

Research Article

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Effects of halving intravaginal sponges and eCG dose on estrus response and fertility in Tahirova ewes during the breeding season

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Abstract: The aim of this study was to compare the effect of halving the intravaginal sponges impregnated with 20 mg of flugestone acetate (FGA; 20 or 10 mg) and a dose of equine chorionic gonadotropin (eCG; 600 or 300 IU) on fertility in Tahirova ewes. A total of 200 Tahirova ewes were randomly assigned to 1 of 2 groups during the breeding season. Half of the ewes (n = 100) were treated with whole intravaginal sponges (WS, 20 mg of FGA) and the other half (n = 100) with halved sponges (HS, 20 mg of FGA) for 14 days. The ewes in both groups were further randomly divided into 2 subgroups (of 50 ewes each). Half of the ewes in each group received a 600 IU dose and the other half received a 300 IU dose of eCG at sponge withdrawal via IM route. As a result, 4 treatment groups, WS 600, WS 300, HS 600, and HS 300 were established. All the ewes were injected with PGF, a (0.294 mg, IM) a day prior to sponge withdrawal. Blood samples were collected from 40 randomly selected ewes (20 from the WS group and 20 from the HS group) on the 1st, 4th, 7th, 10th, and 13th day after sponge insertion. Serum progesterone profiles of ewes treated with whole and halved FGA sponges were similar on all sampling days. The FGA dose (whole or halved sponge) affected the pregnancy rate and the dose of eCG (600 or 300 IU) affected the fecundity significantly, while it did not affect the pregnancy rate. The data also demonstrate that FGA dose × eCG dose interaction affects estrus and lambing rates significantly. In the whole sponge groups, ewes treated with 600 IU of eCG had significantly lower lambing rates, but a significantly higher fecundity rate compared to the ewes injected with 300 IU of eCG. While the fecundity rates were similar in the 600 IU eCG groups (WS600 and HS600), estrus, pregnancy, and lambing rates were significantly higher in the HS600 group compared to the WS600 group. It was concluded from the study that the sponges containing 20 mg of FGA can be halved for a more economical estrus synchronization at the farm level in Tahirova ewes, resulting in higher pregnancy rates, but halving the recommended dose of eCG (600 IU) could decrease the fecundity rate.

Key words: Tahirova ewes, synchronization, halved sponge, eCG, progesterone

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Tahirova koyunlarında vajinal süngerleri ikiye keserek uygulamanın ve eCG dozunun fertilite üzerindeki etkileri

Özet: Bu çalışmada, Tahirova koyunlarında 20 mg flugeston asetat ihtiva eden intravajinal süngerleri ikiye kesip kullanmanın (FGA; 20 veya 10 mg) ve equine chorionic gonadotropin (eCG) dozunu yarıya indirmenin (eCG; 600 veya 300 IU) fertilite üzerindeki etkilerini araştırmak amaçlandı. Üreme sezonunda toplam 200 Tahirova koyunu tesadüfen 2 gruptan birine ayırıldı. Koyunların yarısına (n= 100) 14 gün süre ile tam süngerler yerleştirilirken (WS grubu, 20 mg FGA) diğer yarısına (n= 100) ikiye kesilmiş süngerler uygulandı (HS grubu, 20 mg FGA). Daha sonra her 2 gruptaki koyunlar tesadüfi olarak 2 alt gruba ayrıldı (her bir grupta 50 koyun). Her 1 alt grubun birindeki koyunlara sünger çıkımında kas içi yolla 600 IU diğerine ise 300 IU eCG uygulandı. Böylece WS 600, WS 300, HS 600 ve HS 300 grupları oluşturuldu. Tüm koyunlara sünger çıkımından 1 gün önce PGF, a (0.294 mg, IM) uygulandı. Rastgele seçilmiş 40 koyundan (WS grubundan 20 ve HS grubundan 20) sünger verlestirildikten 1, 4, 7, 10 ve 13 gün sonra kan örnekleri alındı. Tam ve ikiye kesilmiş (yarım) sünger uygulanan koyunların serum progesteron seviyeleri tüm örnek alım günlerinde benzer bulundu. Flugeston asetat (tam veya yarım sünger) gebelik oranını önemli derecede etkiledi, eCG dozunun ise (600 veya 300 IU) gebelik oranını üzerinde etkisi olmazken doğurganlık oranını önemli düzeyde etkiledi. Tüm sünger gruplarında 300 IU eCG uygulanan koyunlar ile karşılaştırıldığında, 600 IU eCG uygulanan koyunların doğurganlık oranı önemli derecede daha yüksek bulunurken kuzulama oranı ise önemli düzeyde daha düşük bulundu. Altıyüz IU eCG uygulanan grupların (WS600 ve HS600) doğurganlık oranı benzer bulunurken HS600 grubunun östrus, gebelik ve kuzulama oranları WS600 grubundan önemli düzeyde daha yüksek bulundu. Bu çalışmadan özet olarak, Tahirova koyunlarında 20 mg FGA ihtiva eden vajinal süngerlerin sahada daha ekonomik östrus senkronizasyonu yapmak amacıyle ikiye kesilerek kullanılabileceği ve bu şekilde daha yüksek gebelik oranları elde edilebileceği, fakat tavsiye edilen eCG dozunun (600 IU) yarıya indirilmesinin doğurganlık oranını azaltabileceği sonuçları çıkarıldı.

Anahtar sözcükler: Tahirova koyunları, senkronizasyon, yarım sünger, eCG, progesteron

Introduction

The Tahirova breed was developed in 1964 by crossing Turkey's native sheep (Kıvırcık) with East Friesian rams. Tahirova, which is a milk-meat type sheep bred, can not only be milked for 9-10 months per lactation, giving more milk, but it also retains the traditional flavor of Kıvırcık meat. These characteristics enabled the Tahirova breed to spread throughout the Marmara and Aegean regions of Turkey (1).

Fertility following a synchronized estrus is often depressed partly due to the poor synchronization of this induced estrus and ovulation (2). Of the most important factors leading to this depressed fertility following the use of synthetic progestagens, is the dose level and method of preparation or impregnation (3). There are suggestions that optimal fertility, following synchronization with progesterone, can be achieved with lower doses of progestagen, by halving the intravaginal sponges (4). Crosby et al. (5) On the other hand, postulate that a high level of progestagen followed by its rapid withdrawal is a necessary prerequisite for acceptable fertility. The use of gonadotropin is routinely incorporated into the intravaginal device synchronization protocols used in does and ewes during anestrus to induce ovulation. However, there are many factors that can influence the effect of eCG in controlled breeding (6), including the dose of progestagen (7) and eCG (8), the duration of progestagen treatment (9), the season (10), and repeated treatments (11). The effectiveness of an estrus synchronization protocol is measured by its ability to elicit a fertile, tightly synchronized estrus response in a majority of treated females (12). The procedure should be cheaper, need less labor, and be safe to humans.

The aim of this study was to compare the effect of halving the intravaginal sponges impregnated with 20 mg of flugestone acetate (FGA; 20 or 10 mg) and a dose of equine chorionic gonadotropin (eCG; 600 or 300 IU) on the estrus response, pregnancy, lambing, and fecundity rates in Tahirova ewes.

Materials and methods

In this study, 200 Tahirova ewes, 3 to 5 years in age, weighing 40 to 45 kg were used. The experiment was conducted during the breeding season (July to October). The flock was managed under semi intensive conditions on a commercial farm in Tekirdağ, Turkey, 40°59'N, 27°31'E, elevation 140 m. The climate in Tekirdağ is characterized by warm to hot summers and cold winters. Rainfall is approximately 678 mm per annum.

The ewes were kept under natural field conditions, with access to good quality grass and each animal received 1 kg of grain barley daily; half a kg in the morning and half in the evening during the breeding season. All ewes began treatment on the same day and the sponge insertion day was defined as day 0. The experimental design was a 2×2 factorial to study the effects of 2 doses of FGA (20 or approximately 10 mg; by halving the sponges) and 2 doses of eCG (600 or 300 IU) on estrus, pregnancy, lambing, and fecundity rates.

A total of 200 Tahirova ewes were randomly assigned to 1 of 2 groups during the breeding season. Half of the ewes (n = 100) were treated with whole intravaginal sponges (WS) containing 20 mg of flugestone acetate (FGA, Chronogest[°] CR, Intervet, Turkey) and the other half (n = 100) with halved sponges (HS, 10 mg of FGA) for 14 days. The ewes in both groups were further randomly divided into 2 subgroups (of 50 ewes each). Half of the ewes in each group received a 600 IU dose and the other half received a 300 IU dose of eCG (Chronogest, Intervet, Turkey) at sponge withdrawal via IM route, resulting in 4 different treatment combinations, namely WS600, WS300, HS600, and HS300 (n = 50 per group). All the ewes were injected IM with $PGF_{2}\alpha$ (Tiaprost tromethamol, 0.294 mg, Iliren, Intervet, Turkey) a day prior to the sponge withdrawal.

The intravaginal sponges were cut into 2 with a pair of scissors and a piece of thread was attached to each half before being inserted to the HS group. Blood samples were collected from 40 randomly selected ewes (20 from the WS group and 20 from the HS group) on the 1st, 4th, 7th, 10th, and 13th day after sponge insertion. Serum was harvested and

stored at -20 °C until analyzed. The concentration of progesterone (P4) in the serum was determined by radioimmunoassay using a commercial kit (DSL-3900, Progesterone, DSL, USA). The sensitivity of the assay was 0.13 ng/mL. Intra- and interassay coefficients of variation were 5.5% and 8.7%, respectively.

Ewes were observed for estrus signs with the aid of fertile rams (3 to 5 years old) for 1 h every morning and evening, starting at the sponge withdrawal day, for 4 consecutive days. Ewes standing to be mounted were considered at estrus and mated. Ewe fertility was monitored in terms of pregnancy rate (number of ewes lambing/ewes showing estrus and mated), lambing rate (number of ewes lambing/all ewes included in treatments), and fecundity rate (number of lambs born/number of ewes lambing).

Statistical analysis

The effects of the FGA dose (whole = 20 mg or halved = 10 mg), the dose of eCG (600 or 300 IU) and the FGA dose \times dose of eCG interaction on estrus, pregnancy, lambing, and fecundity rates were analysed and compared between the 4 groups by GLM procedures with the SPSS program (Version 10.0) for Windows (MS). Estrus, pregnancy, lambing, and fecundity rates were compared using a chi-square test. The mean progesterone concentrations between groups were analyzed by ANOVA-repeated measures (GLM procedure of SPSS, Version 10.0).

Results

The FGA dose (whole or halved sponge) affected the pregnancy rate (P < 0.05) and the dose of eCG (600 or 300 IU) affected the fecundity significantly (P < 0.01), while it did not affect the pregnancy rate. The FGA dose × eCG dose interaction effects estrus (P < 0.05) and lambing (P < 0.01) rates significantly (Table 1). In the whole sponge groups, ewes treated with 600 IU of eCG had significantly lower lambing rates, but a significantly higher fecundity rate compared to the 300 IU eCG injected ewes. Estrus, pregnancy, lambing, and fecundity rates of half sponge groups (HS600 and HS300) were similar. These rates were similar in the WS300 and HS300 (300 IU eCG

	Main effects				c: ·C		
Trait	Sponge		eCG		Significance		
	Whole	Halved	600 IU	300 IU	Sponge	eCG	Sponge × eCG
Estrus rate (%)	91.0	96.0	92.0	95.0	n.s.	n.s.	*
Pregnancy rate (%) ¹	86.5	95.8	89.7	92.6	*	n.s.	n.s.
Lambing rate (%) ²	79.0	92.0	83.0	88.0	**	n.s.	**
Fecundity rate (%) ³	152.5	152.9	164.5	140.9	n.s.	**	n.s.

Table 1. The effects of FGA dose, eCG dose, and FGA dose × eCG dose interaction on estrus, pregnancy, lambing, and fecundity rates.

¹: Number of ewes lambing/all ewes mated

²: Number of ewes lambing/all ewes included in treatments (n = 50)

³: Number of lambs born/ number of ewes lambing

*: P < 0.05, **: P < 0.01 and n.s.: Not significant

injected) groups (P > 0.05). While the fecundity rate were similar in the 600 IU eCG groups (WS600 and HS600), estrus (P < 0.05), pregnancy (P < 0.05), and lambing rates were significantly higher in the HS600 group compared to the WS600 group (Table 2). While the estrus rates were similar in ewes both treated with whole and halved sponges in the 300 eCG groups, the ewes treated with halved sponges had a significantly higher estrus incidence than those of the whole sponge ewes in the 600 eCG groups. The blood progesterone concentration of the whole and half sponge group ewes were similar on the 1st, 4th, 7th, 10th, and 13th day after sponge insertion (P > 0.05, Figure).

Discussion

Results of the present study confirm and extend the observations that estrus can be synchronized successfully by using halved intravaginal sponges as reported previously (4,13,14). The data also demonstrates that the FGA dose \times eCG dose interaction effects estrus and lambing rates significantly. The FGA dose (whole or halved sponge) affected the pregnancy rate and the dose of eCG (600 or 300 IU) affected the fecundity significantly, while it did not affect the pregnancy rate.

While the estrus rates were similar in ewes treated with both whole and halved sponges in the 300 eCG

WS600 WS300 HS600 HS300 Groups n = 50n = 50n = 50n = 5086.0^b 96.0^{ab} 94.0^{ab} Estrus rate (%) 98.0^a Pregnancy rate (%) (35/43) 81.4^y (44/48) 91.7xy (48/49) 98.0^x (44/47) 93.6xy Lambing rate (%) (35/50) 70.0^{b*} (48/50) 96.0^{a*} (44/50) 88.0^a (44/50) 88.0^a 160.4 ab 145.5 bc Fecundity rate (%) 168.6^a 136.4^c

Table 2.Estrus, pregnancy, lambing, and fecundity rates of ewes treated with whole sponges plus 600 (WS600)or 300 IU eCG (WS300), and halved sponges plus 600 (HS600) or 300 IU eCG (HS300).

Within rows, means without common letters are different (^{abc}: P < 0.05, ^{xy}: P < 0.01, *: P < 0.01).

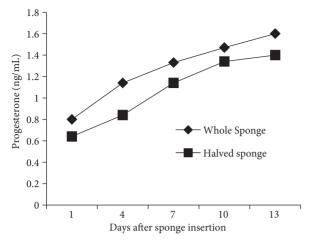


Figure. Mean (±s.e.) serum progesterone profiles of ewes with whole (n = 20) or halved (n = 20) sponges. Group \times Day did not differ significantly for progesterone on the 1st, 4th, 7th, 10th, and 13th day after sponge insertion (P > 0.05).

groups, the ewes treated with halved sponges had a significantly higher estrus incidence than those of the whole sponge ewes in the 600 eCG groups. Similarly, Greyling et al. (13) failed to find differences in estrus incidence in ewes following treatment with intravaginal sponges impregnated with 40 or 60 mg of medroxy progesterone acetate, followed by 300 of IU eCG.

Previously, it has been demonstrated that the progestagen dose used in sponges to synchronize estrus in ewes, after a 14 day treatment, was not entirely utilized. Greyling et al. (13) found that only 23.4% (or 13.2 mg) of the progestagen from the whole sponge and 50.7% (or 18.7 mg) from the halved sponges was absorbed by the animal during the treatment period. Similarly, Simonetti et al. (15) observed that differences among the initial doses (40, 50, and 60 mg) of MAP correlated with differences among the residual MAP remaining in the sponges following treatment, but not with the absorbed levels. In studies investigating the effects of progestagen dose on fertility at synchronized estrus, treatments with 30 or 40 mg of MAP impregnated sponges have resulted in conception rates similar to (14,15), or higher than (4,13), the commercially available 60 mg MAP sponges. However, the reasons why the reduced doses of MAP favor fertility are not very clear.

It has been shown that ewes treated with lower progestagen doses exhibited estrus earlier (16), and the reason could be attributed to the existence of lower residual progestagen following sponge withdrawal (13,15). In the present study, although not significant statistically, the serum progesterone concentrations on the 1st, 4th, 7th, 10th, and 13th day after sponge insertion were found to be slightly lower (P > 0.05) in the halved sponge group than those of the whole sponge group (Figure). However, the half sponge treatment increased the pregnancy and lambing rates significantly. There are no precise data explaining how the half sponges created this effect. Depending on references, some possible explanations might be given. Currently, the most reasonable explanation can be that depending upon the differences in the impregnated FGA dose, blood progestagen levels during the treatment or residual progestagen amounts following sponge withdrawal, could cause little differentiation in the pre-ovulatory LH surge design (the peak serum LH concentrations, LH surge frequency, and/or amplitude etc.), which may have resulted significant variations in ovulation and fertility. As we could not measure LH in our study, we could not prove this. However, parallel to our hypothesis, Greyling et al. (13) found that the halved sponge group has slightly higher mean LH concentrations (15.3 \pm 10.1 vs. 12.2 \pm 5.1 ng mL⁻¹, P > 0.05) and significantly higher conception and lambing rates (P < 0.01) compared with the whole sponge group. In previous experiments, subluteal concentrations of progesterone (1-2 ng/ mL) were effective in suppressing the occurrence of spontaneously occurring preovulatory surges of LH (17). In a recent study, progesterone was shown to have these inhibitory effects at much lower concentrations. Hatler et al. (18) demonstrated that estradiol cypionate (ECP) induced mean baseline luteinizing hormone (LH) concentrations were 0.93, 0.71, 0.64, and 0.55 ng/mL, respectively (P > 0.05), the percentage of cows with ECP-induced LH surge were 100%, 80%, 40%, and 0%, respectively (P < 0.01), and in cow groups, the mean progesterone concentrations in follicular phase after $PGF_{2\alpha}$ induced luteolysis were 0.11, 0.45, 0.78, and 1.20 ng/ mL. Similarly, they found that in groups, the mean progesterone concentrations in follicular phase were

0.13, 0.30, 0.70, and 1.20 ng/mL, and the percentages of cows that ovulated were 100%, 42.9%, 0.0%, and 0.0% respectively (P < 0.01).

Equine chorionic gonadotrophin (eCG) is currently the most widely used hormone to improve fertility in ewes. Its effect is dose dependent (8). It has been reported that 250 (19) or 300 IU (20) of eCG did not influence the fertility or the prolificacy rate in ewes. It has also been suggested that such low doses are not sufficient to stimulate additional follicular development (19). Similarly, in the present study, the eCG dose (300 vs. 600 IU) was observed to have a significant effect on the fecundity rate and a 600 IU dose treatment resulted in higher fecundity rates (P < 0.01). When half sponges were used, pregnancy, lambing, and fecundity rates were similar in the 300 and 600 IU eCG groups. However, with whole sponges, ewes receiving 300 IU of eCG had significantly higher lambing rates (88.0% vs. 70.0%; P < 0.01) and lower fecundity rates (136.4% vs. 168.6; P < 0.05) than those of 600 IU eCG group.

The results of the present study show that, while the FGA dose (whole vs. halved sponges) itself had a significant effect on pregnancy (P < 0.05) and lambing (P < 0.01) rates, the eCG dose (300 vs. 600 IU) influenced the fecundity rate significantly (P <

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0.01). Sponges impregnated with 45 or 20 mg of FGA applied for 14 days, plus 600 IU of eCG at sponge withdrawal, was a routinely employed treatment in sheep. When the WS600 and HS300 groups were compared, it was observed that the WS600 ewes had a significantly lower lambing rate (P < 0.05) but considerably higher fecundity rate (P < 0.05). On the other hand, when the WS600 and HS600 ewes were compared, the fecundity rate was similar in both groups (168.6% and 160.4%, respectively; P > 0.05), but ewes in the HS600 group had significantly higher estrus, pregnancy, and lambing rates.

In conclusion, the results of the present study suggest that for a more economical estrus synchronization under field conditions, the 20 mg FGA impregnated sponges can be split into 2 and used successfully followed by 600 UI of eCG, achieving higher pregnancy and lambing rates. However, halving the recommended dose of eCG (600 IU) seems to significantly reduce the fecundity rate in Tahirova ewes.

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