

Effect of recombinant follicle stimulating hormone (rFSH) on some fertility parameters in Awassi ewes synchronized with PGF₂α in the breeding season

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Summary: The present study was aimed at the investigation of the effects of Recombinant Follicle Stimulating Hormone (rFSH) on fertility in Awassi ewes synchronized with PGF₂α in the breeding season. The fifty thoroughbred Awassi ewes used in the study were injected twice with 0.150 mg of Tiaprost (PGF₂α, Iliren®) at a 10-day interval. Following the second PGF₂α injection, the ewes were randomly distributed into two equal groups. The ewes included in the first group were not subjected to any additional injection and were referred to as the PGF₂α group (Group 1, n=25). The animals included in the second group (Group 2, n=25) were administered 10 IU Follitropin (rFSH, Puregon®), diluted 1:10 with physiological saline, 24 hours before the second PGF₂α injection. Ewes were tested for estrus by a fertile teasing ram three times a day (06.00, 14.00 and 20.00 h), for one hour each time. Ewes exhibiting estrus were mated naturally. Pregnancy was determined by transrectal ultrasonography on 40. d after mating. Estrus was observed at a rate of 88.0 % in Group 1 and 92.0 % in Group 2. The rate of pregnancy was 100 % in the first group and 95.6% in the second group. The rate of lambing was 100 % and 95.5 % in Groups 1 and 2, respectively. Furthermore, twin pregnancy rates were 4.5 % in the first group and 57.1% in the second group. While differences between the groups were determined to be significant (p<0.001), no significant difference was detected for pregnancy, estrus or lambing rates. In conclusion, it was determined that the administration of rFSH to Awassi ewes in the breeding season, 24 hours before the second PGF₂α injection, led to an increase in twin pregnancy rates. Thereby, this protocol can be used as an alternative synchronization method to increase fertility. It has been concluded that further studies are needed to determine the effect of different doses of rFSH on fertility in ewes.

Key words: Awassi ewes, fertility, PGF₂α, rFSH

Aşım sezonunda PGF₂α ile senkronize edilen İvesi koyunlarında bazı fertilitte parametreleri üzerine rekombinant follikül stimüle edici hormonun (rFSH) etkisi

Özet: Sunulan çalışmanın amacı, aşım sezonunda PGF₂α ile senkronize edilmiş İvesi Koyunlarında Recombinant Follikül Stimüle Hormonun (rFSH) fertilitte üzerine etkisi araştırmaktır. Çalışmada kullanılan toplam 50 baş safkan İvesi koyuna 10'ar gün ara ile iki defa 0.150 mg dozunda Tiaprost (PGF₂α, Iliren®) enjeksiyonları yapıldı. İkinci PGF₂α enjeksiyonları yapıldıktan sonra koyunlar rastgele iki eşit gruba ayrıldı. Birinci gruptaki koyunlara ilave bir enjeksiyon yapılmadan (Grup 1, n=25) PGF₂α grubu, ikinci gruptaki (Grup 2, n=25) koyunlara ise ikinci PGF₂α enjeksiyondan 24 saat önce 1/10 oranında serum fizyolojik ile sulandırılıp 10 IU Follitropin (rFSH, Puregon®) enjeksiyonu yapıldı. Östruslar (Saat 06.00, 14.00 ve 20.00) günde üç kez 1'er saat süreyle fertil bir arama koçu tarafından test edildi. Östrus gösteren koyunlara doğal aşım yaptırıldı. Gebelikler doğal aşımı takip eden 40. günde transrektal ultrasonografi ile tespit edildi. Östruslar Grup 1'de % 88.0, Grup 2'de % 92.0 oranında gözlemlendi. Gebelik oranı ise birinci grupta % 100 elde edilirken bu oran ikinci grupta % 95.6 olarak elde edildi. Kuzulama oranı Grup 1'de % 100 Grup 2'de ise % 95.5 olarak gerçekleşti. İkizlik oranları birinci grupta % 4.5, ikinci grupta % 57.1 olarak gerçekleşti. Gruplar arasında farkın önemli olduğu saptanırken (p<0.001), gebelik, östrus ve kuzulama oranları açısından önemli bir fark saptanmadı. Sonuç olarak, aşım sezonundaki İvesi Koyunlarında ikinci PGF₂α enjeksiyondan 24 saat önce rFSH uygulamalarının ikizlik oranını arttırdığı görülmüştür. Böylece bu protokol, fertilitteyi arttırmak amacıyla alternatif bir senkronizasyon metodu olarak kullanılabilir. Koyunlarda rFSH'nin farklı doz uygulamalarının fertilitte üzerine etkisini belirlemek için daha fazla çalışmaların yapılma kanaatine varılmıştır.

Anahtar sözcükler: İvesi koyun, fertilitte, PGF₂α, rFSH.

Introduction

Estrus synchronization programmes in ewes commonly involve the synchronization of luteal regression using PGF₂α treatment (5) double injection of

PGF₂α or its analogues, 11 days (10-14 days) apart (2, 38). PGF₂α induces regression of the corpus luteum and has been used for synchronization of estrus in sheep yielding acceptable (67–86%) lambing rates (21). PGF₂α

is used to synchronize ewes for artificial insemination or natural mating but its application to synchronize ovulation in TAI programmes has resulted in lower pregnancy rates (22). Because high percentage of ewes show estrus symptoms after second administration of PGF_{2α}, this occurs in a wide range of time, around 120 h, which precludes the use of AI in ewes (30).

The most widely used procedures for synchronization and induction of estrus are 11-day (8-12 days) treatment with FGA or MAP- impregnated intravaginal sponges and intramuscular injection of PMSG and PGF_{2α} or its analogues 48 h before sponge withdrawal (18). The administration of eCG in estrus synchronization has been widely used in ewes, usually in conjunction with progesterone. Development of antibodies against eCG after repeated synchronization cause major decreases in fertility, lambing and synchronization rates especially when fixed time AI is applied (6, 17).

Ovulation rates in ewes are increased by injection of gonadotropins such as equine chorionic gonadotropin (eCG), follicle stimulating hormone (FSH), human chorionic gonadotropin (hCG) (8, 12, 26, 40).

Follicular waves resulting from endogenous peaks in FSH secretion emerged and grew in the presence of the growing largest follicle of the follicular waves induced by exogenous FSH (16).

The studies of Dattena et al. (14) and D'Alessandro et al. (13) showed that a single injection of FSH dissolved in (Polyvinylpyrrolidone) PVP induced a superovulatory response similar to that obtained using standard multiple-dose protocols in ewes. D'Alessandro et al. (13) suggested that a single injection of porcine FSH in PVP given 24 h before sponge removal was effective in ewes. The best results were obtained using a single FSH treatment (10 IU) at the end of progesterone treatment in ewes at the beginning of the breeding season (7).

The injection of exogenous gonadotropin is commonly used to enhance the fertility of humans and domestic species (15). The common hormone preparations (31) used for this purpose are gonadotropins extracted from the pituitary tissue of sheep or pigs, the plasma or urine of pregnant women (hCG), eCG, or the urine of post-menopausal women (hMG).

Recombinant FSH preparations are produced by inserting the genes encoding for the alpha and beta subunits of FSH into expression vectors, that are transfected into Chinese hamster ovary cell (24).

Lemke et al. (27) reported that recombinant gonadotropins might be effective alternatives to pituitary or placenta-derived gonadotropins during non-breeding and breeding season superovulation protocols. However, there is no study on the effect of rFSH on the fertility of Awassi ewes in breeding season.

The aim of this study was to investigate the effect of rFSH injection on fertility of Awassi ewes synchronized with PGF_{2α} during breeding season.

Materials and Methods

The study was carried out from September to October of 2007 at the Animal Research Station, Harran University, Şanlıurfa, located at 37° 07'N latitude and 38° 49'E longitudes, with an elevation of 518 m above sea level. A total of 50 cyclic Awassi ewes, 2-5 years of age and weighing 45-55 kg body weight, were used in this study. Ewes were weighed and body condition scored on the day when the first injection of PGF_{2α} was given (on day 0). Body condition scores were evaluated according to Russel et al. (39) as 1 being emaciated and 5 being obese. The ewes were allowed to graze on natural pasture (08.00, 11 00, 13.00, and 21.00 h) and kept overnight in pens. In addition to pasture, each ewe received 250 g barley per day. Water and mineral licks were available *ad libitum*.

A total of fifty thoroughbred Awassi ewes were treated with the PGF_{2α} 10 day protocol consisting of two i.m. doses of a PGF_{2α} analogue (0.150 mg of Tiaprost per dose, Iliren[®], Intervet, Turkey) administered 10 days apart. The ewes were randomly distributed into two equal groups. The ewes included in the first group were not subjected to any additional injection and were referred to as the PGF_{2α} group (Group 1, n=25). The animals included in the second group (Group 2, n=25) were administered 10 IU Follitropin (rFSH, Puregon[®], 50 IU Per ampoule/ml, Organon, Turkey), diluted 1:10 with physiological saline, 24 hours before the second PGF_{2α} injection. The dose of the rFSH preparation (10 IU) used in this study was determined according to the study of Boscós et al. (7), who administered FSH to the ewes synchronized with MAP intravaginal sponges and have shown that the most effective dose of the FSH was 10 IU. Ewes were tested for estrus by a fertile teasing ram three times a day (06.00, 14.00 and 20.00 h), for one hour each time. Ewes exhibiting estrus were mated naturally.

Pregnancy was determined 40 d after the mating by transrectal ultrasonography using a Scanner LC 100 (Pie Medical scanner LC 100 Vet, Netherlands) provided with a 6 MHz linear probe.

The following parameters were recorded:

- Estrus response : (synchronized number of ewes /number of ewes) x 100.
- Pregnancy rate : (number of pregnant ewes/number of ewes mated) x 100.
- Lambing rate : (number of ewes lambing/number of ewes mated) x 100.
- Single rate : (number of single lambing/total number of lambing) x 100.
- Twinning rate : (number of twin lambing/total number of lambing) x 100.

Statistical analyses were run with SPSS software package (Version 13.0 for Windows; SPSS Inc., Chicago, IL) (41). The results were analyzed with *Chi-Square* test to compare estrus response, pregnancy, lambing, single and twinning rate between the two groups. Differences were considered significant at a level of $P < 0.05$.

Results

Results of the present study are shown in Table 1. Estrus behavior was observed to be within 120 h after the second $\text{PGF}_{2\alpha}$ injection in 88.0 % (22/25) of Group 1 ewes and in 92.0 % (23/25) of Group 2 ewes. The proportion of ewes that exhibited estrus after estrous synchronization did not differ ($P > 0.05$) between Group 1 and 2.

Pregnancy rates were 100.0 %, and 95.6 % in Groups 1 and 2, respectively. Lambing rates in Groups 1 and 2 were 100.0 % and 95.5 %, respectively. There were no statistically significant differences ($P > 0.05$) for the pregnancy and lambing rates, between the treatment groups, as demonstrated in Table 1. Single and twinning rates in Groups 1 and 2 were obtained 95.5 % (21/22), 42.9 % (9/21) and 4.5 % (1/22), 57.1 % (12/21), respectively. The differences between groups were statistically significant.

Table 1. Some reproductive parameters in Awassi ewes after two doses of $\text{PGF}_{2\alpha}$ administered 10 days apart or $\text{PGF}_{2\alpha} + \text{rFSH} + \text{PGF}_{2\alpha}$ in breeding season.
Tablo 1. Aşım sezonunda 10 gün ara ile uygulanan çift doz $\text{PGF}_{2\alpha}$ ve $\text{PGF}_{2\alpha} + \text{rFSH} + \text{PGF}_{2\alpha}$ enjeksiyonlarından sonra İvesi Koyunlarında bazı reproduktif parametreler.

Parameter	Group 1 ($\text{PGF}_{2\alpha} + \text{PGF}_{2\alpha}$)	Group 2 ($\text{PGF}_{2\alpha} + \text{rFSH} + \text{PGF}_{2\alpha}$)
Number of ewes (n=50)	n=25	n=25
Estrus response (%)	88.0 (22/25)	92.0 (23/25)
Pregnancy rate (%)	100.0 (22/22)	95.6 (22/23)
Lambing rate (%)	100.0 (22/22)	95.5 (21/22)
Single rate (%)	95.5 ^a (21/22)	42.9 ^b (9/21)
Twinning rate (%)	4.5 ^c (1/22)	57.1 ^d (12/21)

Means in the same row with different letters (a,b,c,d) differ significantly ($P < 0.001$)

Discussion and Conclusion

The hypophysial gonadotropin follicle-stimulating hormone (FSH) plays an important role in the regulation of oocyte maturation (35), and a key component for growth of ovulator follicles in ewes (32, 36). The follicle-stimulating hormone (FSH) is a heterodimeric protein composed of two subunits, α and β , which are non-covalently linked (37).

Single-chain analogs of conventional gonadotropins are produced by transfecting Chinese hamster ovary cells with a gene construct that links the coding portions

of the α and β subunit genes (20). The follicular response induced by a single injection of the chimeric protein also reflects the long-lived nature of the single-chain gonadotropin analogs in sheep (9). Lemke et al. (27) demonstrated that the single-chain analogs of the human gonadotropins were also active in sheep.

FSH has been shown to enhance the in vitro fertilization and embryonic development of immature oocytes of sheep, cattle and other mammals (11). It has been shown that commercially available FSH preparations vary in the content of FSH and LH. High LH content or low FSH: LH ratio interferes with fertilization of ova and embryo viability (7, 28, 34).

In the last few years several authors have reported the effect of recombinant gonadotropins devoid of LH activity on human and bovine embryonic development (3, 4). Therefore the rFSH preparation without intrinsic LH activity was used in the present study.

In the present study, estrus was successfully induced and synchronized by two $\text{PGF}_{2\alpha}$ doses at 10-days interval and administrated 10 IU of rFSH 24 h before the second $\text{PGF}_{2\alpha}$ injection in the breeding season. In this study, the estrus response was 88% and 92.0% in the groups 1 and 2, however the difference between groups was not significant.

Boscos et al. (7) observed 90.7 % and 92.5 % estrus response in ewes treated at the beginning of the breeding season with MAP intravaginal sponge for 12 days and a single i.m. treatment of 10 IU FSH or 400 IU eCG, at the time of sponges removal, respectively. They also obtained an estrus response of 62.5 % and 37.5 % in the ewes treated with 5 IU or 2.5 IU FSH, respectively. Tsakalof et al. (42) obtained 100 % estrus synchronization in Chios crossbred ewe lambs after a single treatment of 500 IU FSH, administrated at the end of a 15-day progesterone treatment. Cardwell et al. (10) reported an estrus synchronization success of 81 % and 88 % in the ewes received only norgestomet implant for 10 d or implant plus i.m. injection of PMSG (500 IU) at implant removal.

The estrus response obtained in this study with $\text{PGF}_{2\alpha}$ plus FSH was higher than that reported by Menchaca et al. (33) who obtained 80 % estrus response in ewes 25 and 48 h after treatment with two $\text{PGF}_{2\alpha}$ doses at 7-day interval. In the present study the estrus response in two groups were comparable to those reported by other researchers, who used progesterone sponge devices (19, 23). The findings of the present study suggested that FSH treatment 24 hours before the second $\text{PGF}_{2\alpha}$ injection in estrous synchronization did not alter the estrus response in Awassi ewes.

In the present study pregnancy rate in the ewes treated with rFSH was not different from that the ewes synchronized with two $\text{PGF}_{2\alpha}$ doses at 10-days interval. The pregnancy rates (100 % two $\text{PGF}_{2\alpha}$ doses at 10-day

intervals, 95.6 % in the PGF_{2α}+rFSH+PGF_{2α} protocol) for the synchronized estrus were higher than those for Akkaraman (84.6%) crossbred ewes (5) and for Awassi ewes (85.7 %) in another study (19). The results suggested that two PGF_{2α} doses at 10-days interval, rFSH administration 24 h before second PGF_{2α} injection resulted in acceptable pregnancy rates for hand mated programmers during the breeding season in ewes.

Lambing rates were not significantly different between both groups. The mean lambing rate obtained in this study (100.0 % for Group 1 and 95.6 % for Group 2) was similar to Berrichon ewes (93.8%) but higher than in Chios ewes (63.0 %) treated with a purified FSH (10 IU) after sponges applications (7). In the present study, twinning rates were significantly (P<0.001) increased by rFSH treatment in Group 2 (57.1 %) compared to two PGF_{2α} doses with 10-days interval which received only PGF_{2α} (4.5 %). In addition, Ahmad et al. (1) reported that ovulation rates were increased in anestrous ewes treated with 25 mg of progesterone at the time of ram introduction and a single injection of 8 mg of FSH-P in propylene glycol 12 h before injection of 20 mg of prostaglandin F_{2α} i.m. on d 14 after the rams were introduced. This was probably due to an increase in ovulation rate and in concentrations of circulating steroids, which might affect embryo quality and uterine environment at the time of implantation (25). Because FSH is used in superovulation regimens (14, 29), McNatty et al (32) shows that ewes about to have twin ovulations tend to have higher concentrations of FSH in the luteal phase, but lower concentrations in the follicular phase, than about to have single ovulations.

The results of this study showed that the administration of rFSH 24 h before second PGF_{2α} injection increased the twinning rate in Awassi ewes. Therefore this protocol could be used as an alternative synchronization method for increasing the fertility in Awassi ewes. Further studies are required for determining the effect of rFSH on the fertility administrated in different doses and other sheep breeds.

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