

USING FGA SPONGE + GnRH FOR IMPROVING FERTILITY IN GOATS DURING THE BREEDING SEASON

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ABSTRACT

The fertility of 1/2 Damascus-Baladi goats was evaluated when treated with FGA-sponge plus injection of GnRH. Thirty does were divided into 3 equal groups: A, B and C. All groups were synchronized during the breeding season with FGA-sponges for 15 days then single intra-muscular injection of 4 µg GnRH on day 13, 14 and 15 from the time of sponge insertion was tried with groups A, B and C, respectively. Estrous behavior was observed on 83.3% of the does in the three treatment groups (A, B and C). All does in group A exhibited estrus (100%) which was higher ($P < 0.05$) than groups B and C (80% and 70%, respectively). All does in the three treatment groups showed estrus 24 to 36 h after sponge withdrawal (overall mean 30.5 ± 0.9 h); most does exhibited estrus at 28 h ($P < 0.05$). Percentages of does showed estrus 24, 28, 32 and 36 h after sponge withdrawal were 8, 40, 32 and 20%, respectively. Estrus duration was longer for does in group A (31.9 ± 3.8 hr) ($P < 0.05$) than groups B and C (24.6 ± 3.7 and 23.0 ± 3.7 hr, respectively). Does in group C had the lowest ($P < 0.05$) conception rate (50%) compared to groups A and B (70% and 80%, respectively). Does in group B showed the highest conception rate and litter size (80% and 1.6 ± 0.2 , respectively), suggesting that treatment with single intra-muscular injection of 4 µg GnRH 24 h before FGA-sponge removal could give a good result of estrus synchronization, superovulation and conception.

Keywords: *Goats; GnRH; estrous activity; fertility; progesterone.*

INTRODUCTION

Controlling goat reproduction using hormonal treatment to induce/synchronize estrus has many advantages. Synchronization of kidding over a limited period allows producers to give optimum care for the mothers and kids and in turn reduction of kid mortality. Moreover, producers will be able to efficiently use complementary techniques that might help for better reproductive management that including artificial insemination (AI) and embryo transfer (ET) which could facilitate introducing genetic material either transferred domestically or internationally.

The method most commonly used to control production during the breeding season is the progestagen impregnated intervaginal sponge, left in situ for 12 to 14 days (Gordon, 1997). Intravaginal progestagen-sponge, followed by pregnant mare's serum gonadotrophin (PMSG) injection were given to does to synchronize estrus during the normal breeding season (Menegatos *et al.*, 1995), to induce estrus out of season (Karatzas *et al.*, 1997), and to improve ovulation rate (Greyling and Van Niekerk, 1990).

In addition, the traditional treatments of time artificial insemination (TAI) in goats that consist of a long progestagen (progesterone or a synthetic analogue) exposure (11–17 days) associated with an intramuscular injection of equine chorionic gonadotrophin (eCG) given at the end of the treatment (Corteel *et al.*, 1988) might induces subluteal serum progesterone concentrations in sheep (Vinoles *et al.*, 1999) and goats (Rubianes *et al.*, 1998) toward the end of the treatment. Several studies in sheep (Leyva *et al.*, 1998) have associated the subluteal concentration of progesterone with some abnormalities in follicular development, ovulation, oocyte health, luteal function and/or fertility. Accordingly, the short-term protocol has been developed for sheep and goats to avoid prolonged progesterone exposure (Menchaca and Rubianes, 2004). Such protocol uses a short progestin exposure (i.e. 5–7 days) associated with a prostaglandin F2 α injection at the beginning of the treatment with a small dose of eCG (200–350 IU) administrated at the end of progestin exposure. A greater pregnancy rate has been obtained using the short-term protocol in goats when performing a single artificial insemination after detection of estrus or at fixed-time 54 h after the end of treatment (Rubianes *et al.*, 2001). Although using PMSG or eCG gave a high level of synchronization and fertility, they have a poor response in the second treatment due to the presence of anti-PMSG or anti-eCG antibodies in goats that had been treated previously (Baril *et al.*, 1996 and Drion *et al.*, 2001).

Knowledge of time, rate and synchrony of ovulation after treatment is important to establish a suitable schedule for fixed time AI. Freitas *et al.* (1997) stated that variation between animals in timing of estrus after synchronization explain the low rate of fertility in goats inseminated at a predetermined time after sponge withdrawal.

The objective of this study was to evaluate the level of estrus synchronization and fertility rate of goats treated with intravaginal FGA-sponge for 15 days plus GnRH injection at various times after sponge insertion (day 13 , 14 or 15).

MATERIALS AND METHODS

1. Animals and hormonal treatment

Thirty 1/2 Damascus-Baladi does of mixed ages (3-5 years) and body weight ranging from 37 to 57 kg were randomly divided into 3 equal groups (A, B and C). Does in all treatment groups A, B and C were synchronized during the breeding season (September-January) with intra-vaginal progestagen sponge (Chronogest Intervet Laboratories, Cambridge; UK) for 15 days. A single intra-muscular injection of 4 µg buserelin (Receptal Hoechst -UK ltd; UK) was given in different times to does of groups A, B and C on days 13, 14 or 15, respectively. Sponges were removed from does in all groups on day 15.

All animals were healthy and clinically free of diseases. Animals were fed according to nutrient allowances of goats (NRC, 1981). Water and salt mineral blocks were available all the time.

2. Estrous detection and insemination

Estrus of does in groups A, B and C was detected with the aid of a teaser buck twice a day (8:00 am and 4:00 pm) from the time of sponge insertion, then every 4 h from the time of sponge withdrawal. Teasing lasted for 20 minutes per session. Does which were observed to be receptive to the buck were considered in estrus. All does in the treatment groups were inseminated with fresh semen 2 days after sponge withdrawal (day 17). Semen was collected from three Damascus bucks where only ejaculates of good initial motility (80-90%) and sperm concentration (at least 2.5×10^9 / ml) were used. Semen was diluted (1 part semen: 4 part extender) according to **Evans and Maxwell (1987)**. Insemination was carried out using a simple insemination pipette and a vaginal speculum. A total of 1 ml semen containing at least 500×10^6 motile spermatozoa was used for insemination.

The time of the onset of estrus and estrous duration were recorded for the three treatment groups. Fertility was determined by non-return to serve by bucks (up to 64 days after insemination). At the end of the experiment, conception rates (No. of does conceived/ Total No. of does used in the experiment) and litter sizes (No. of kids/ No. of does kidded) were calculated.

3. Blood sampling and hormonal analysis

Blood samples were collected for hormone evaluation from 15 does, five from each treatment group, on the day of sponge insertion (day 0) and then at days 5, 10, 12, 14, 15, 16, 17, 28 and 35. The blood samples were

left to clot at room temperature for at least 4 h. The clots were removed and sera were cleared by centrifugation at 1500×g for 20 min and stored at -20 °C until progesterone hormone assayed.

Progesterone levels were determined using a radioimmunoassay kit (DSL- USA, catalog No. 3900). The assay is based on competition reaction with sensitivity 0.1 ng/ml and coefficient of variation 4.8 and 9.2% for the intra- and inter-assay, respectively (Meizger, 1992). The concentrations were detected using automatic Mini-Gama counter (LKB 1275, USA).

4. Statistical analyses

Data of estrus duration and litter size were analyzed using SAS (1999). Data of progesterone concentrations were analyzed by procedure of mixed repeated measurements of SAS. The significant differences among the treatments groups were tested by Duncan's Multiple Range test.

RESULTS AND DISCUSSION

Results showed that estrous behavior was observed in average on 83.3% of the does in all groups (Table 1). Does of group A showed estrus with a significant higher percentage (100%) than does in group B and C (80 & 70 %, $P < 0.05$). This indicate that earlier injection of GnRH (48 h before sponge withdrawal) seemed able to stimulate many does to commence estrus. LeBlanc et al. (1998) and Jobst et al. (2000) reported that administration of GnRH or its analogous one week prior to PGF2 α improved the rate and precision of synchronization of subsequent estrus in lactating dairy cows. It also increased the size of ovulatory follicle and raised plasma estrogen concentration at estrus (Wolfenson *et al.*, 1994). Twagiramungu *et al.* (1995) reported that application of GnRH resets the follicular wave cycle, leading to selection of a dominant follicle 1- 2 days after GnRH treatment. Moreover, El-Amrawi *et al.* (1993) recorded estrus behavior in 100% of does treated with FGA-sponge for 17 days followed by 400 IU PMSG injection on the day of sponge withdrawal during the breeding season. Chemineau, (1985) reported that FGA pretreatment enhanced the incidence of estrus at first ovulation in Creole does (55% vs 100%).

In the present study, most does in group A exhibited estrus 28 h after sponge withdrawal, while does in group C showed estrous behavior earlier (22 h) ($P < 0.05$) than does in group A (28.3 h) and B (26.3 h) (Table 1). The overall mean of the time of the onset of estrus from sponge withdrawal (25.5 ± 3.6 h) for the three treatment

groups agrees with that found by **Mavrogenis (1988)** in Damascus goats treated with FGA-sponge + PMSG. Does in group C showed estrous behavior earlier ($P < 0.05$) than does in groups A and B. Our results fall within the range (12-48 h) detected by **Corteel (1975)** after the end of FGA-sponge + PMSG treatment during the breeding season.

Moreover, does in group A showed significantly longer estrus duration (31.9 h) ($P < 0.05$) than that of groups B and C (24.6 & 23.0 h) (Table 1). **Mori and Kano (1984)**, and **Greyling and Van Niekrek (1990)** reported that synchronization with PGF2 α , or administration of progestagen together with PMSG lengthens estrous duration by about 6 h compared with natural cycles. Such prolongation was only observed in does of group A. These results agree with the findings of **Devendra and Burns (1983)**. It seems that the mean duration for standing estrus varies between animals and from one estrus to another (**Akusu and Egbunike, 1990** and **Teleb et al., 2003**). On the other hand, **Hafez (1993)** reported that duration of estrus is species-dependent and varies slightly from one female to another, within the same species.

Table 1. Total number of does exhibited estrus, mean values of the onset of estrus* and estrus duration in does of the three treatment groups.

| Does groups | Total number of does | Total number and % of does exhibited estrus | Onset of estrus (hr)* (Mean \pm SE) | Duration of estrus (Mean \pm SE) |
|-------------|----------------------|---|---------------------------------------|------------------------------------|
| A | 10 | 10 ^a (100%) | 28.3 \pm 3.7 ^a | 31.9 \pm 3.8 ^a |
| B | 10 | 8 ^b (80%) | 26.3 \pm 3.6 ^{ab} | 24.6 \pm 3.7 ^b |
| C | 10 | 7 ^b (70%) | 21.9 \pm 3.6 ^b | 23.0 \pm 3.7 ^b |
| Overall | 30 | 25 (83.3%) | 25.5 \pm 3.6 | 26.5 \pm 3.7 |
| <i>P</i> < | - | 0.05 | 0.05 | 0.05 |

^{a, b} Means with different superscripts are significantly different.

* Calculated from time of sponge withdrawal.

Conception rates and litter sizes of does in groups A, B and C are presented in Table 2. The overall mean of does conceived was 66.67%. Litter size was significantly higher in group B (1.6) than groups A and C (1.0 & 1.2) ($P < 0.05$). In addition, the highest conception rate was recorded in does of group B (80%) compared to A (70%) and C (50%). Conception rates detected in the present study are in harmony with those reported by **Ishwar and Pandey (1992)** and **Kusina et al. (2000)**. They detected that progesterone concentrations before natural estrus or during prostaglandin-based synchronized estrus do not alter fertility in goats.

The overall litter size for does in this study was lower than that observed in Damascus goats by **Devendra and Burns (1983)** and **Shalaby et al. (2000)**. It is suggested that the plane of nutrition, body weight of the dam and system of management may affect kidding

percentage and litter size (Shalaby *et al.*, 2000). Furthermore, Galina *et al.* (1995) hypothesized that the number of kids per kidding may be partially affected by the age of the dam and sequence of parturition.

The higher conception rate and litter size (80% and 1.6 ± 0.2 , respectively) recorded in does of group B than group A (70% and 1.0 ± 0.2 , respectively) and C (50% and 1.2 ± 0.2 , respectively) indicate that administration of GnRH 24 h before sponge withdrawal increases ovulation rate, and consequently the incidence of multiple births. In agreement to what observed in group B, Beck *et al.* (1996) detected fertility rate of 89% during the breeding season in sheep, when 4 µg Buserlin + 100 µg cloprostenol was used. Webb *et al.* (1992) and Beck *et al.* (1996) reported that GnRH induces LH surge causing ovulation or luteinization of the ovarian follicles. Moreover, Walker *et al.* (1989) found that administration of GnRH prior to insemination in ewes increased yield of fertilized ova, initiated a new follicular wave development and improved the number of ovulations (Cognie, 1990). In contrast, Baril *et al.* (1998) and Wildeus (1999) observed that conception rate decreased after synchronization of estrus with progestin in goats. Greyling and Van der Nest (2000) and Kusina *et al.* (2000) found no differences after synchronization. The low fertility observed in groups A and C may be due to that the interval from the onset of estrous to the time of insemination is too long or too short, with ova arriving oviduct after death of spermatozoa or before time of insemination.

Table 2. Conception rate and litter size of does in the three treatment groups.

| Does groups | Total number of does | Total number of does conceived | Conception rate (%) | Total number of kids born | Litter size (Mean±SE) |
|-------------|----------------------|--------------------------------|---------------------|---------------------------|-----------------------|
| A | 10 | 7 | 70 ^a | 7 | 1.0±0.2 ^a |
| B | 10 | 8 | 80 ^a | 13 | 1.6±0.2 ^b |
| C | 10 | 5 | 50 ^b | 6 | 1.2±0.2 ^a |
| overall | 30 | 20 | 66.67 | 26 | - |
| P < | - | - | 0.05 | - | 0.05 |

^{a, b} Means with different superscripts are significantly different.

Serum progesterone concentrations in does of the three treatment groups A, B and C during the estrous cycle followed sponge insertion (day 0) and GnRH injection until the day of insemination (day 17) are shown in Figure 1. Progesterone levels were similar in does of the three groups. Progesterone concentrations showed an increase after GnRH injection with the highest values observed at day 17th in does of groups A and C (0.43 ± 0.27 and 0.23 ± 0.08 ng/ml, respectively) and at day 14th in does of group B (0.42 ± 0.25 ng/ml). Ryan *et al.* (1994) and Ullah *et al.* (1996) found that progesterone concentration increased, decreased, or

remained unchanged during luteal phase following GnRH administration in dairy cows.

In the present study both progestagen + GnRH was administrated. Treatment with GnRH alone may results in high proportion of premature luteal regression; in the present study previous priming of progesterone using FGA-sponges could eliminate this effect. Haresign *et al.* (1996) reported that the effect of progesterone may be also exerted directly at the level of the follicle to alter its ability to respond to gonadotrophin stimulation, thereby ensuring normal luteal development after ovulation. Aboul-Ela *et al.* (2004) and El-Sobayil *et al.* (2007) observed that postpartum estrus activity increased with the increase of progesterone concentration pre- and post-estrus incidence in goats and buffaloes. They suggested that progesterone has a priming effect on the onset of postpartum estrus activity.

Serum progesterone concentrations in all groups were significantly different ($P < 0.001$) between does that conceived and those that failed to conceive after insemination (Figure 2). Progesterone concentrations tended to increase throughout the gestation period (Mavrogenis, 1988 and Gaafar *et al.*, 2005), reaching the highest levels at day 41 (5.2 ng/ml).

CONCLUSION

In conclusion, the physiological basis of GnRH and the low economic cost of FGA sponge + GnRH protocol make it suitable to be used to synchronize estrus and ovulation with acceptable conception rate. Administration of GnRH 24 h before sponge withdrawal increases conception rate, and consequently the incidence of multiple births.

Further study using larger number of animals is recommended to validate the efficiency of administrating GnRH 24 h before sponge withdrawal. In addition, using ultrasonography or laparoscopy is needed to determine the ovulation rate. Measuring LH profile throughout the experiment is also important to detect ovulation time.

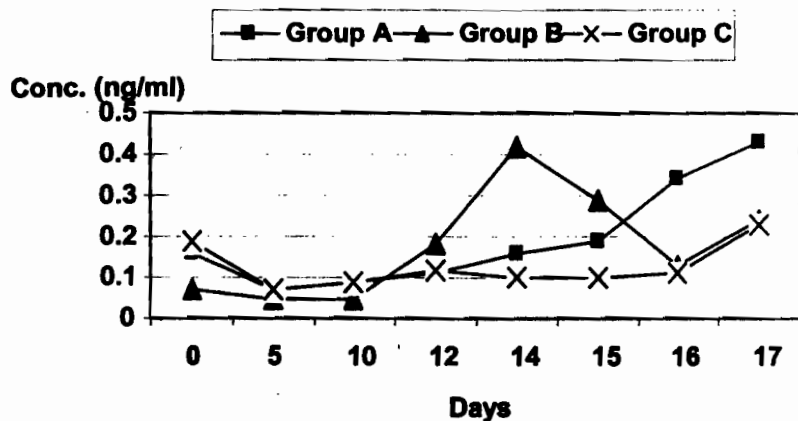


Fig. 1. Mean values of serum progesterone concentrations (ng/ml) followed sponge insertion (day 0) and GnRH injection in does of groups A (on day 13), B (on day 14) and C (on day 15) until days of insemination (day 17).

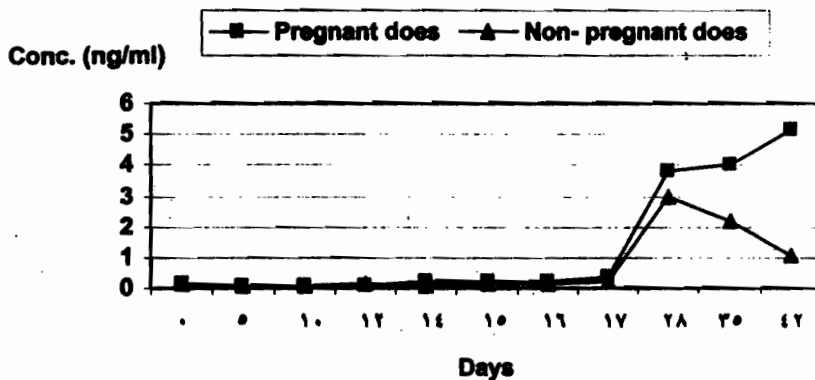


Fig 2: Mean values of serum progesterone concentrations (ng/ml) in pregnant and non- pregnant does in the 3 treatment groups (A, B and C) from time of sponge insertion (day 0) until day 42.

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استخدام الإسفنجات المهبلية (FGA) + GnRH لتحسين خصوبة الماعز
خلال موسم التناسل

دعاء فاروق طلب، طارق العشموى

قسم بحوث الأغنام و الماعز - معهد بحوث الانتاج الحيوانى

تم تقييم الخصوبة فى عدد ٣٠ معزة خليط (دمشقى- بلدى) بعد معاملتها
بالإسفنجات المهبلية +GnRH . قسمت الماعز إلى ٣ مجاميع متساوية (أ، ب، ج).
عوملت الماعز فى المجاميع الثلاث خلال موسم التلقيح بالإسفنجات المهبلية
(FGA) لمدة ١٥ يوم، ثم حققت الماعز بالمجاميع (أ، ب، ج) بـ ٤ ميكروجرام
GnRH فى اليوم ١٣، ١٤ و ١٥ من وقت وضع الإسفنجات، على التوالى.

أظهرت النتائج إن نسبة حدوث الشياح فى الماعز فى المجاميع الثلاث هى
٨٣,٣%. حيث شاعت جميع الماعز بالمجموعة أ (١٠٠%) مقارنة بـ ٨٠ و ٧٠%
بالمجاميع (ب، ج). أظهرت الماعز فى المجاميع الثلاث الشياح ٢٤-٣٦ ساعة من
نزع الإسفنجات (متوسط 30.5 ± 0.9 ساعة)، حيث ظهر الشياح فى معظم الماعز
بعد ٢٨ ساعة من نزع الإسفنجات. كما أظهرت النتائج إن طول فترة الشياح أطول
فى ماعز المجموعة أ (31.9 ± 3.8 ساعة) مقارنة بـ 24.6 ± 3.7 و 23.0 ± 3.7
ساعة فى المجاميع ب و ج، على التوالى.

أوضحت النتائج إن ماعز المجموعة (ج) كانت الأمل فى الخصوبة (٥٠%)
مقارنة بـ (٧٠، ٨٠%) فى المجاميع أ و ب، على التوالى. وكانت ماعز
المجموعة (ب) الأعلى فى نسبة الخصوبة وعدد المواليد فى البطن الواحدة (٨٠%)
و 1.6 ± 0.2 ، على التوالى).

كانت نتائج استخدام المعاملة بالإسفنجات المهبلية لمدة ١٥ يوم مع حقن ٤
ميكروجرام GnRH ٢٤ ساعة قبل نزع الإسفنجات إيجابية حيث أعطت نتائج جيدة
لتوحيد الشياح وكذلك تعدد التبويض و عدد الولادات فى البطن الواحدة.