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RESEARCH ARTICLE

Effects of FGA Sponge and Ovsynch Based Protocols on Reproductive Performance of Fat-tailed Ewes During the Breeding Season

Recai KULAKSIZ [1] Ömer UÇAR [2] Ali DAŞKIN [3]

[1] Division of Reproduction and Artificial Insemination, Faculty of Veterinary Science, University of Kafkas, TR-36300 Kars - TURKEY
[2] Division of Reproduction and Artificial Insemination, Faculty of Veterinary Science, University of Atatürk, TR-25240 Erzurum - TURKEY
[3] Division of Reproduction and Artificial Insemination, Faculty of Veterinary Science, University of Ankara, TR-06110 Ankara - TURKEY

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Summary

The effects of different synchronisation prototocols on the reproductive parameters of fat-tailed ewes during the breeding season were studied herein. Seventy-one multiparous fat-tailed ewes were randomly divided into four treatment groups, as follows; Group 1 (long-term FGA): for a total of 20 ewes, 20 mg FGA sponges inserted intravaginally for 14 d, plus an i.m. injection of 400 IU PMSG upon the sponge withdrawal, Group 2 (short-term FGA): for 18 ewes, the insertion of sponges for 8 d, plus the PMSG injection on day 8 upon the sponge withdrawal, Group 3 (Ovsynch): for 17 ewes, an i.m. injection of 0.004 mg GnRH on day zero, followed by the injection of 125 µg $PGF_{2\alpha}$ on day 7 and the second GnRH injection on day 9, and Group 4 (short-term FGA plus Ovsynch): for 16 ewes, the FGA sponge inserted along with the first GnRH injection (as day zero), $PGF_{2\alpha}$ injection on day 7 upon the sponge withdrawal, and the second GnRH injection on day 9. The results showed that; time to onset of oestrus ($P<0.001$), duration of oestrus ($P<0.05$), the rates of pregnancy ($P<0.01$) and lambing ($P\leq0.001$) differed between the synchronisation groups. Overall, findings of onset/duration of oestrus and pregnancy/lambing rates suggest that; the standard Ovsynch synchronisation programme was markedly inferior to the long- and short-term FGA sponge protocols in fat-tailed ewes during the breeding season.

Keywords: *Fat-tailed ewe, Oestrus synchronisation, FGA, Ovsynch*

Aşım Mevsimindeki Yağlı Kuyruklı Koyunlarda FGA ve Ovsynch Temelli Senkronizasyon Protokollerinin Üreme Performansı Üzerine Etkileri

Özet

Sunulan çalışmada, aşım sezonundaki yağlı-kuyruklu koyunlarda farklı senkronizasyon protokollerinin reprodüktif parametreler üzerine etkileri araştırıldı. Bu amaçla, toplam 71 baş birden fazla doğum yapmış yağlı-kuyruklu koyun rasgele 4 deneme grubuna ayrıldı: Birinci gruptaki koyunlara (n=20, uzun süreli FGA), 20 mg FGA içeren süngerler intravaginal olarak yerleştirildikten sonraki 14. günde uzaklaştırılarak, 400 IU PMSG intramuskuler olarak enjekte edildi. İkinci gruptaki koyunlara (n=18, kısa-süreli FGA), 20 mg FGA içeren süngerler intravaginal olarak yerleştirildikten sonraki 8. günde uzaklaştırılarak, 400 IU PMSG intramuskuler olarak enjekte edildi. Üçüncü gruptaki koyunlara (n=17, Ovsynch grubu), Ovsynch protokolü (0. günde 0.004 mg GnRH, 7. günde 125 µg $PGF_{2\alpha}$ ve 9. günde 0.004 mg GnRH) uygulandı. Dördüncü gruptaki koyunlara (n=16; kısa-süreli FGA + Ovsynch) ise, 20 mg FGA içeren süngerlerin intravaginal olarak yerleştirilmesiyle birlikte 0.004 mg GnRH enjekte edildi. Süngerlerin 7 gün sonra uzaklaştırılmasını takiben 125 µg $PGF_{2\alpha}$ ve 9. günde 0.004 mg GnRH uygulandı. Senkronizasyon grupları arasında, son uygulama-östrus aralığı ($P<0.001$), östrus süresi ($P<0.05$), gebelik ($P<0.01$) ve doğum oranları ($P\leq0.001$) açısından önemli farklar olduğu gözlendi. Sonuç olarak; standart Ovsynch protokolünün, üreme sezonundaki yağlı-kuyruklu koyunlarda klasik kısa ve uzun süreli FGA içeren vajinal sünger uygulamalarına göre gebelik ve doğum oranlarında önemli ölçüde azalmaya yol açtığı kanısına varıldı.

Anahtar sözcükler: *Yağlı kuyruklu koyun, Östrus senkronizasyonu, FGA, Ovsynch*

İletişim (Correspondence)
+90 474 2426807/5220
recaikulaksiz@gmail.com

INTRODUCTION

Numerous methods have been used to for improving the reproductive performance in sheep. For this, some researchers have focused on animal husbandry practices (e.g. flushing or the ram effect), while others adopted the administration of certain agents [1-3].

Oestrus synchronisation has been practised in sheep industry for almost a half century [4]. The methods are based on the control of the lifespan of corpus luteum (by using prostaglandin or progestagen). The most common techniques are progestagen devices, inserted for 12-14 d, followed by an administration of eCG [5]. Administrations of intravaginal progestagen (FGA or CIDR), for 10-16 d, followed by intramuscular (i.m.) injection of eCG, have been successfully used [6-8]. Progestagens appear to be the most practical hormones of choice. However, a prolonged time of administration could result in low conception rates [9-11], as likely to be attributable to the impaired sperm transport *in vivo* [12]. Meanwhile, short-term protocols possibly allow for facilitating the managerial tasks, minimising the vaginal discharge and infection risks, and thus increasing the fertility rates. Indeed, short periods of sponge administration, for as short as 5-7 d, have been successful in sheep regardless of breeding season [10,13].

Alternatively, a GnRH-based $PGF_{2\alpha}$ protocol has also been reported to be effective in ewes during the breeding season [13,14]. Furthermore, their combination with the Ovsynch protocol has also been used in cattle [15]. Likewise, this protocol was also used recently in ewes [16]. Physiological aspects of Ovsynch protocol may be described as follows: on day zero, the ovulation occurs followed by the progression of corpus luteum or, alternatively, intrafollicular luteinisation takes place after the first GnRH injection. On day 5, the injection of $PGF_{2\alpha}$ induces luteolysis, while the injection of second GnRH on day 7 evokes the ovulation. Indeed, Deligiannis et al.[16] reported that the majority of animals eventually became pregnant following the artificial insemination performed 36-62 h after the second GnRH. Furthermore, Holtz et al.[17] comparing progesterone sponge and Ovsynch protocols in goats concluded that the latter protocol could also be an alternative choice with reasonable outcome.

In the literature, there exist only a little information on the effect of Ovsynch protocol or its combination with FGA-impregnated intravaginal sponges in fat-tailed ewes. Therefore, the present study was performed to investigate the reproductive efficiency of Ovsynch and or FGA protocols for oestrus synchronisation during the natural breeding season in fat-tailed Akkaraman and Awassi ewes.

MATERIAL and METHODS

Locations and Animals Used

This study was conducted during the breeding season at the Experimental Research and Practice Farm, Faculty of Veterinary Science, Ankara University, Ankara, Turkey. Ewes were in dry period (not lactating) and clinically healthy.

A total number of 71 fat-tailed ewes (40 Akkaraman and 31 Awassi breed) were used. The animals were 2-5 years old with body weight between 45-55 kg and body condition score of 2.5-3.0 based on the method described by Ucar et al.[18]. The ewes were kept in open pen (2.5 meter square per ewe) with adequate watering, and offered alfalfa hay *ad libitum* together with a commercial concentrate supplement (400-500 g/head).

Oestrus Synchronisation Protocols

The ewes were randomly divided into four treatment groups, as follows:

Group 1 (Long-term FGA sponge plus PMSG injection): For a total of 20 ewes (11 Akkaraman and 9 Awassi), 20 mg FGA, Fluorogestone acetate, sponges (Chronogest® CR, Intervet, Istanbul, Turkey), inserted intravaginally for 14 d, followed by i.m. injection of 400 IU PMSG, pregnant mare serum gonadotropin (Chronogest/PMSG, Intervet) upon the sponge withdrawal.

Group 2 (short-term FGA sponge plus PMSG injection): For a total of 18 ewes (10 Akkaraman and 8 Awassi), 20 mg FGA sponges inserted intravaginally for 8 d, followed by i.m. injection of 400 IU PMSG upon the sponge withdrawal.

Group 3 (Ovsynch): For a total of 17 ewes (10 Akkaraman and 7 Awassi), i.m. injection of 0.004 mg GnRH analogue, Buserelin (Receptal®, Intervet) on day zero followed by an i.m. injection of 125 µg $PGF_{2\alpha}$ analogue, Cloprostenol (Estrumate®, DIF; Istanbul, Turkey) 7 d later, and finally a second injection of 0.004 mg Buserelin on day 9.

Group 4 (short-term FGA plus Ovsynch): For a total of 16 ewes (9 Akkaraman and 7 Awassi), we implemented the FGA sponge inserted along with the injection of 0.004 mg Buserelin (as day zero), followed by an injection of 125 µg Cloprostenol on day 7 upon the sponge withdrawal and, finally the second injection of Buserelin on day 9.

Oestrus Detection, Mating and Fertility

Oestrus signs were monitored four times a day for 15 min each time as described by Ucar et al.[8]. The signs were observed following each protocol for 18-72 h with intervals of 6 h. Teaser rams were used for detection of ewes on heat.

Once the ewes were detected in oestrus, they were then separated from the rest of flock and hand-mated with fertility-proven rams (n=4, for each breed) used rotationally. Duration of oestrus was recorded as the time starting from the onset of oestrus signs until the rejection of mounting ram, as the end of receptivity. The pregnancy rates as well as the rates of lambing and twinning were recorded after parturition. Ongoing pregnancies were determined by considering those ewes that have previously showed oestrus, mated, and non-returned (no oestrus/no mating again) within the subsequent oestrus cycle (between day 14-21 post-mating/synchronisation).

Statistical Analyses

The present data (mean ±SEM) from the onset of oestrus signs and duration of oestrus were analysed by one-way analysis of variance (ANOVA). The analyses of oestrus responses, pregnancy rate as well as lambing and twinning rates were performed using Chi square test. A 95% confidence interval was used. Differences between the synchronisation groups were considered statistically different, using the least significant difference ($P<0.05$). Minitab statistical software programme (MINITAB, Version 11.2; Minitab Inc., Pennsylvania, USA) was used for all statistical analyses.

RESULTS

According to our preliminary statistical analyses (ANOVA), considering the breed as co-factor (Akkaraman *vs.* Awassi), there were no significant differences in any of the reproductive parameters studied. Therefore, all the animals from the two breeds were considered as 'fait-tailed ewes'.

Considering the effect of different oestrus synchronisation groups *(Table 1)*, the onset of oestrus signs, duration of oestrus, pregnancy and lambing rate of ewes were differed significantly between the groups.

The onset of oestrus signs was significantly shorter in the long-term FGA-PMSG group (Group 1) as compared to those in other groups. Also, the duration of oestrus was significantly shorter in the Ovsynch group (Group 3), as compared to those in others.

Additionally, the rates of pregnancy and lambing were significantly lower in the Ovsynch group as compared to those in the long-term FGA-PMSG (Group 1) and short-term FGA-PMSG groups (Group 2). The concerned rates of Ovsynch and short-term FGA plus Ovsynch groups (Group 4) both were also significantly lower than those in the long-term and short-term FGA groups

Finally, for the twinning rates, the values in the Ovsynch protocol were numerically lower as compared to those in others.

DISCUSSION

Our findings indicated that the standard Ovsynch programme was inferior to the conventional FGA-PMSG based on the classical reproductive traits in ewes during the breeding season.

The oestrus rate was relatively higher (87.5%) in the short FGA plus Ovsynch-treated ewes than those (75-82.4%) in other groups. So far, a high oestrus response (75-100%) has been recorded with the FGA-PMSG [19-21]. Indeed, using the FGA-PMSG (control) herein, the oestrus rate was 75%, similar to those (73-77%) in Menze and Dorset ewes [19,22]. A higher (up to 100%) rate was also reported in other breeds [21,23]. Different results might be related to differences in the PMSG dose, the source of progesterone, breed type, etc. The oestrus rate with the short FGA-PMSG was 77.8%, similar to 83.3-88% in Awassi [24] and Ossimi ewes [25]. However, Martemucci and D'Alessandro [26], using the Ovsynch in cross-bred Altamurana ewes in non-breeding season reported a rather lower oestrus rate

Table 1. *The effects of different oestrus synchronisation protocols upon the reproductive traits in fat-tailed ewes*
Tablo 1.*Yağlı kuyruklu koyunlarda farklı senkronizasyon protokollerinin reprodüktif parametreler üzerine etkileri*

Parameters Studied	Synchronisation Groups≠				Statistics		
	Group 1 (n=20)	Group 2 (n=18)	Group 3 (n=17)	Group 4 (n=16)	F-Ratio	P Value	Significance
Oestrus, %	75.0±9.9	77.7±10.1	82.3±9.5	87.5±8.5	0.32	0.812	N.S.
Interval to onset of oestrus*, h	37.2±1.7 [a]	49.7±1.3 [b]	50.5±1.3 [b]	51.0±0.8 [b]	23.77	0.000	P<0.001
Duration of oestrus*, h	27.2±0.8 [b]	27.0±0.8 [b]	24.0±1.2 [a]	27.8±0.8 [b]	3.30	0. 027	P<0.05
Pregnancy rate*, %	93.3±6.6 [b]	85.7±9.7 [b]	42.8±13.7 [a]	57.1±13.7 [a]	4.51	0. 007	P<0.01
Lambing rate*, %	93.3±6.6 [b]	85.7±9.7 [b]	35.7±13.3 [a]	50.0±13.9 [a]	6.24	0.001	P≤0.001
Twinning rate*, %	33.3±12.6	35.7±13.3	7.1±7.1	21.4±11.4	1.31	0.281	N.S.

≠ ***Group 1:*** *" long-term FGA-PMSG";* ***Group 2:*** *"Short-term FGA-PMSG";* ***Group 3:*** *"Ovsynch";* ***Group 4:*** *"Short-term FGA + Ovsynch"; * The number of treated ewes and those in oestrus were 57 only; a total of 14 ewes (of 5, 4, 3 and 2 ewes from Group I-V, respectively) with no signs of oestrus were excluded from the analyses; [a,b] Means (± SEM) having different superscripts within the same row are significantly different from each other (P<0.05);* ***F-ratio:*** *Frequency ratio;* ***P value:*** *Probability value;* ***Significance:*** *Statistical significance;* ***N.S.:*** *not significant (P>0.05)*

(33%), as compared to that in our study (80%). The low rates of oestrus reported might be due mainly to the physiological status and breed type of ewes used therein.

Interval to onset of oestrus upon the sponge withdrawal was markedly shorter in the long-term treatment as compared to others. Herein, the oestrus interval with the long-term FGA-PMSG was 37.2 h, similar to 38.1-44.7 h in Awassi and Dorper ewes [27,28]. Small differences between the durations might be related to a smaller number of animals, shorter duration of sponge administration and a higher dose of PMSG used in the latter [29]. Herein, the interval was 49.7 h after the short-term FGA, similar to 46.2 h in a previous study [30]. However, higher times of 69-70 h [24,25] were also reported elsewhere. Martemucci and D'Alessandro [26], using the Ovsynch reported that the interval was 59 h, while it was 50 h herein. Small differences of oestrus intervals might be due to the individual ewes in different breeds and to different management conditions used.

The duration of oestrus was shorter (24 h) in ewes treated with the Ovsynch, as compared to the other protocols. The durations of oestrus with both the FGA protocols were similar to those in previous studies [27,29]. However, the durations of oestrus observed (24-27.9 h) were shorter than the 34.9 h in Awassi ewes [24], but longer than the 18.7 h in Dorper ewes [28]. Differences might be related mainly to the season as well as to other co-factors (e.g. protocol, feeding, etc.).

The present results of long- or short-term FGA were not markedly different based on the pregnancy, lambing and twinning rates. Likewise, Ustuner et al.[24] reported that both the long- and short-term resulted in similar fertility (22% vs. 20%, resp.) in Awassi ewes during the season. By contrast, the short-term (6 d) treatment resulted in a higher pregnancy rate due likely to the ovulation of newly recruited growing follicles [10]. Therein, however, the rates of pregnancy and lambing with both durations did not differ widely. Meanwhile, pregnancy and lambing rates of Ovsynch, either alone or its combination with short-term FGA, were markedly lower than the FGA-sponge administrations, regardless of its duration. In this respect, we simply consider that, the conventional Ovsynch protocol originally developed for cattle species [15] may be inappropriate for sheep. This could simply be attributable to abrupt species differences in the seasonality and durations of oestrus cycles (21 d *vs.* 16-17 d) for cattle and sheep, respectively [2]. Apparently, the standard Ovsynch protocol should be modified for the ewe, as having different follicular and luteal dynamics. In a broader sense, the variations might also be due to different synchronisation protocols, the type of hormones and breed used [3,8]. In this respect, we considered that progesterone-primed treatment would mimic the luteal phase likely to be followed by the propagation of follicular development upon the sponge withdrawal. Indeed, the FGA-PMSG presumably ensure both an increase in the number of females in oestrus [2,8] and the reduction of short cycles especially in late breeders, as leading them to enter into the breeding season more efficiently. In another study, however, similar lambing rates (47-50%) were reported with either the Ovsynch alone or its combination with the short-term FGA [16,31]. This was also the case herein.

The higher pregnancy/lambing rates observed in the FGA-PMSG based groups may be due to the PMSG action. In fact, the Ovsynch protocol mainly acts as the source of exogenous doubling GnRH (mainly provoking the endogenous LH release), while the PMSG acts mainly as the source of exogenous FSH that seems to be more advantageous over the LH *per se*. It was thought that the standard Ovsynch (injections on day zero-7-9) used in ewes might be inappropriate, as reported elsewhere [16]. Hence, it would be logical that both different days of the injections and the administrative sequence of GnRH (before or after the FGA) should be studied further [16]. However, progesterone plus PMSG might be well enough for stimulating the ovarian cycle, eventually leading to a successful lambing at early, mid- or late in the breeding season [3].

Overall, the findings showed that different synchronisation protocols could markedly alter the onset/interval (shortest with the FGA-PMSG) and duration of oestrus as well as the rates of pregnancy and lambing (lowest with the Ovsynch) in fat-tailed ewes during the breeding season. In that, however, there was only a numerical (but not significant) improvement in reproductive parameters (e.g. lambing) as compared to the standard Ovsynch protocol studied herein. Hence, not only the protocol itself but also the follicular/luteal dynamics should be studied further for a better understanding of its *in vivo* regulatory mechanisms of exogenous hormones in sheep. Nevertheless, our study would provide some clues for the future synchronisation trials in sheep. In this respect, we might recommend some modifications (such as studying the durations between GnRH and $PGF_{2\alpha}$ administrations) of present synchronisation protocols to achieve further improvements in the ultimate fertility. Additionally, future investigations of synchronisation with a higher number of animals from other breeds should also comprise an early determination of the plasma progesterone levels in- and outside the breeding seasons.

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