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RESEARCH ARTICLE

Effects of Long and Short-Term Progestagen Treatments Plus GnRH Followed by TAI on Fertility Parameters in Lactating Hair Goats during the Transition Period

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Summary

The aim of this study was to determine the efficiency of the progestagen containing intravaginal sponges used for 6 or 12 days at timed artificial insemination (TAI) in Hair Goats during the transition period, the effect of GnRH administrations at the time of TAI on fertility was also investigated. In this study 104 lactating hair goats aged between 2-5 years old minimally primiparous was used as animal materials. All of the goats received 30 mg fluorogestone acetate (FGA) impregnated vaginal sponges. The sponges were inserted into vagina for 6 days in the short-term (ST, n=52) group also for 12 days in the long-term (LT, n=52) group. The goats received 400 IU PMSG and 0.075 mg of cloprostenol at the time of sponge removal. The goats in both the ST and LT groups timed artificially inseminated 48 h after sponge withdrawal with fresh cooled semen. At the time of artificial insemination, the subgroups ST1 (n=24) and LT1 (n=22) were maintained as controls, the subgroups ST2 (n=24) and LT2 (n=23) received 5 mcg of buserelin acetate. Pregnancy and twinning rates of the ST1, ST2, LT1 and LT2 groups were 37.5%, 41.6%, 40.9%, 47.8% and 22%, 30%, 11%, 18%, respectively. The litter sizes were 122%, 130%,111% and 118% in the ST1, ST2, LT1 and LT2 groups, respectively. The parturition rates were 100% in all treatment groups. Pregnancy rates, twinning rates and litter sizes were not statistically different among the groups (P>0.05). The results of this study indicated that, a TAI protocol without detection estrus seems to be a suitable administration for short and long progesterone sponges aplications in lactating hair goats during the transition period. In addition, progesteron and AI without estrus detection procedure may be useful alternative to traditional synchronization program using progesteron and AI after estrus detection. Also, GnRH addition to progesterone sponges did not improve reproductive paramaters.

Keywords: *Goat, Timed artificial insemination, Fluorogestone acetate, GnRH*

Geçiş Döneminde Laktasyondaki Kıl Keçilerinde Kısa ve Uzun Süreli Progestagen Uygulamalarıyla Sabit Zamanlı Suni Tohumlamayla Birlikte GnRH Kullanımının Fertilite Parametreleri Üzerine Etkisi

Özet

Çalışma, geçiş döneminde laktasyondaki keçilerde 6 veya 12 gün süreli kullanılan progestagen içeren intra-vaginal sünger ugulamalarının sabit zamanlı tohumlamalardaki etkinliği aynı zamanda sabit zamanlı tohumlamaların GnRH ile kombine edilmesinin fertilite üzerine etkilerini belirlemek amacıyla yürütüldü. Araştırma yaşları 2-5 arasında değişen, en az bir doğum yapmış 104 baş Kıl Keçisi üzerinde yürütüldü. Keçilerin tamamına 30 mg fluorogeston asetat (FGA) içeren sünger uygulandı. Süngerler kısa dönem (ST, n=52) grubunda 6 gün, uzun dönem grubunda (LT, n=52) ise 12 gün süreyle vaginada tutuldu. Süngerlerin çıkartıldığı gün keçilere 400 IU PMSG ve 0.075 mg cloprostenol kas içi enjekte edildi. Keçilerin tamamı süngerler çıkartıldıktan 48 saat sonra soğutulmuş taze sperma kullanılarak tohumlandılar. Tohumlama zamanında ST grubundaki keçiler ST1 (n=24) ve ST2 (n=24), LT gruplarındaki keçiler ise LT1 (n=22) ve LT2 (n=23) olmak üzere kendi aralarında rastgele iki alt gruba ayrıldılar. ST1 ve LT1 kontrol grubu olarak bırakıldı, ST2 ve LT2 grubundaki keçilere tohumlama anında 5 mcg buserelin asetat uygulandı. ST1, ST2, LT1 ve LT2 gruplarında sırasıyla gebelik oranları %37.5, %41.6, %40.9, %47.8, ikizlik oranları %22, %30, %11, %18, yavru verimleri ise %122, %130, %111, %118 olarak saptandı. Bütün çalışma gruplarında doğum oranları %100 olarak tespit edildi. Gruplar arasında gebelik oranı, doğum oranı, ikizlik oranı ve yavru verimleri arasında farklılık önemli değildi (P>0.05). Çalışmanın sonuçları kıl keçilerinde geçiş döneminde östrüs tespiti yapılmadan uygulanan sabit zamanlı tohumlamalarda hem kısa hem de uzun dönem vaginal sünger uygulamalarının kullanılabileceğini göstermektedir. Buna ek olarak progesteron kullanılarak yapılan östrüs tespitsiz sabit zamanlı tohumlamanın geleneksel östrüs tespiti yapılarak uygulanan suni tohumlama uygulamalarına iyi bir alternatif olabileceği kanısına varıldı. Aynı zamanda tohumlama anında yapılan GnRH uygulamalarının reprodüktif parametreleri artırmadığı kanısına varıldı.

Anahtar sözcükler: *Keçi, Sabit zamanlı tohumlama, Florogeston asetat, GnRH*

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INTRODUCTION

Goats are seasonally polyoestrous animals, of which the sexual cycle ranges between 19-24 days. In the northern hemisphere, where Turkey is located, goats enter the breeding season either at the end of summer or at the beginning of autumn [1]. The duration of the oestrus phase is 24-36 h and ovulation occurs 30-36 h after the onset of oestrus [2].

In small ruminants, oestrous synchronization enables the insemination of a large number of animals resulting in a shorter period of time. Thereby, the holding may spare a shorter period of time for insemination and benefit from a more efficient labour force. As the timing of antiparasitic treatment, vaccination and feed ration alterations coincides with the breeding season; oestrous synchronization facilitates process management. Furthermore, oestrous synchronization enables the scheduling of births to time periods, when the demand for animal products increases and prices display an upward trend, thereby providing market advantage to the farmer [2-4].

The most common method used for oestrous synchronization in small ruminants during both the breeding and non-breeding seasons is treatment with progestagen containing intravaginal sponges [5]. It has been reported that, in sheep and goats, vaginal sponges can be applied for a period of 6-14 days [6-8]. It has been indicated that, short-term intravaginal sponge treatment for 5-8 days during both the breeding and non-breeding seasons yields success in the induction and synchronization of oestrus in goats [9-11]. Also the short term applications were found to be more useful for the flexiblity of the usage in the field conditions [12].

In small ruminants, artificial insemination enables better use of breeder males of highly productive breeds, and thereby, contributes to the success of genetic improvement programmes [13,14]. Furthermore, in these species, oestrous synchronization facilitates the organization of artificial insemination operations and reduces costs. Timed artificial insemination (TAI) eliminates the necessity for detecting oestrus, yet, it is reported that satisfactory fertility rates can be obtained only with hormonal treatment. By means of hormonal treatment, control is established on both follicular development and luteal activity for the synchronization of ovulation [15].

It is reported that the combined use of GnRH and its analogues with other hormones in synchronization programmes of farm animals is effective in the synchronization and induction of ovulation [16,17]. Walker et al.[18] and Epplestone et al.[19] reported that in sheeps, the GnRH administrations 24-44 h after the progestagen treatment is effective in synchronization of oestrous but did not increase the fertility. Sarıbay et al.[20] suggested that GnRH administration to goats 48 h after intravaginal sponge withdrawal did not increase pregnancy rates.

In Hatay province, the farmers requires estrus synchronization through the transition period in goats to advance the start and decrease the length of the breeding period, increase profit through earlier production (milk, kids), exessive benefit from pasture, reduction of peri-natal mortality. Most of the reproductive research was conducted on the artificial manipulation of estrus by using hormones either during the anoestrous period or during the natural breeding season [2,3,6,11,12,20]. However, the information including the efficiency of synchronization regimes on natural breeding and anestrus seasons are exposed, the information regarding synchronization efficiency and fertility of TAI in goats induced by hormonal treatments during the transition period (between the breeding to the non-breeding season) is non-existent. Also, the interval from the sponge removal to estrus response and the LH surge time was found to be similar in anestrus and seasonal goats these parameters were detected to be longer in transition period [21]. GnRH was considered to shorten these periods and increase the fertility. For these reasons, it was aimed to determine the efficiency of the progestagen containing intravaginal sponges used for 6 or 12 days in TAI in goats during the transition period, the effect of GnRH administrations at the time of TAI on fertility was also investigated.

MATERIAL and METHODS

This research was carried out in at mid July of 2010 in Hatay Province. Hatay is located in the eastern Mediterranean region of Turkey (36°53" North latitude and 35°40" East longitude) during the transition period (June to September). Throughout the study, the average annual temperature was 35ºC at daytime and 25ºC at night-time.

The present study was conducted on 104 healthy hair goats, aged between 2-5 years old and had no history of fertility problems. Throughout the study period, the goats were milked once a day in the mornings and were grazed on the pasture between 08.00-18.00 h. The goats also offered 150 g of concentrate feed/goat/day in the evenings. Mineral salt and water were offered ad l ibitum.

With an aim to induce or synchronize oestrus, all of the goats were treated with intravaginal sponges impregnated with 30 mg of FGA (Crono-gest, Intervet, Turkey). The goats were randomly allocated to two equal groups. Mean body weight of the groups were 48.6±4.3 kg, 49.0±4.0 in the ST and LT groups respectively.

In the group, which received short-term treatment (ST, n=52), the intravaginal sponges were left in place for a

period of 6 days, whilst in the long-term treatment group (LT, n=52), the sponges were left in place for a period of 12 days. Furthermore, on the day the intravaginal sponges were removed, the animals were administered with 400 IU of PMSG (Cronogest PMSG, Intervet, Turkey) and 0.075 mg of cloprostenol (Dalmazin, Vetaş, Turkey) by intramuscular route.

The semen was collected from three bucks by an electroejaculator (Ruakura Ram Probe, Manufactured For Shoof International Ltd, New Zealand) which produced a 15 volt electricity. After restraining the buck, the lightly lubricated probe is inserted into the rectum, positioned on the midline and far enough forward to partially overlay the seminal vesicles and ampulle. As soon as ejaculation occurs the power was turned off and the probe was removed [22].

Each ejaculate was immediately evaluated to determine the motility of the semen. The percentage of sperm motility was evaluated using a light microscope with heated stage. Dilution was performed in one step by addition of sterilized skimmed cow milk (Pınar, Turkey), containing 1.000 µg procain penicillin and 1.250 µg of dihydrostreptomycin sulfate (Dipenisol, Bayer, Turkey) per ml of diluent. The semen was stored in straws which contained $1X10^8$ motile cells/0.25 ml straw at 4-6°C in the refrigerator up to 12 h from collection. The viability (motility) of the semen sample was evaluated prior to use for insemination [23].

The goats in both the ST and LT groups were intra-cervically inseminated with cooled semen ($1X10^8$ motile cells/0.25 ml straw) 48 h after the withdrawal of the sponges. The intra- cervical inseminations were carried out using a vaginal speculum and an insemination catheter labelled for cattle. The catheter was introduced and advanced deep enough to deposit the semen into the cervix [13].

Prior to artificial insemination, the goats included in the ST and LT groups were randomly allocated to two sub-groups, hereinafter referred to as ST1 (n=24) + ST2 (n=24), and LT1 (n=22) + LT2 (n=23), respectively. The subgroups ST1 and LT1 were maintained as control, whilst the sub-groups ST2 and LT2 were administered with 5 mcg of buserelin acetate intramuscularly (Receptal, Intervet, Turkey) at the time of artificial insemination. The artificial inseminations were performed intracervically by the same operator within 2 h.

Reproductive parameters were evaluated according to the formulations given below:

Pregnancy rate: *(number of pregnant goats/total number of goats in the group) x 100*

Parturition rate: *(number of goats exhibiting parturition/ number of pregnant goat) x 100*

Twinning rate: *(number of twin kid parturition/number of pregnant goats) x 100*

Litter size: *(number of kids/number of goats exhibiting parturition) x 100*

In all animals, pregnancy was diagnosed by trans-abdominal ultrasonography using a sector array 5-7.5 mHz transducer at approximately 50 d following TAI.

Pregnancy rates, parturition rates, twinning rates and litter sizes were assessed using the chi-square (X^2) test [24].

RESULTS

During the study, 2 goats (3.8%) in group (ST), 6 goats (11.5%) in group (LT) were excluded from the study due to losing the the intravaginal sponges. Furthermore 3 animals, (one in LT two in ST groups) were not assessed due to the diseases.

In the subgroups ST1, ST2, LT1 and LT2, pregnancy rates were 37.5%, 41.6%, 40.9% and 47.8%, respectively, whilst the parturition rates was 100% in all groups. Twinning rates were 22%, 30%, 11% and 18% and litter sizes were 122%, 130%, 111% and 118% in the subgroups ST1, ST2, LT1 and LT2, respectively. No statistical difference was obtained for pregnancy, parturition and twinning rates or litter sizes among the groups (P>0.05), and it was shown in *Table 1*.

Table 1. *Pregnancy, parturition, twinning rates and litter sizes of the subgroups*
Tablo 1. *Alt grupların gebelik, doğum, ikizlik oranları ve yavru verimleri*

Parameters (%)	**Subgroups**			
	ST1 (n=24)	**ST2 (n=24)**	**LT1 (n=22)**	**LT2 (n=23)**
Pregnancy rate (%)	37.5 (9/24)	41.6 (10/24)	40.9 (9/22)	47.8 (11/23)
Parturition rate (%)	100 (9/9)	100 (10/10)	100 (9/9)	100 (11/11)
Twinning rate (%)	22 (2/9)	30 (3/10)	11 (1/9)	18 (2/11)
Litter size	122 (11/9)	130 (13/10)	111 (10/9)	118 (13/11)

(P>0.05)

DISCUSSION

During the study, the rate of lost intravaginal sponges was determined as 3.8% (2/52) and 11.5% (6/52) in the ST and LT groups, respectively. Wildeus [25] reported that the rate of sponge loss should be lower than 10% in sheep and goats. The rate of sponge loss determined in the LT group (11.5%) in the present study was slightly higher than the rates Wildeus [25] reported. It has been reported that, the extended duration of intravaginal sponge treatment increases the risk of the sponges loss [9,26].

In the present study, the pregnancy rates of the subgroups ST1 and LT1 were determined as 37.5% and 40.9%, respectively (P>0.05). Menchaca and Rubianes [27] obtained 49.4% pregnancy rate in goats treated with intravaginal sponges (60 mg MAP) for 5 days and were inseminated with cooled semen ($200x10^6$ motile cells/straw) 48 h after sponge withdrawal. The dose of cooled semen in this research was $100x10^6$. The pregnancy rate; achieved by Rubianes et al.[28] was 45%, following the artficial insemination with fresh semen that was applied 48 h after the cessation of progestagen treatment in goats treated with progestagen containing sponges for 6 days. Cooled semen was used in this study. The pregnancy rate of the goats reported by Dogan et al.[29] was 71.5% in goats which were treated with intravaginal sponges (40 mg, FGA) for 11 days also combined with PMSG (500 IU) and cloprostenol (125 mcg) and inseminated twicely 36 and 48 h following the sponge removal. The goats in this study was in breeding season and were not lactating. The goats in our study were both inseminated once and were in lactation. Additionally, this study was carried out during the transition period. The pregnancy rates determined in the study, in ST1 (37.5%) and LT1 (40.9%) groups followed by TAI were lower than the rates previously reported by the abovementioned researchers [27-29]. The differences may be due to the dose and storage of semen [27], the number of inseminations [29], lactation [29], the transition period [21] and TAI without detection of estrous.

It has been indicated that, short-term intravaginal sponge treatment for 5-8 days during both the breeding and non-breeding seasons yields success in the induction and synchronization of oestrus in goats [9-11]. Ozer and Dogruer [12], reported that the form and the duration of application of progestagens did not effect any fertility parameters, also the short term applications were found to be more useful for the flexiblity of the usage under field conditions. The results of the study showed that short and long term applications did not effect the fertility parameters and no statistical difference was obtained among the groups (P>0.05).

Pierson et al.[21] reported that the intervals from sponge removal to estrus and the LH surge were not significantly different between the breeding and non-breeding seasons. Only in the transition period, these intervals were found to be significantly longer. In efforts aimed at synchronization, the administration of GnRH shortens the LH surge, renders the occurrence of the LH peak more regular and increases the rate of ovulation by shortening the period between the withdrawal of intravaginal sponges and ovulation [16,30,31]. In sheeps the GnRH administrations 24-44 h after the progestagen treatment synchronized estrous but did not increase the fertility [18,19]. Medan et al.[32] reported that in which implants containing norgestomet were placed into the ear of goats for 11 days in the non-breeding season and the animals were administered with 125 mg of cloprostenol 24 h prior to implant removal with one group having been administered with 10.5 mcg of GnRH 24 h after implant removal, the pregnancy rates in the group administered with GnRH and the group that did not receive GnRH as 70% and 57.5%, respectively. On the other hand, Reyna et al.[33] reported that oestrous synchronization in sheep by means of intravaginal sponge treatment (30 mg, FGA) for 12 days in the non-breeding season associated with the administration of 400 IU of PMSG and finally, 40 mcg of GnRH 36 h after sponge withdrawal, yielded similar pregnancy rates in the animals administered with GnRH (23%) and those maintained as controls (21%). The findings obtained in the present study for the effects of the combination of GnRH administration at TAI on pregnancy rates in goats were in agreement with the findings obtained by Reyna et al.[33] in sheep, but were different from the findings reported by Medan et al.[32] in goats. The difference may have arisen from the type of progestagen used, the timing of GnRH administration and the GnRH dose administered and the hormonal status occurring in the transition period. Sarıbay et al.[20] reported that, in goats, which were treated with intravaginal sponges (30 mg, FGA) for 14 days in the non-breeding season and were administered with 500 IU of PMSG and 0.075 mg of cloprostenol 48 h prior to sponge withdrawal with one group having been administered with 5 mcg of GnRH 48 h after sponge withdrawal, the pregnancy rates of the groups that received GnRH and did not receive GnRH were 38.9% and 33.3%, respectively. Furthermore, the researchers suggested that [18,19] suggested that GnRH administration to sheep 24-44 h after intravaginal sponge withdrawal could improve the synchronization of ovulation, but did not increase pregnancy rates. Husein and Kridli [34] determined that the administration of 50 mcg of GnRH to sheep 28 h after intravaginal sponge withdrawal did not increase fertility.

In the present study, the pregnancy rates, twinning rates and litter sizes of the ST1, ST2, LT1 and LT2 subgroups were determined as 37.5%, 22%, 122%; 41.6%, 30%, 130%; 40.9%, 11%, 111%; and 47.8%, 18%, 118%, respectively. The difference at these fertility parameters among the groups was statistically insignificant (P>0.05).

The results of this study indicated that, a TAI protocol

without detection estrus seems to be a suitable administration for short and long progesterone sponges aplications in lactating hair goats during the transition period. In addition, progesteron and AI without estrus detection procedure may be useful alternative to traditional synchronization program using progesteron and AI after estrus detection. Also, GnRH addition to progesterone sponges did not improve reproductive paramaters.

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