that the modified application of eCG and PGF2α evaluated in the present study will attract attention in the future owing to its ease of application, low cost, and lack of negative complications, such as vaginitis.

Oestrus synchronizations correctly designed and successfully implemented in sheep farming can increase lamb production, which is the most important factor in profitability. This leads to a profitable and sustainable business structure. Swellum et al. found that the 6-day CIDR application (6-day group) was more profitable than other periods. Their profit was 676.91 USD for the group (n = 20) (Swelum et al. 2018). In another study by the same researchers, the profit obtained by one-time application (n = 19) was 216.61 USD (Swelum et al. 2019). In a study conducted in Pakistan, the researchers achieved a profit of 18.36 USD and 45.52 USD per sheep in 2013 and 2019, respectively (Khojastekey et al. 2020). The lower profitability rates compared to the present study are caused by the small number of animals, the cost of the application method, and country-wise differences in lamb income. In the oestrous synchronization study conducted by Rosasco et al. (2019), the highest return per sheep was obtained (99.33 USD) (Rosasco et al. 2019). This figure is similar to the income per ewe obtained in the present study (127.9 USD).

In conclusion, the results of the present study showed that similar benefits can be obtained with a modified use of PGF2a during the transition period in Kangal sheep because of residual formation and complications of P4-supported synchronizations, such as vaginitis.

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