

## The effect of different progesterone protocols on the reproductive efficiency of ewes during the non-breeding season

Hussein Ahmed Amer\*, and Abubakr Maher Hazzaa

*Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*

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### ABSTRACT

The effect of intra-vaginal Fluorogestone-Acetate (FGA) sponges (a progesterone analogue) +/- eCG (equine chorionic gonadotropin) on the fertility of anestrus ewes was assessed. Ninety-six ewes were divided into 4 groups (24/group). The ewes were exposed to treatment for 6 days (FGA or FGA+eCG groups) and for 12 days (FGA or FGA+eCG groups). Sponges were withdrawn (day 0) and eCG (500 IU) or saline was injected. Ewes were examined for estrus and bred by fertile rams. Pregnancy was diagnosed by progesterone levels and ultrasound scanning. Estrous expression differed only between the FGA-eCG (12 days) and FGA (6 days) groups ( $P < 0.05$ ). Intervals from day 0 to onset of estrus were shorter ( $P < 0.05$ ) in the FGA-eCG (12 days) than those in the FGA (12 days) and FGA (6 days) groups. Progesterone levels were basal from day -10 through day 5 ( $< 0.1$  ng/mL) and increased gradually thereafter until day 20. Progesterone remained elevated through day 20 in 50, 75, 66.7 and 91.7% ewes treated for 6 days (FGA and FGA-eCG) or 12 days (FGA and FGA-eCG). Mean values between eCG-treated versus non eCG-treated ewes were significant ( $P < 0.01$ ). From 68 pregnant ewes, 62 lambed at full term (150 days). No significant differences in pregnancy, lambing rate and number of newborns were found between eCG groups (6 or 12 days). Pregnancy rate from the first treatment was 70.8%. In conclusion, intra-vaginal FGA sponges with a 12 day+eCG regime could adequately improve the reproductive performance of anestrus ewes. From the economic side, it is possible to replace them with a 6 day FGA+eCG regime but with lower efficiency.

**Key words:** intra-vaginal, fluorogestone-acetate, sponges, anestrus, ewes, progesterone, pregnancy

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### Introduction

Many protocols of estrous synchronization have been developed in the last few decades. The most widely used protocol was based on the use of intra vaginal progestagen sponges inserted for 12 to 14 days followed by eCG at sponge removal and ram introduction

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\*Corresponding author:

Hussein Ahmed Amer, Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, El-Zeraa str. 114, 1134-Zagazig, Egypt, Phone:+2 55 2367711; E-mail: mer\_vet@hotmail.com

(GORDON, 1975; HANSEL and BEAL, 1979; ZARKAWI, 2001; HUSEIN and HAMIT, 2005). Despite the successful outcome from implementing such a protocol, reduced fertility rates still exist in some research studies (KRUIP and BRAND, 1975; FIZGERALD et al., 1985; HUSEIN and KRIDI, 2002). Reduced fertility rates have been attributed to many factors including heredity, breed, seasonality, age, environment, nutrition, diseases, semen quality, female reproductive status and hormonal treatment (WEBB et al., 1994; LEWIS et al., 1996; BECK et al., 1996; HUSEIN et al., 1996, 1998; YAVUZER, 2005). Two other interesting factors may be important. First, the circulating levels of P4 and release during the 12-day period in which the pessaries are in place decline over time. Typically, P4 peaks within two days of pessary administration and then decreases gradually with time during the remaining period (HUSEIN et al., 1998; HUSEIN and KRIDI, 2002; YAVUZER, 2005). The decline in P4 concentrations during the period of insertion may be due to the wash-out of the sponge by vaginal fluid (WHEATON et al., 1993). Therefore, the amount of P4 absorbed and P4 levels at the time of sponge removal in a 12-day treatment may not be sufficient to maintain normal patterns of follicular growth (GORDON, 1975). Low P4 concentrations have been associated with the formation of persistent follicles, prolonged luteal function and reduced fertility in cattle (THATCHER et al., 1996) and sheep (JOHNSON et al., 1996). Second, vaginal contamination is created following and during the process of sponge insertion. In addition, the use of P4 pessaries for long periods is laborious and more time is needed to follow up pessary retention, a treatment that has also been associated with accumulation of offensive vaginal mucus and a bad smell upon pessary removal (WHEATON et al., 1993).

Our intention was to minimize the period over which progestagen sponges are inserted, from 12-14 days to only 6 days. Short periods of progestagen sponge treatment for as little as 5 to 7 days have been reported to be successful in inducing and synchronizing estrous in sheep during both the breeding and non-breeding seasons (FIZGERALD et al., 1985; BECK et al., 1993; VINOLES et al., 2001; ATAMAN et al., 2006). Progesterone priming for as little as 2 days is sufficient to ensure normal luteal function in sheep, but the final stages of follicular growth normally require about 4-5 days (MCLEOD and HARESIGN, 1984). Reducing the period of sponge insertion may maintain higher P4 levels upon removal of pessaries and may reduce the chance of vaginal contamination. Therefore, this study is in the light of the supposition that FGA treatments for 6 or 12-days will produce similar reproductive performance of ewes bred during the non-breeding season.

The objective of this study was to evaluate the effects of intra-vaginal administration of FGA sponges for 6 or 12 days +/-eCG regimes on the reproductive performance of ewes during the anestrous season.

## Materials and methods

*Animals.* Ninety-six, 2.5 to 5.5 year old Rahmani ewes, weighing  $45.3 \pm 4.8$  kg ( $M \pm SD$ ) with a body condition score of 2.5 to 3 (scale=0 lowest to 5 highest) according to ROMDHANI et al. (2004), were used in the study conducted from May to July. The experiments were performed at a sheep private farm in Abu-Kabeer, Sharkia Province. All ewes had previously lambed and their last lambs had been weaned by them. Ewes were fed 1.0 kg wheat straw and 0.5 kg concentrate mixture per ewe daily and trace minerals salt blocks and water were available *ad libitum*. Ewes were treated previously by prophylactic doses of external and internal parasites.

*Experimental design.* Ewes were randomly assigned in equal numbers to 4 treatment groups ( $n=24$ /each group). The first treatment included 40 mg intra vaginal fluorogestone acetate (FGA, 40 mg progesterone analogue, Ceva Animal health, Ltd, France) sponges inserted for 6 days versus 12 days. In addition, 500 IU equine chorionic gonadotropin (eCG, Ceva Animal Health, Libourne Cedex, France) versus saline (control) at the time of sponge removal were injected. Ewes in the 6 or 12 day treatments were classified into FGA +/- eCG groups. All treatments were timed to end together (day 0) at which time the sponges were withdrawn and eCG or saline was injected. Seven harnessed fertile rams which had been isolated from these ewes joined them upon sponge removal. All animals were run together in a single pen and the ewes were checked for breeding at 6 hour intervals for 5 days. Pregnancy was diagnosed on day 28 using ultrasound [Pie-medical systems, Genius-240 with 6-8 MHz rectal probe].

*Reproductive parameters.* Following the FGA sponge removal, some reproductive parameters were recorded. These parameters included:

1-Incidence of estrous: the number of ewes detected by rams within 72 hours following FGA sponge removal.

2-Onset of estrous: estrous occurring within 72 hours following sponge removal.

3-Pregnant ewes: pregnancy based upon P4 profiles on day 20 and ultrasonography on day 28.

4-Lambd ewes: number of ewes lambed.

5-Number of newborns: number of lambs born from mating at first service.

*Blood sampling and hormonal assay.* Base line blood samples were collected immediately before sponge insertion on day -10 from ewes in the 12-day FGA-treated groups and on day -4 from ewes in the 6-day FGA-treated groups. Blood samples were also collected from all ewes daily between days 0 and 5, and then on alternate days thereafter until day 20 to compare P4 concentrations among groups for estrus detection and pregnancy diagnosis. All blood samples (5 mL each) were drawn via jugular venipuncture into heparinized tubes (5 IU/mL). Blood samples were centrifuged within 30 minutes of

collection at 3000 g for 15 minutes. Plasma was pipetted into 1.5 mL Eppendorf tubes using sterilized plastic disposable Pasteur pipettes and then stored at -20 °C until assayed for P4 using radioimmunoassay in a single run (RIA, Coat-A-Count kits; Diagnostic Products Corporation, DPC, Los Angeles, CA, USA). Sensitivity was 0.1 ng mL<sup>-1</sup> and intra-assay coefficient of variation was 3.1%.

*Statistical analysis.* Data were analyzed by using SAS/ATAT-ANOVA procedures (SAS, Institute Inc. SAS guide to personal computers, 4<sup>th</sup> ed. SAS Inst. Inc. version 6 Cary NC, USA., 1996). Data in text, tables and figures are presented as means ± SEM, unless otherwise noted. The incidences of estrous and pregnancy and lambing rates were analyzed using the Chi-square test. The pregnancy rate was defined as the number of ewes that became pregnant based upon sustained P4 levels of >0.1 ng mL<sup>-1</sup> between days 15 and 20 and confirmed on day 28 by ultrasonography and lambing 145 to 155 days following day 0.

## Results

Estrous expression following FGA sponge withdrawal occurred in 80/96 (83.3%) ewes, indicating the effectiveness of FGA sponges administered for 6 or 12 days in combination with the ram effect in sensitizing ovarian activity and ensuring acceptable estrous expression rate in the anestrous season. Estrous was observed in 66.7, 83.3, 83.3 and 100% of ewes in 6 days (FGA and FGA-eCG) or 12 days (FGA and FGA-eCG) groups, respectively. Estrous expression rate differed ( $P < 0.05$ ) between 12-day FGA-eCG and 6-day FGA groups (Table 1, Fig. 2). The effect of treatment on estrous expression was evident when eCG was incorporated into the synchronizing protocol which occurred in 75% of ewes not receiving eCG and in 91.7% of ewes when eCG was incorporated.

Table 1. Reproductive responses following sponge removal in ewes treated at 6 days or 12 days FGA +/-eCG and their effect on lambing rate and number of newborns

Parameters	Experimental groups				Total
	6 days		12 days		
	FGA	FGA-eCG	FGA	FGA-eCG	
n (ewes)	24	24	24	24	96
Incidence of estrus	16 (66.7) <sup>b</sup>	20 (83.3) <sup>ab</sup>	20 (83.3) <sup>ab</sup>	24 (100) <sup>a</sup>	80 (83.3)
Onset of estrus (h)	42.9 ± 3.3 <sup>b</sup>	37.4 ± 3.1 <sup>ab</sup>	42.9 ± 2.9 <sup>b</sup>	32.9 ± 2.8 <sup>a</sup>	39.0 ± 3.0
Pregnancy rate	12 (50) <sup>b</sup>	18 (75) <sup>ab</sup>	16 (66.7) <sup>ab</sup>	22 (91.7) <sup>a</sup>	68 (70.8)
Lambing rate	8 (33.3) <sup>bdc</sup>	18 (75) <sup>a</sup>	14 (58.3) <sup>ac</sup>	22 (91.7) <sup>ac</sup>	62 (64.6)
Number of newborns	10 <sup>b</sup>	20 <sup>ab</sup>	16 <sup>ab</sup>	26 <sup>a</sup>	72
Non-pregnant	12 (50)	6 (25)	8 (33.3)	2 (8.3)	28 (29.3)

<sup>a,b</sup> Values within row with different superscripts differ ( $P < 0.05$ ). <sup>c,d</sup> Values within row with different superscripts differ ( $P < 0.01$ ); <sup>a,b,c,d,e</sup> Numbers or values within row with similar superscripts are similar ( $P < 0.1$ ).

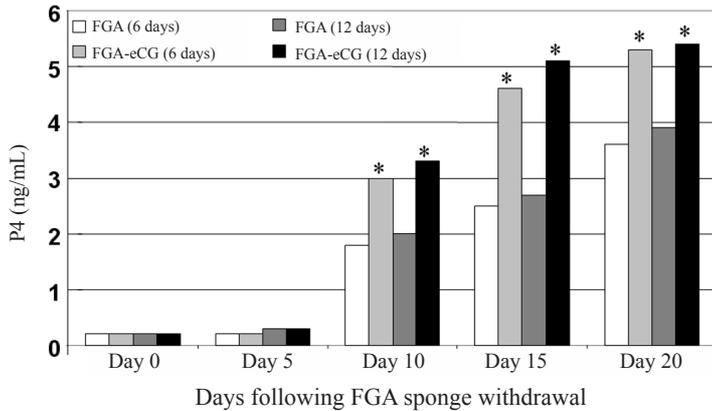


Fig. 1. Plasma progesterone (P4) profiles from day 0 until day 20 following FGA sponge withdrawal in ewes treated with 6-day (FGA or FGA-eCG) and 12-day (FGA or FGA-eCG). P4: Progesterone. \*P<0.01

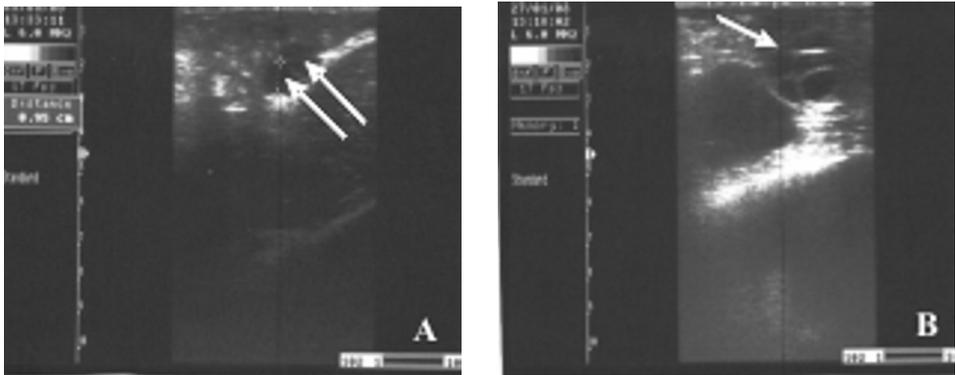


Fig. 2. Ultrasound scanning of treated ewes showing double follicular growth in 6-day FGA-sponges+eCG regime (A) and multiple follicular growth in 12-day FGA-sponges+eCG regime (B)

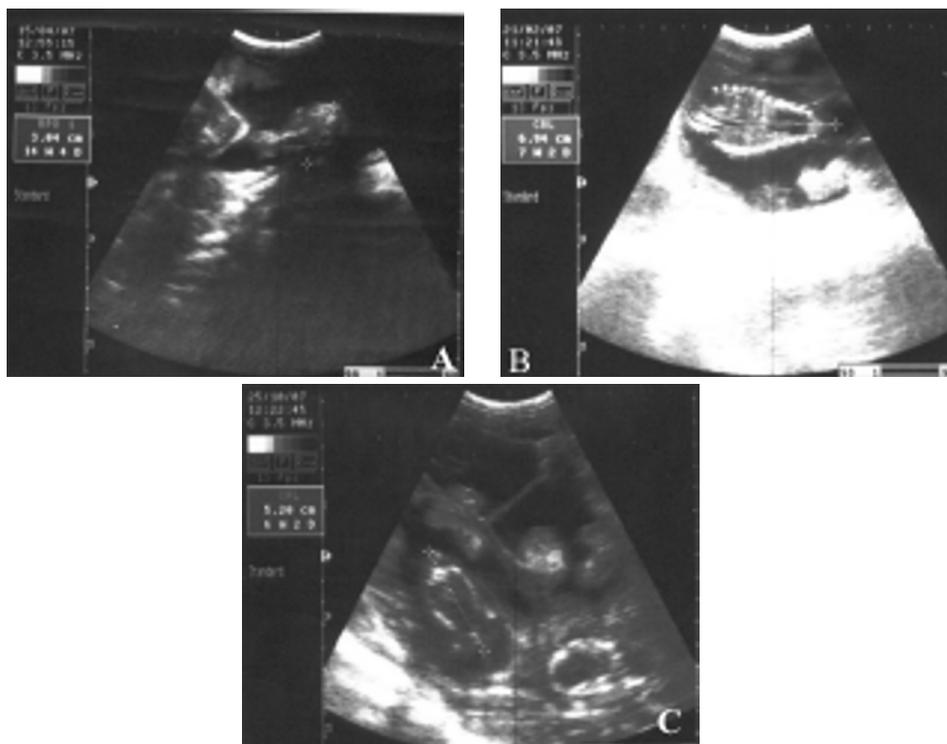


Fig. 3. Ultrasound scanning showing pregnancy in treated ewes, either single pregnancy in 6-day FGA-sponges+eCG regime (A and B) or twin pregnancy in 12-day FGA-sponges+eCG regime (C)

Intervals from 0 h to onset of estrus were shorter ( $P<0.05$ ) in 12-day FGA-eCG ( $32.9 \pm 2.8$ h) than those in the 12-day FGA ( $42.9 \pm 2.9$  h) and 6-day FGA ( $42.9 \pm 3.3$  h) groups. No differences were found in intervals to onset of estrus between the groups treated with eCG or between the 12-day FGA, 6-day FGA and 6-day FGA-eCG groups (Table 1). Overall intervals to onset of estrus occurred earlier ( $P<0.01$ ) in eCG-treated than in non-eCG treated ewes with no differences ( $P>0.5$ ) between the 12-day FGA and the 6-day FGA treated ewes.

Initial plasma P4 concentration was basal among groups and averaged  $0.1 \pm 0.02$  ng/mL<sup>-1</sup>, indicating the absence of cyclicity and seasonal anestrous (Fig. 1). After day 0, P4 concentration remained low ( $0.16 \pm 0.02$  ng/mL<sup>-1</sup>) until day 5 and all ewes ovulated during this period based upon a subsequent rise in P4 levels. Differences in P4 concentrations between day 0 and 5 were not significant. Progesterone concentration increased gradually

thereafter from day 5 until day 20. Mean plasma P4 concentrations between day 5 and 20 differed ( $P < 0.01$ ) significantly and were  $1.8 \pm 0.1$ ,  $3.3 \pm 0.2$ ,  $2.3 \pm 0.1$  and  $3.8 \pm 0.3$  ng/mL<sup>-1</sup> for the 4 groups, respectively. Mean values between eCG-treated versus non eCG-treated ewes were significant ( $P < 0.01$ ). Maximum P4 concentrations were reached between day 11 and 20 among ewes of the 4 groups and differences were not significant between eCG-treated groups or non-treated groups.

Progesterone remained elevated through day 20 in 50, 75, 66.7 and 91.7% of ewes in 6 days (FGA and FGA-eCG) or 12 days (FGA and FGA-eCG), respectively. These ewes were diagnosed pregnant based on ultrasonography on day 28. Pregnancy rate was higher ( $P < 0.05$ ) in the 12-day FGA-eCG group and greater than the 12-day FGA and 6-day FGA-eCG with no difference between the 6-day FGA and FGA-eCG groups (Table 1, Fig. 3). The ewes with lower P4 profiles after day 15 were diagnosed non-pregnant (28) based on ultrasonography. Of the 68 ewes that became pregnant, 62 lambed ( $150 \pm 2$  days) following day 0 and were 33.3, 75, 58.3 and 91.7% ewes of 6-day (FGA and FGA-eCG) or 12-day (FGA or FGA-eCG), respectively. Significant pregnancy loss only occurred in ewes that did not receive eCG treatment. Ewes receiving FGA sponges for 12 days had higher overall pregnancy and lambing rates than those treated for 6 days, respectively. Likewise, pregnancy and lambing rates were improved in eCG-treated versus non eCG-treated ewes. The overall pregnancy (70.8%) and lambing (64.6%) rates were obtained.

The number of newborns per ewes exposed was greater ( $P < 0.05$ ) in 12-day FGA-eCG than 6-day FGA with no differences between the 12-day FGA-eCG group and the 12-day FGA or 6-day FGA-eCG and between the 6-day FGA group and the 12-day FGA or 6-day FGA-eCG (Table 1).

## Discussion

Estrous expression following FGA sponge withdrawal indicates the effectiveness of FGA sponges administered for 6 or 12 days in combination with the ram effect in sensitizing ovarian activity and ensuring acceptable estrous expression rate in anestrus season. Estrous expression rate differed between the 12-day FGA-eCG and 6-days FGA groups. The effect of treatment on estrous expression was evident when eCG was incorporated into the synchronizing protocol which occurred in 75% of ewes not receiving eCG and in 91.7% of ewes when eCG was incorporated. It has been shown that the occurrence of estrous in ewes treated with synthetic progestagen in combination with eCG varied from 47 to 80% (CUNNINGHAM et al., 1980). The absence of estrous in the remaining ewes may be attributed to inadequate estradiol secretion, which may be reflected by silent estrous since ovulation occurred in all ewes based upon P4 profiles.

The onset of estrous was shorter in 12-day FGA-eCG than in the 6 or 12 day FGA groups. The delay in the 6-day or 12-day FGA is attributed to the absence of eCG

treatment in these two groups. No differences were found in the intervals to the onset of estrous between the groups treated with eCG or among the 12-day FGA, 6-day FGA and 6-day FGA-eCG groups. Overall intervals to onset of estrous occurred earlier in eCG-treated than non-eCG treated ewes with no differences between the 12 day FGA and the 6 day FGA treated ewes. The shorter intervals in the eCG-treated groups are attributed to the action of eCG on follicular growth by mediating faster pituitary endocrine responses and estradiol secretion. Treatment with eCG has been shown to advance the intervals to estrous and ovulation in sheep (KILLEEN and MOORE, 1970; CARDWELL et al., 1998; HUSEIN et al., 1998). These effects are consistent with gonadotropic action of eCG in sheep.

Initial plasma P4 concentrations on days -12, -4 and on day 0 were basal among groups indicating the absence of cyclicity and seasonal anestrus. The present study was conducted in May during which time cyclic activities in Awassi ewes cease (EPSTEIN, 1982; EPSTEIN, 1985). Similarly, the seasonal absence of estral behavior has been previously reported under similar circumstances (HUSEIN and KRIDI, 2002; 2003). After day 0, P4 concentration remained low until day 5 and all ewes ovulated during this period based upon a subsequent rise in P4 levels. Progesterone concentration increased gradually thereafter from day 5 until day 20, and reached maximum between day 11 and 20 in the ewes. Higher levels of P4 in the eCG-treated ewes during this period may largely be attributed to higher ovulation rates and formation of extra corpora lutea due to the gonadotropic activity of eCG (GREYLING et al., 1988; ROBINSON and SCARAMUZZI, 1994).

In the present study, the ewes in which P4 levels remained elevated were diagnosed pregnant based on ultrasonography on day 28. However, the pregnancy rate was higher in 12 day FGA-eCG group than the other groups. Of the 68 ewes that became pregnant, 62 lambed ( $150 \pm 2$  days) following day 0. A significant amount of pregnancy loss only occurred in ewes that did not receive eCG treatment. Ewes receiving FGA sponges for 12 days had higher overall pregnancy and lambing rates than those treated for 4 days, likewise; in eCG-treated versus non eCG-treated ewes. The overall pregnancy (70.8%) and lambing (64.6%) rates obtained in the present study were greater than those (52.2 and 30.4%) previously reported (HUSEIN and KRIDI, 2002). Improved pregnancy and lambing rates among ewes that had sponges for 6 or 12 days and eCG treatment at sponge withdrawal may be attributed to the incorporation of eCG into the synchronization protocols, since the two FGA regimens produced similar reproductive performance when eCG was used. The use of eCG has been shown to improve reproductive response of ewes out-of-season (MAXWELL and BARNES, 1986; HUSEIN et al., 1998). These observations imply that the administration of a FGA-sponge regime for 6 days in the anestrus season must be accompanied by eCG treatment.

Although the use of FGA sponges alone for 6 days was successful in inducing estrous among ewes, pregnancy and lambing rates and the number of lambs born per ewe exposed were lower in groups treated with FGA alone without eCG than treatments in which eCG was incorporated. eCG has been widely used in estrous synchronization programs and is known typically to stimulate ovarian activity and improve fertility rates of the ewe during the anestrus season (DUTT, 1953; KILLEEN and MOORE, 1970; MAXWELL and BARNES, 1986). In the present study, the number of lambs born per ewe exposed was greater in the 12-day FGA-eCG than 6-day FGA.

The results of the present study are in agreement with those reported previously (FIZGERALD et al., 1985; BECK et al., 1993; ATAMAN et al., 2006). However, FIZGERALD et al. (1985) used 60 mg MPA sponges for 7 days and 20 mg PGF<sub>2</sub>- at sponge removal and this was as effective as the results obtained using a 14 day MPA-sponge treatment. Another authors (BECK et al., 1993) showed that a 5-day P4 treatment and PGF<sub>2</sub>- at sponge removal produced similar results to those obtained using 8-day P4 treatment without PGF<sub>2</sub>- treatment. More recently, the effectiveness of a 7-day FGA-eCG treatment in synchronizing estrous and producing high pregnancy and lambing rates was reported (ATAMAN et al., 2006). In contrast, incorporation of eCG into a short-term progestagen treatment had a deleterious effect on pregnancy rates. Examination of previous data showed that the periods of short-term progestagen treatment varied between 5 and 8 days with successful reproductive performance (FIZGERALD et al., 1985; BECK et al., 1993; VINOLES et al., 2001; ATAMAN et al., 2006). In addition, follicular growth and maturation in sheep normally require more than 4 days (MCLEOD and HARESIGN, 1984). Therefore, the period of FGA treatment in the present study was reduced to 6 days. However, intervals from 0 h to onset of estrous were shorter than what occurred in the 1<sup>st</sup> treatment with FGA sponges and the overall pregnancy and lambing rates were 28.6% and 17.9%. The lower pregnancy and lambing rates that occurred in the present study may be attributed to some fertility problems in the farm. In addition, seasonal reproduction in sheep is mainly regulated by the photoperiod through melatonin secretion along with other environmental factors such as temperature, nutrition, and social relationships (ARENDRT, 1998).

In conclusion, the use of intra-vaginal FGA sponges in a 12 day+eCG regime could adequately improve the reproductive performance of ewes during the anestrus season, with the possibility of replacing it with a 6 day FGA+eCG regime but with lower efficiency.

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**SAŽETAK**

Procijenjen je učinak intravaginalnih spužvica s fluorogeston-acetatom (FGA, analog progesterona) sa i bez konjskoga korionskoga gonadotropina (KG) na plodnost anestrinih ovaca. Ukupno je 96 ovaca bilo podijeljeno u četiri skupine po 24 ovce. Jednoj skupini ovaca stavljene su spužvice s FGA ili FGA+KG tijekom šest dana, a drugoj skupini tijekom dvanaest dana. Spužvice su bile izvadene 0. dana te je ovcama dan KG (500 IJ) ili fiziološka otopina. Sve su pretražene na znakove estrusa te pripuštene rasplodnim ovcama. Gravidnost je bila dijagnosticirana na temelju određivanja razina progesterona i ultrazvučnog nalaza. Znakovi estrusa razlikovali su se samo između skupine ovaca koja je dobivala FGA i KG tijekom 12 dana i skupine koja je dobivala FGA tijekom šest dana ( $P > 0,05$ ). Intervali od 0. dana do pojave estrusa bili su kraći ( $P < 0,05$ ) u skupini koja je dobivala FGA-KG tijekom 12 dana u odnosu na skupinu koja je dobivala FGA tijekom 12 dana i skupinu koja je dobivala FGA tijekom šest dana. Razine progesterona bile su bazalne u razdoblju od -10. do 5. dana ( $< 0,1$  ng/mL) i postupno su se povećavale do 20. dana. Povišene razine progesterona u razdoblju od 20 dana zabilježene su u 50%, 75%, 66,7% i 91,7 % ovaca obrađivanih tijekom 6 dana (FGA i FGA-KG) ili tijekom 12 dana FGA i FGA-KG-om. Razlike u srednjim vrijednostima između skupina koje su dobivale KG u odnosu na ovce koje nisu dobile KG bile su značajne ( $P < 0,01$ ). Od ukupno 68 gravidnih ovaca, 62 su se ojanjile u terminu od 150 dana. Nisu ustanovljene značajne razlike u tijeku gravidnosti, stopi i broju ojanjene janjadi između skupina KG (šest ili 12 dana). Postotak oplodnje iznosio je 70,8%. Zaključno se može reći da postupak dvanaestodnevne primjene spužvica s konjskim korionskim gonadotropinom može poboljšati reproduktivske rezultate u anestrinih ovaca. S gospodarskog stanovišta taj se postupak može zamijeniti postupkom šestodnevne primjene FG+KG, ali s lošijim rezultatima.

**Cljučne riječi:** intravaginalna primjena, fluorogeston-acetat, spužvice, anestrus, ovce, progesteron, gravidnost

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