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RESEARCH ARTICLE

Short-acting Deslorelin Implant (Ovuplant®) could not Sufficiently Induce Fertile Cycles of Coloured Mohair Goats in Suckling (Anoestrous) Season [1]

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Summary

It is known that the pituitary FSH and LH stores are dramatically depleted during the anoestrous in seasonal breeders. Hence, the aim of this study was to evaluate the effect of deslorelin implant (a GnRH analogue) to induce oestrus in suckling goats. Suckling goats (n=21) of coloured Mohair breed were randomly assigned into two trial groups: Goats in Group I (n=11, Ovuplant®) received subcutaneously the implant (2.1 mg deslorelin), while animals in Group II (n=10, control) received no treatment. Along with the implant administration, serum progesterone (P_4) concentrations were determined (every other day up to 12 d) by RIA method. Within 4 days of implant administration, there were significantly higher ($P<0.001$) rates (100%) of pro-oestrus signs (attractiveness without mating) in Group I, than those in controls (zero %). The signs sustained for 9 days without oestrus (mating) in the vast majority (10/11) of implant-treated animals. But, an exceptional goat was in receptive oestrus commenced from the third day following the onset of pro-oestrus. Even so, there was no pregnancy post-mating. The P_4 concentrations between the groups remained below 1 ng/ml, regardless of implant treatment. Findings suggest that; i) for the P_4 concentrations, there was no marked difference between implant-treated and control groups, as remaining below 1 ng/ml all, and ii) short-acting deslorelin implant could not induce fertile oestrus cycles in coloured Mohair goats during the suckling (anoestrous) season.

Keywords: *Deslorelin, Oestrus Synchronisation, Suckling, Goat*

Kısa-Etkili Deslorelin İmplant (Ovuplant®) Uygulaması Emzirme (Anöstrus) Sezonundaki Renkli Tiftik Keçilerinde Fertil Östrusu Yeterince Uyaramaz

Özet

Hipofiz FSH ve LH depolarının, mevsimsel östrus gösteren ırklarda anöstrus sezonu boyunca etkili bir biçimde azaldığı bilinmektedir. Dolayısıyla, bu çalışmanın amacı laktasyondaki keçilerde östrusu uyarmak için deslorelin implant (bir GnRH analoğu) etkisini araştırmaktır. Toplam 21 hayvandan oluşan renkli Tiftik keçisi laktasyon döneminde rastgele iki farklı gruba ayrıldı: Grup I'deki keçilere (n=11, Ovuplant®) derialtı implant (2.1 mg deslorelin) verilirken, Grup II'deki hayvanlara (n=10, kontrol) ise herhangi bir uygulama yapılmadı. İmplant uygulamasıyla birlikte, RIA metoduyla serum progesteron (P_4) konsantrasyonları belirlendi (günaşırı 12. güne kadar). İmplant uygulamasından sonraki 4 gün içerisinde önemli ($P<0.001$) düzeyde pro-östrus (teke ilgisi var, çiftleşme yok) belirtileri ortaya çıktı (Grup I: %100, kontrol: %0). Anılan belirtiler, implant uygulanan hayvanların büyük çoğunluğunda (10/11) 9 gün boyunca devam etti. Ancak, bu keçilerden biri pro-östrus başlangıcını izleyen 3. günde östrus göstererek erkeği kabul ettiyse de, çiftleşme sonrası gebelik şekillenmedi. Öte yandan, serum P_4 konsantrasyonu implant uygulamasından bağımsız olarak her iki grupta 1 ng/ml'den daha az bulundu. Elde edilen bulgulara göre; i) P_4 konsantrasyonu yönünden, implant uygulamasına bakılmaksızın gruplar arasındaki farkın belirgin olmayıp, her iki grupta 1 ng/ml düzeyinin altında kaldığı, ve ii) kısa etkili deslorelin implant uygulamasının laktasyondaki (anöstrus) renkli Tiftik keçilerinde fertil östrus sikluslarını uyarmada yetersiz olduğu kanısına varıldı.

Anahtar sözcükler: *Deslorelin, Östrus Senkronizasyonu, Emzirme, Keçi*

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INTRODUCTION

Goats are seasonally polyoestrous animals. They can be regarded as fall breeders with sexual activity occurring between September and January in temperate regions in northern latitudes [1]. Similarly, goats mate between September and December in Eastern Anatolia, Turkey [2]. Breeding outside the normal mating season may be desirable for animals that; **i)** have failed to conceive, **ii)** have previous history of abortion, or **iii)** are from a given commercial flock where the maintenance of a continuous production is preferred throughout the year [3].

For oestrus induction in goats, some methods have been indicated during the anoestrus season. Of these, abrupt introduction of sexually-active bucks [4] and artificial alteration of photoperiod [5] are among the strategies used widely.

Progestagens are the primary hormones to induce ovulation. However, it is possible to obtain successful pregnancy using intravaginal sponges or subcutaneous norgestomet ear implants in seasonally anoestrus goats [6]. Apparently, they could be used alone, but some administrations were supplemented with $PGF_2\alpha$ and eCG for a superior outcome [7-11]. The eCG alone was reported to be inadequate to ensure satisfactory synchrony in deep anoestrous [12]. By contrast, Karaca et al.[13] observed in deep anoestrus season that 80% of goats treated with eCG only for 6 days, exhibited standing oestrus, ultimately resulting in 60% pregnancy.

In mares, analogues of hCG and GnRH are widely used for stimulation [14-16]. For controlling the time of ovulation, the use of hCG more than once has led to lower activity of ovarium due to corresponding antibody formation. Considering its additional shortage of market supply, more intensive studies were conducted in the 1990's [17-19]. It is presumed that single dose administration of GnRH would not be sufficient enough for the ovulation expected due to the pre-ovulatory LH peak sustaining for a prolonged time in mares [17,20-22]. For this, novel GnRH analogues have been manufactured for controlled release for a short-time. One of them is the Ovuplant® device, containing 2.1 mg Deslorelin.

Deslorelin implant can be used for long durations and it acts without side-effects in goats [23]. By long-time use, it ensures down-regulation of gonadotropic GnRH receptors in hypophysis, thereby suppressing the reproductive functions successfully in dogs [24-27] and bucks [23]. Likewise, the implant may be used for; **i)** transient contraception or suppressing aggression in wild carnivores [24], **ii)** controlling population increase of macropodides (kangaroo and wallabies) and koala, as more humane alternative than the fatal control techniques [28,29], **iii)** suppressing aggressive mating behaviour of endangered male sea lions [30] and finally **iv)** controlling the oestrus cycle or its reversible suppression allowing for the AI at a desirable time in heifers and cows in beef enterprises [31,32].

In the literature, however no study using the implant could be found in goats during suckling (anoestrous) season. In an exceptional study of Yıldız et al.[23], the implant was used only for its transient suppressive effects in sexually-active bucks. Therefore, the objective of this study was to evaluate the effectiveness of short-acting GnRH implant for inducing oestrus in coloured Mohair goats during suckling season.

MATERIAL and METHODS

Animals

Twenty-one multiparous, lactating coloured Mohair goats in suckling (anoestrous) season were used. Prior to the study, goats were housed in indoor shelters during winter and had kidding in mid-March onwards. After kidding, the kids remained with mothers and they were allowed to graze together on pasture in May.

Animals were housed indoors at night and medium quality grass hay with water provided *ad libitum*. Instead of milking, goats were suckled by the kids. The bucks, as normally remain together with females continuously, were separated starting from the first day of implant administration. Animals were exposed to natural lighting/summer conditions (with 11.7 h day-light in June 2010) in Van province (altitude 1.727 m), Turkey.

Experimental Design

Study was conducted in June, as the suckling (anoestrous) season. In this region, natural breeding season normally starts in September. Suckling goats (n=21) were randomly assigned into two groups. Goats in Group I (n=11, treatment) were inserted with Ovuplant® (GnRH - Peptech Animal Health, Australia), short-acting GnRH analogue containing 2.1 mg deslorelin, under the neck skin. Animals in Group II (n=10, control) received no implant.

Blood Collection

For determination of P_4 levels, blood samples were collected by jugular venepuncture into the plain tubes at the beginning of deslorelin treatments on June 6th (as Day zero) and continued on Days 2, 4, 6, 8, 10 and 12. Samples were retained at 4°C overnight, and sera were harvested in the following day by centrifugation (3.000 × *g* for 10 min). Sera were stored at -24°C until the analysis by RIA (Radioimmunoassay). A commercially available Test Kit (Progesterone RIA, DSL-USA) was used, at a sensitivity of 0.12 ng/ml, according to the user's manual.

Determination of Pro-oestrus, Oestrus and Pregnancy

Starting from Day 2 of the implant treatment, goats in both groups were introduced to bucks twice daily for duration of 30 min each to detect the signs of standing oestrus, if any. Oestrus detections by teaser bucks (with

previously proven-fertility) were continued for two weeks. Females were considered to be in pro-estrous when they stood nearby the teaser buck, had tail flagging but did not allow for mating persistently. Goats were considered to be in oestrus only when they stood for a mounting buck and allowed for mating. The oestrous goats were hand-mated and the dates of mating were recorded.

Pregnancies were determined by trans-rectal real-time ultrasonography, with a 5 MHz trans-abdominal transducer (HONDA HS -1500, Japan), 30 d after the first mating.

Statistical Analysis

Data from the oestrous signs (including those of pro-oestrus), pregnancy rates and progesterone concentrations of goats in treated and control groups were analysed by regression analysis using MINITAB [33]. Differences of means (±SEM) between the experimental groups were considered significant when P<0.05.

RESULTS

The rates of pro-oestrus, oestrus as well as pregnancy rates following the implant treatment in suckling Mohair goats are given in *Table 1*.

During the 4 days of implant administration, there were significantly higher (P<0.001) rates (100%) of pro-oestrus signs in Group I than those (zero %) in Group II. But, in the vast majority of implant-treated goats (10 out of 11), the signs continued for 9 days without any reliable sign of oestrus (mating). Nevertheless, a single goat in that group was in receptive oestrus afterwards, as commenced from the third day of pro-oestrus. Even so, there was no pregnancy following mating.

The mean values (±SEM) of serum P_4 levels following the implant treatment in anoestrous goats are given in *Table 2*. The mean P_4 levels on alternating (successive) days were similar (P>0.05) between the treated and control groups up to Day 12. The concentrations were consistently low, as all remained below 1 ng/ml, regardless of implant treatment.

DISCUSSION

In this study, Deslorelin implant as GnRH analogue was used for oestrus induction during suckling season in early summer (just before the 21st June, as critical date for commencement of short-days for daylight) in coloured Mohair goats. However, it failed to induce oestrus satisfactorily, such that only 9.09% (one out of 11) of goats showed oestrus (mating), yet no pregnancy occurred afterwards. Although all the implant-treated goats showed dramatically higher rate of pro-eostrus (100 *vs.* zero %), it

Table 1. *The rates of pro-oestrus, oestrus as well as pregnancy rate following the short-acting deslorelin implant treatment in suckling (anoestrous) Mohair goats*
Tablo 1. *Emzirme (anöstrus) dönemindeki Tiftik keçilerinde kısa-etkili deslorelin implant uygulaması sonrası pro-östrus, östrus ve gebelik oranları*

Parameters Studied	Experimental Groups		Significance
	Ovuplant® (n=11)	Control (n=10)	
Pro-oestrus, %	100± 0.0 [b]	0 [a]	P<0.001
Oestrus, %	9.09 ±9.09	0	NS
Pregnancy (30 d) %	0	0	*

*[a,b] Means (±SEM) within the same row having different superscripts differ significantly (P<0.05), * No statistical analysis could be made because of the identical values obtained for each group,* ***NS:*** *not significant (P>0.05)*

Table 2. *Mean levels (±SEM) of serum progesterone (P_4) following the short-acting deslorelin implant treatment in suckling (anoestrous) goats*
Tablo 2. *Emzirme (anöstrus) dönemindeki keçilerde kısa-etkili deslorelin implant uygulaması sonrası ortalama (±SEM) serum progesteron (P_4) düzeyleri*

P_4, ng/ml	Experimental Groups		Statistics	
	Ovuplant® (n=11)	Control (n=10)	P value	Significance
Baseline	0.22±0.11	0.06±0.02	0.198	NS
Day 2	0.06±0.04	0.05±0.02	0.767	NS
Day 4	0.01±0.00	0.02±0.01	0.306	NS
Day 6	0.06±0.03	0.05±0.03	0.849	NS
Day 8	0.19±0.11	0.05±0.02	0.259	NS
Day 10	0.14±0.05	0.05±0.03	0.152	NS
Day 12	0.01±0.00	0.05±0.02	0.055	NS

NS: *not significant (P>0.05)*

did not lead to oestrus except in one, following the third day of pro-oestrus. In this respect, at first, we presumed that the excessive daylight (11.7 h herein) supply might have suppressed the cyclic activity [5,34,35]. Indeed, according to national meteorological reports achieved, the average daylight lengths in our province are 4.8 h and 11.9 h for winter and summer seasons, respectively. Apparently, animals receive almost three-times longer daylight exposures during summer, as compared to those in winter. Furthermore, it is also likely that the insufficient luteal activity associated with silent ovulation or luteinisation of follicles might lead to poor outcome [13,36] following a given synchronisation protocol applied.

As mentioned earlier, the releases of pituitary FSH and LH are reduced by 50% during anoestrous season [34]. Hence, goats have no cyclic activity outside the mating season and the P_4 levels remain less than 1 ng/ml [3]. During the oestrous cycle, however the mean daily plasma concentrations range from non-detectable levels (in oestrous stage) up to 5.1 ng/ml (at mid-cycle). High P_4 level continues for approximately 12 d (the duration as also used herein for measurements) and the mean time required for plasma P_4 to rise, from the basal levels to higher than 2 ng/ml, is 5.48 days [37]. Additionally, Rivera et al.[36] noted that, the levels below 1 ng/ml during periovulatory days and anovulatory periods fluctuate from 1 to 12 ng/ml during the luteal phases. Herein, the levels consistently remained well below than 1 ng/ml by Day 12 regardless of treatment. The levels of P_4 well below than 1 ng/ml could be considered as critical (threshold) value for the 'active state' of ovarian function (other than in oestrous period) in ruminants [38]. Apparently, the persistence of low levels may represent the ovarian inactivity and/or insufficient stimulation. In this respect, Chemineau [39] reported in goats that, on a monthly basis, the cause of relatively higher rate (87%) of ovulation, as compared to those (82%) of oestrous females, were due to silent ovulations. It may also be presumed that the long half-life of exogenous gonadotropic hormones such as eCG, with 26 h half-life (21) may also lead to undesirable luteinisation and anovulation of follicles [40].

Undoubtedly, the lactational status (ongoing milking/suckling) would increase the energy demand that is also inevitably increased during the lactation, depending on milk yield [41]. This physiological 'energetically heavy' lactation state may also adversely affect the responsiveness of ovarium to exogenous gonadotropic stimulus given [42]. Indeed, Yildiz et al.[43] observed that, regardless of the exogenous stimuli by introducing sexually activated rams or ewes to the females, the endogenous LH pulse frequency has been affected more profoundly by the body energy reserves in anoestrous ewes. Moreover, regardless of the hormonal treatment by progesterone plus eCG after the natural breeding season in ewes, the nutritional status (body condition) was the key effector of reproductive performance, the litter size achieved [44]. Apparently, these reports underline the critical importance of energy reserves for an optimum reproductive outcome [45].

Conclusively, the present results indicate that; i) there was no marked difference between implant-treated and untreated animals for the P_4 concentrations, as remaining below 1 ng/ml all, and ii) short-acting deslorelin implant (Ovuplant®) could not sufficiently induce fertile oestrous cycles of coloured Mohair goats during the suckling (anoestrous) season in early summer conditions in Van Province, Turkey.

To our knowledge, the present study is the first report about the effect of short-acting Deslorelin implant on the ovarian cyclicity in suckling Mohair goats.

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