

Reproductive Performance After hCG or GnRH Administration of Long-Term Progestagen Treatment of Fat Tailed Ewes During Seasonal Anoestrus

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ABSTRACT

This research was carried out to evaluate the effect of hCG or GnRH two days after long-term progestagen treatment on the reproductive performance of Mehraban ewe during seasonal anoestrus under field conditions. A total of 75 ewes pretreated with intravaginal progestagen sponges left in situ for 14 days and all ewes were treated with Pregnant Mare Serum Gonadotrophin at the time of sponge removal. Two days after removal of sponge, the ewes in two groups were injected (im), either with hCG (hCG group) or with GnRH analogue (GnRH group) and the other group remained untreated and served as control. Blood samples were collected on day 19 after sponge removal from all animals to measure serum concentration of progesterone in order to pregnancy diagnosis. The reproductive performance of the Mehraban ewes in all groups was assessed by lambing data. The pregnancy rate of ewes was highest in the hCG group (86.4%), compared to the control (60%; $P<0.05$) and GnRH (69.2%; $P>0.05$) groups, respectively. The percentage of lambing rate of the ewes which lambed from mating at the induced oestrus was highest in the hCG group (85.7%), when compared to the control (58.3%; $P<0.05$) and GnRH (66.7%; $P>0.05$) groups. The highest proportion of twin lamb birth rate was recorded in the hCG group (61.1%), compared to the control (57.1%; $P>0.05$) and GnRH (31.3%; $P<0.05$) groups. The hCG group recorded significantly greater mean single and twin lamb birth weight compared to the control group ($P<0.05$). It can be concluded that hCG injection, two days after sponge removal, increases prolificacy and can improve reproductive performance of Mehraban ewes outside the breeding season, which is in high importance for sheep holders from economical point of view.

Key Words: Ewe, anoestrus, progestagen, hCG, GnRH, prolificacy

ÖZET

ANÖSTRUS DÖNEMİNDE YAĞLI KUYRUK KOYUNLARA UZUN SÜRELİ PROGESTAGEN UYGULAMASINI TAKİBEN YAPILAN hCG VEYA GnRH ENJEKSİYONUNUN REPRODÜKTİF PERFORMANS ÜZERİNE ETKİSİ

Bu çalışmada saha şartlarında bakılan Mehraban ırkı koyunlara anöstrus döneminde uzun süreli progesteron uygulamasının bitiminden iki gün sonra uygulanan hCG veya GnRH hormonlarının reproduktif performans üzerine etkinliğini araştırıldı. Kullanılan 75 adet koyunun vajinasına progestagen emdirilmiş süngerler yerleştirildikten sonra

14 gün beklendi, süngerlerin çıkarıldığı günde tüm koyunlara gebe kısrak serum gonadotropin hormonu uygulandı. Sünger çıkarılmasından iki gün sonra çalışma koyunlarından ilk gruba hCG (im) enjeksiyonu yapıldı (hCG grup) ve diğer gruba GnRH analogu enjekte edildi (GnRH grup), çalışma grupları dışında kalan koyunlar kontrol grubu olarak ayrıldı. Sünger çıkarılmasından 19 gün sonra serum progesteron konsantrasyon tayini ile gebelik kontrolü yapılması için tüm hayvanlardan kan alındı. Tüm gruplarda yer alan Mehraban koyunlarının reproduktif performansları kuzulama oranları değerlendirilerek belirlendi. Gebelik oranında gruplar içinde en yüksek değer hCG grubunda (%86,4) tespit edilirken bunu sırasıyla kontrol (%60; $P<0,05$) ve GnRH (%69,2; $P>0,05$) grubu takip etmektedir. Östrusu uyarılan ve çiftleştirilen koyunlardan hCG grubunun (%85,7) kuzulama oranı, GnRH grubu (%66,7; $P>0,05$) ve kontrol grubu (%58,3; $P<0,05$) ile karşılaştırıldığında daha yüksek bulunmuştur. Grupların ikiz doğurma oranları karşılaştırıldığında en yüksek oran hCG grubunda (%61,1) tespit edilirken, kontrol grubunda (%57,1; $P>0,05$) ve GnRH grubunda (%31,3; $P<0,05$) daha düşük oranlar tespit edildi. Tek ve ikiz kuzuların doğum ağırlıkları karşılaştırıldığında hCG grubunda doğan yavruların doğum ağırlıkları kontrol grubundan belirgin şekilde yüksek olduğu gözlenmiştir ($P<0,05$). Sonuç olarak sezon dışı dönemde Mehraban ırkı koyunlara sünger çıkarılmasından 2 gün sonra uygulanan hCG enjeksiyonunun elde edilen yavru sayısını ve üreme performansını artırıcı etkisi bulunmuştur, elde edilen bu artışlar koyun yetiştiricileri için ekonomik açıdan çok önemli bir noktadır.

Anahtar Kelimeler: Koyun, anöstrus, progestagen, hCG, GnRH, verimlilik

Introduction

Numerous pharmacological and management techniques have been implemented to improve the reproductive efficiency of sheep during seasonal anestrus. The use of Gonadotropin releasing hormone (GnRH) in combination with other hormones has been reported in several studies and has considerable effect on reproductive performance of ewes during non-breeding season (Amer and Hazzaa, 2009; Dogan and Nur, 2006; Moeini et al., 2009; Safdarian et al., 2006; Ungerfeld and Rubianes, 2002; Zarkawi et al., 1999). Preimplantation embryonic losses have long been recognized as a major cause of reduced reproductive efficiency in domesticated livestock (Edey, 1979; Windsor, 1995) and principal cause of these losses in sheep may be luteal function inadequacy (Ashworth et al., 1989; Nancarrow, 1994; Wilmut et al., 1986). The failure of the ewe's corpus luteum (CL) to function normally may be caused by inadequate gonadotrophin stimulation during the periovulatory period which can lead to lower preovulatory LH surge. Lower LH surge may also affect follicular maturation and consequently oocyte quality, thus result in the production of lower quality embryos with lower viability.

Human chorionic gonadotropin (hCG), which is similar to LH in function, can induce oocyte maturation and ovulation (Kinser et al., 1983; Schmitt et al., 1996) and increase luteal

weight and endogenous synthesis of progesterone (P_4) from the corpus luteum in sheep (Farin et al., 1988; Nephew et al., 1994). Treatment of ewes lambs with hCG at the time of mating increases conceptus growth. Placentation was also improved in hCG treated animals, as the number of placentomes and lambs born was increased in the breeding season (Khan et al., 2003). An injection of hCG has been administrated at artificial insemination or breeding for improving the reproductive performance of ewes (Gomez-Brunet et al., 2007; Moeini et al., 2009). Zamiri and Hosseini (1998) reported an increased of prolificacy in ewes which received hCG twenty-four hours after the second injection of PGF 2α compared to the control group (Zamiri and Hosseini, 1998).

GnRH and its analogues induce ovarian follicular development and corpus luteum function indirectly, via the release of pituitary LH and FSH (Twagiramungu et al., 1995) and may increase the multiple birth rate and litter size in ewes (Karaca et al., 2009). As ovulation in ewes occurs during a period of time, GnRH might be used to enhance percentages of ewes that have ovulations in response to introduction of rams (Jordan et al., 2009). These authors compared the effects of pretreatment with GnRH and P_4 on the reproductive response of ewes introduced to rams during seasonal anoestrus.

The aim of the present study was to determine the effects of an injection of hCG or GnRH two days after sponge withdrawal on the reproductive efficiency of fat tailed Mehraban ewes in a commercial flock during the seasonal anoestrus. The animals ovulate between 36 to 72 h after removal of the controlled release device based on the advice on correct administration of vaginal sponge catalogue. The hypothesis being that hCG or GnRH injection 48 h after long-term progesterone treatment could improved lambing rate in the ewe during seasonal anoestrus.

Materials and Methods

This study was conducted during the non-breeding season from March to July 2010 (Spring), in Komijan, Markazi province, Iran. Komijan is located at a latitude of 34° 43' N and longitude 49° 19' E. Its altitude is 1750 m above sea level. A total of 75 Mehraban fat tailed ewes, 2–5 years of age (mean body weight 59 kg and BCS 2.51 [1 = extremely emaciated, 5 = obese]) were divided into three groups including hCG (n = 22), GnRH (n = 28) and untreated control ewes (n = 25). The animals were managed under the same conditions on the farm. They were kept under natural field conditions, having access to good quality grass and water and maintained in good health.

Oestrus was synchronised in all groups using intravaginal progestagen sponges (20 mg Cronolone, Chronogest, Intervet, UK) left *in situ* for 14 days. At the time of sponge removal all ewes were treated with pregnant mare serum gonadotrophin (400 IU PMSG-Folligon, Intervet, Holland; i.m.). Twenty two of ewes received human chorionic gonadotropin (250 IU hCG-Chorulon, Intervet, Holland; i.m.) and twenty eight of ewes were treated with gonadotropin releasing hormone (4.2 µg GnRH-Vetocept; Buserelin; i.m.) two days after sponge withdrawal in the treatment groups and twenty five of ewes were allocated as the control group (no treatment and injected with distilled water as placebo). Then, after injection all ewes were exposed to eight rams of proven fertility for 51 days (3 cycles).

Blood samples were collected on day 19 after sponge removal from all animals via jugular venipuncture for measuring serum progesterone. Serum progesterone concentration ≥ 2.5 ng/mL on day 19 after sponge removal was considered indicative of pregnancy (Boscos et al., 2002). Serum was separated by centrifugation at $1500 \times g$ for 15 minutes and stored at -21°C until P_4 was assayed. Progesterone concentrations were determined using ELISA kit (DRG Instruments GmbH, Germany), which detects the concentrations as low as 0.045 ng/ml and has coefficients of variation of 6.86 and 5.59% for intra- and inter-assays, respectively. The reproductive performance of the ewes in all groups was assessed by lambing data including prolificacy (number of lambs born/number of ewes lambed) or litter size and lamb birth weights and lambing rate which were recorded at lambing. Ewes lambed for two periods, many of them, which conceived at the first oestrous cycle after treatment lambed at first period. At the second period, those ewes lambed which conceived at the second oestrous cycle (natural estrus).

Data of one ewe of hCG, four ewes of GnRH and one ewe of untreated control group were deleted for analysis, because of different causes such as die and sale after taking the blood samples. The results were statistically analyzed using the SPSS statistical software (Version 15.0, SPSS Inc, Chicago, Illinois). The lamb birth weight and litter size of lamb at birth were compared among groups by one-way ANOVA and Bonferroni as Post Hoc test. Differences between the proportion of pregnancy and lambing rate, single and twin birth rate of control, GnRH and hCG treatment groups during seasonal anoestrus were compared by Chi-square test. Values of $P \leq 0.05$ were considered as significant data.

Results

The percentage of pregnant (serum $P_4 \geq 2.5$ ng/ml) and non-pregnant (plasma $P_4 < 2.5$ ng/ml) ewes of the study was statistically different between the hCG and control groups.

The pregnancy rate of ewes following the induced oestrus (first oestrus) after treatment was highest in the hCG group (86.4%), compared with the control (60%; $P < 0.05$) and GnRH (69.2%; $P > 0.05$) groups, respectively.

Number of ewes lambing was 14, 16 and 18 at the first (Table 1) and 10, 8 and 3 at the second period of lambing in the control, GnRH and hCG groups which conceived at the first (induced) and second (natural) oestrous cycle, respectively.

Table 1 sets out the percentage of lambing rate for hCG treatment group which was higher than control group during seasonal anoestrus ($P < 0.05$; Table 1). The hCG group recorded a significantly higher percentage of twin lamb birth rate, compared with the GnRH group

($P < 0.05$; Table 1). The proportion of twin lamb birth rate was higher in the hCG group; however this difference was not significant between hCG and control group (61.1% vs. 57.1%; $P > 0.05$; Table 1).

The effect of treatment with GnRH and hCG on day 2 after spong removal on the mean (\pm SD) of lambing birth weight and litter size are shown in Table 2. The mean (\pm SD) single and twin lamb birth weights of the ewe in hCG group (4930 ± 665.5 and 4186.3 ± 191.3) were significantly higher compared to the control group (4388.7 ± 546.4 and 3969.4 ± 226.8) ($P < 0.05$). In addition, the litter size in hCG group was higher than in control and GnRH groups, but this difference was not significant (Table 2; $P > 0.05$).

Table 1. Percentage of the lambing rate at first period, single and twin birth rate following the induced (first) oestrus for control, GnRH and hCG treatment groups during seasonal anoestrus of ewe.

Table 1. Anöstrus döneminde bulunan GnRH, hCG ve kontrol grubu koyunların ilk dönemdeki kuzulama, tek ve ikiz doğurma oranları.

Parameters	Group		
	Control	GnRH	hCG
Lambing rate%(N)	58.3 (14/24) ^a	66.7 (16/24) ^{ab}	85.7 (18/21) ^b
Lamb birth rate			
Single	42.9 (6) ^{cd}	68.8 (11) ^c	38.9 (7) ^d
Twin	57.1 (8) ^{cd}	31.3 (5) ^c	61.1 (11) ^d
Fecundity	91.7 (22)	87.5 (21)	138.1 (29)

^{ab}: Values within row having different superscripts differ significantly ($P < 0.05$).

^{cd}: Values within row differ tendency toward significantly ($P = 0.08$).

Table 2. Effect of treatment with GnRH and hCG on day 2 after spong removal on the mean (\pm SD) of lambing birth weight and litter size of lamb at birth of ewes which conceived at the first oestrus.

Table 2. Sünger çıkarılmasından 2 gün sonra GnRH ve hCG uygulamasının ilk östrusunda gebe kalan koyunlardan doğan kuzuların ortalama (\pm SD) doğum ağırlıkları ve bir batında doğan yavru sayısı üzerine etkisi.

Parameters	Group		
	Control	GnRH	hCG
Lambing birth weight (g)			
Single	4388.7 ± 546.4^a	4715.6 ± 565.9^{ab}	4930 ± 665.5^b
Twin	3969.4 ± 226.8^a	4052.9 ± 155.9^{ab}	4186.3 ± 191.3^b
Litter size	1.4 ± 0.5	1.3 ± 0.4	1.5 ± 0.5

^{ab}: Values within row having different superscripts differ significantly ($P < 0.05$).

Discussion

In this study, treatment with hCG or GnRH two days after long-term progestagen treatment during seasonal anoestrus was performed to increase the pregnancy and lambing rate (specifically twin lamb birth rate) of fat tailed ewes. The results of the present study supported this hypothesis. The results showed that the lambing rates were 58.3%, 66.7% and 85.7% in the control, GnRH and hCG, respectively.

The pregnancy and lambing rate in ewe are the most important subjects for increasing economical income of sheep holders in sheep production system, which was higher in hCG group compared with control group during seasonal anoestrus. Lambing rate of the ewes treated with hCG was 85.7% following the induced oestrus after treatment in the present study. This is higher than 64.3% (Alkass et al., 1989), 80% (Zarkawi et al., 1999) and 67% (Titi et al., 2010) reported in ewes treated only with intravaginal sponges followed by PMSG injection. Twin lamb birth rate of hCG group was 61.1% in this study which was higher than 30% (Zarkawi et al., 1999) and 47.2% (Karaca et al., 2009) reported in ewes only received long-term progesterone treatment and eCG injection at sponge removal. In the present study, single and twin lamb birth weight of ewe in hCG were higher than control group. The mean of single lamb birth weight was 4.9 kg for hCG which is similar to 5 kg (Zarkawi et al., 1999); but lamb birth weight of twin was 4.2 kg for hCG which is greater than 3.9 kg reported by Zarkawi et al. (1999).

In the study of Beck et al. (1996), the injection of 4 µg GnRH 5 days prior to a 100 µg of prostaglandin F_{2α} (PGF_{2α}) analogue injection improved fertility (88.9%) during breeding season in ewes. The results recorded were comparable with those obtained by two injections of PGF_{2α}, 11-days interval (92.6%). GnRH treatment 5 days prior to injection of PGF_{2α} resulted in high oestrous response and fertility and induced a more compact synchronization rate than double PGF_{2α} injection alone (Ataman and Akoz, 2006). In a study by Karaca et al. (2009), the fertility rate recorded was

higher after exogenous GnRH treatment (10.5 µg buserelin), immediately prior to a short-term (7d) compared to the long-term (12d) progesterone treatment without GnRH treatment (89.6% vs. 71.6%). There was however no significant difference between short-term progesterone treatment with or without GnRH treatment in ewes at the onset of the breeding season (89.6% vs. 87.3%). In a study reported by Husein and Kridli (2003), the pregnancy rate obtained was higher (75% vs. 62.5%) in anoestrous ewes treated with a pro-gestosterone-GnRH-PGF_{2α}, compared to a GnRH -PGF_{2α} treatment. The result of the present study in the GnRH group is in agreement with the long-term progesterone treatment, without GnRH administration in the study of Karaca et al. (2009). It is also in accordance with the study of Husein and Kridli (2003).

According to the results of the present study, treatment with hCG and GnRH two days after long-term progestagen treatment during seasonal anoestrus could result in better reproductive performance of anoestrous ewes. Injection of hCG or GnRH generally induces an increase in LH pulse frequency and causes ovulation. Treatment with hCG or GnRH was performed in order to induce ovulation and the formation of a corpus luteum in the anoestrous ewes. Hence, it was proposed that the number of ewes with ovulated follicles would increase in response to treatment - resulting in high lambing rate. In this study, the proportion of twin lamb birth rate was higher in the hCG group; however this difference was not significant between hCG and control group which may be due to the group size used in the present study. Lack of GnRH effect on the lambing rate (especially twin lamb birth rate) could probably be ascribed to the effect of buserelin (GnRH analogue) may result in less secretion of endogenous GnRH, which is necessary for the formation of perovulatory follicles. In dairy cows it has been reported that buserelin is much more potent than gonadorelin and fertirelin acetate (other GnRH analogues) for the release of LH and FSH (Chenault et al., 1990). Both FSH and LH are released simultaneously in response to GnRH

stimulation. In the presence of a dominant follicle, these hormones may cause follicle maturation and ovulation. However, positive effects of the other GnRH analogues (cystorelin and gonadorelin) on the percentage of ewes with a plasma concentration of P4 greater than 1 ng/ml during seasonal anoestrus have been reported (Jordan et al., 2009).

It has been shown that progesterone priming prior to treatment with GnRH-PGF2 α was effective in oestrous induction, conception rate after induced oestrus and overall lambing rate in anoestrous ewes (Husein and Kridli, 2003). Combination of GnRH with the progesterone programs increased the prolificacy of the ewe (Jordan et al., 2009; Karaca et al., 2009; Titi et al., 2010). The number of lambs born per lambing ewe (litter size) of GnRH group in this study (1.3) was similar to 1.31 obtained by Titi et al. (2010); but the litter size of hCG group was 1.5. These results showed that the incorporation of hCG into the progestagen treatment protocol results in improved reproductive performance of ewes compared with GnRH group. It can be stated that hCG injection after progesterone priming in anoestrous ewes may increase the number of ovulated ewes which result in higher lambing rate and litter size compared to control and GnRH groups.

Moeini et al., (2009) found that the mean birth weights of single and twin's lambs of ewes treated by hCG at artificial insemination (AI) time were greater than control ewes. Their results also showed the higher fertility rate and prolificacy of ewes treated with hCG compared with non-treated ewes, out of breeding season. However, Gomez-Brunet et al. (2007) found that the fertility and prolificacy were not affected by hCG at the time of insemination. They concluded that hCG treatment at the time of AI could be beneficial to increase the fertility from flocks with low fertility rates (Gomez-Brunet et al., 2007). Increased prolificacy was reported in ewes which received hCG twenty-four hours after the second injection of PGF2 α compared to the control group (Zamiri and Hosseini, 1998). Thus, human chorionic gonadotropin with similar function with LH,

can increase the fertility rate and improve the pregnancy rate (Nephew et al., 1994; Schmitt et al., 1996). In conclusion, a combination of hCG or GnRH and progestagen sponges can be effective in the reproductive performance of anoestrous ewe. In addition, the use of hCG may yield better responses than GnRH, when combined with the traditional progestagen sponges and eCG during seasonal anoestrus.

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