



## Synchronization of Estrus in Field Conditions Using Progestagen Sponge, GnRH, and PGF2 $\alpha$ in Barki Ewes during Breeding Season

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### ABSTRACT

This study was conducted to evaluate reproductive performance of Barki ewes subjected to three estrus synchronization programs in field conditions during breeding season. For this purpose, 36 cycling ewes were divided into 4 groups according to the program assigned: Group SeCG (n=10): received intravaginal progesterone sponge (40 mg Fluorogestone acetate, Chrono-gest®, Intervet International) for 7 days, at time of sponge removal, each ewe received i.m injection of 500 IU eCG (Folligon®, Intervet International); Group PG-PG (n=10): received double injections of 250 $\mu$ g Cloprostenol (PGF2 $\alpha$  analogue, Estrumate®, Intervet International) 11 days apart; Group GPG (n=6): received 4 $\mu$ g Buserelin by i.m injection on day 0 (GnRH analogue- Receptal®, Intervet International), on day 5 animals received 250 $\mu$ g Cloprostenol followed by 4 $\mu$ g Buserelin by i.m injection on day 7; Group C (n=10): served as control group that did not receive any treatment. Results showed that estrus induction rate was significantly high in SeCG group that was recorded at 100% (P<0.05), while it was 90%, 60%, and 50% for PG-PG, C and GPG respectively. Estrus synchrony was more uniform in SeCG group as 70% of ewes exhibited estrus around 24-36 hrs from end of treatment. There was no significant difference among groups in pregnancy and lambing rate (p>0.05). Pregnancy rates were 100%, 90%, 88.9%, and 83.3% for GPG, SeCG, PG-PG, and C group respectively. **Conclusion**, from results we conclude that intravaginal progesterone sponge for 7 days+500IU eCG at sponge removal is convenient for estrus synchronization of ewes raised in field conditions during breeding season. Moreover, reproductive and fertility parameters recorded in the current study are acceptable and within values reported previously in farm conditions.

### 1. INTRODUCTION

Barki sheep are one of the most dominant breed in Egypt and subtropical areas, they can adapt to unfavorable environmental conditions such as heat stress and poor nutrition. Barki ewes are known to be seasonal breeders; however some studies reported that they can breed all year round (Aboul-Naga et al., 1991). Estrus and ovulation synchronization are management tools used to increase number of pregnant females and to shorten breeding interval resulting in enhanced reproductive efficiency. Controlling reproduction in sheep can be achieved using several methods; some of these include

administration of hormones that modify the physiological events of estrous cycle. Several protocols have been employed to synchronize estrus in ewes during breeding season; the most widely used method is intravaginal progesterone-releasing devices with or without prostaglandin (PGF2 $\alpha$ ) and equine chorionic gonadotropin (eCG) at the time of device removal (Martemucci and D'Alessandro, 2010b; Martinez et al., 2015). Usually duration of progesterone treatment is between 5 and 14 days with variable fertility rates (Alnimer et al., 2005; Martemucci and D'Alessandro, 2010b); progesterone-based protocols can also be used to induce estrus

during non-breeding season in sheep (Almadaly et al., 2016). gonadotropin-releasing hormone (GnRH) injection 24 hours after sponge removal as alternative to eCG was tested and proven to induce estrus in infertile ewes (Lone et al., 2016). Administration of progesterone or its analogues for estrus synchronization is based on simulating the action of natural progesterone produced during luteal phase of estrous cycle, which controls LH secretion from the pituitary gland (Abecia et al., 2012). Other methods of estrus synchronization use GnRH and/or PGF2 $\alpha$  to control luteolysis and lifespan of corpus luteum (CL) in cyclic ewes (Amiridis et al., 2005; Titi et al., 2008). In developing countries, small-holder farmers have a significant role in animal production, they also provide source of income to household communities. Small animal production in rural areas has the potential to develop into economically viable enterprise; hence more research efforts should be directed to such areas to boost animal fertility and productivity. Noticeably, most of the published studies on estrus synchronization in small ruminants were performed under strict farming environment that meets optimum managerial practices of animal breeding. Therefore, the purpose of this study was to evaluate practicality of estrus synchronization programs in sheep raised in field conditions of rural areas.

## **2. MATERIALS AND METHODS:**

### **2.1. Animals**

This study was conducted on 36 Barki ewes that belong to a farmer in village near Damanhour, El Behira province. Animals were healthy with normal breeding history; ewes were dewormed one week before the beginning of the study. The age of the ewes ranged between 3 to 4 years, and their weight ranged between 40 to 60 kg with body condition score of 2-3. The herd was kept in field in semi-covered pens at ambient temperature and natural day length. Animals had free access to drinking water and green roughages. Experimental procedures used in the current study were approved by local ethical committee of Alexandria University.

### **2.2. Estrous synchronization:**

During breeding season (October-January), ewes under study were divided into 4 groups that were treated with the following protocols:

#### **Group SeCG:**

10 ewes received intravaginal progesterone sponge (loaded with 40 mg FGA-Fluorogestone acetate, Chrono-gest®- Intervet International). Sponges

remained for a period of 7 days. At time of sponge removal, each ewe received i.m injection of 500 IU eCG (Folligon®, Intervet International).

#### **Group PG-PG:**

10 ewes received 2 injections of 250 $\mu$ g Cloprostenol (PGF2 $\alpha$  analogue, Estrumate®, Intervet International) with 11 days interval.

#### **Group GPG:**

6 ewes received 4 $\mu$ g Buserelin by i.m injection on day 0 (GnRH analogue- Receptal®, Intervet International), on day 5 they received 250 $\mu$ g Cloprostenol followed by 4 $\mu$ g Buserelin by i.m injection on day 7.

#### **Group C:**

10 ewes served as control group that did not receive any treatment.

### **2.3. Parameters recorded:**

After the last treatment in each program, 2 fertile rams were introduced to the herd for estrus detection and mating (male to female ratio was 1:5). Rams were color-marked on breast region to identify ewes in estrus; rams were kept for 5 days and estrus detection was performed twice daily, females in estrus were identified by colored rump. Reproductive parameters recorded for each group included: estrus response rate (number of ewes showed estrus/number of treated ewes X100), time of estrus exhibition after end of treatment, pregnancy rate (number of ewes conceived/number of ewes mated X100), lambing rate (number of ewes lambd/number of ewes mated X100), and litter size (number of lambs born/number of lambd ewes).

### **2.4. Statistical analysis:**

Percentages of animals showed estrus, pregnancy/lambing rates, and litter size were compared using the chi-square test using SAS 1990 (SAS, 2002).

## **3. RESULTS:**

As shown in table 1, the highest incidence of estrus ( $P<0.05$ ) was recorded in SeCG group (100 %) followed by PG-PG group (90%). Estrus response rate was the least in GPG group that was recorded at 50 % (3 out of 6 ewes), all exhibited estrus 24-48 hrs from end of treatment. Estrus exhibition was compact and synchronous in SeCG group as 70% of ewes showed estrus 24-36 hrs after end of treatment. Estrus exhibition was less uniform in PG-PG group when compared to SeCG group (only 50% showed estrus at the first 24-36 hrs after treatment). Table 2 shows that no significant difference ( $p>0.05$ ) in pregnancy rate, lambing rate and litter size between groups was found.

**Table (1):** Effect of different estrus synchronization programs on exhibition of estrus signs and response rate:

Programs	n	Distribution of estrus exhibition after end of treatment			
		% (No showed estrus)			
		24-36 hrs	36-48 hrs	48-72 hrs	ERR*
<b>SeCG</b>	10	70 (7)	20 (2)	10 (1)	100 (10)
<b>PG-PG</b>	10	50 (5)	20 (2)	20 (2)	90 (9)
<b>GPG</b>	6	16.7 (1)	33.3 (2)	-	50 (3)
<b>Control</b>	10	20 (2)	20 (2)	20 (2)	60 (6)

\*ERR: total estrus response rate between groups is significant (P<0.05)

**Table (2):** Effect of different estrus synchronization programs on pregnancy/lambing rates, and litter size:

Programs	n	pregnancy rate	Lambing rate	Litter size
		% (n)	% (n)	
<b>SeCG</b>	10	90 (9)	88.9 (8)*	2 (16/8)
<b>PG-PG</b>	9	88.9 (8)	100 (8)	2 (16/8)
<b>GPG</b>	3	100 (3)	100 (3)	1 (3/3)
<b>Control</b>	6	83.3 (5)	100 (5)	2 (10/5)

\*One ewe aborted

Differences in pregnancy rate, lambing rate, and litter size between various groups are non-significant (p>0.05).

#### 4. DISCUSSION:

The aim of the current work was to test the usefulness of estrus synchronization programs as one of the modern reproductive techniques in sheep flock kept in field conditions. For this purpose, 4 groups of ewes were subjected to 3 different protocols of estrus synchronization, and then fertility parameters were compared to a control group. Our results showed that using progesterone sponge protocol “SeCG” achieved the highest estrus response rate (100%) that was more compact in comparison to other groups. Similar result was reported by Almadaly and co-workers as they reported 100% estrus induction rate using PRID for 6 days in Rahmani ewes (20 mg FGA+250µg PGF2α 24hrs before sponge removal+500IU eCG at sponge removal) during non-breeding season (Almadaly et al., 2016). Using medroxyprogesterone acetate “MAP” sponge for as short as 4 days combined with PGF2α and GnRH also produced 100% estrus induction rate in Awassi ewes (Husein and Kridli, 2003). Martemucci and D'Alessandro recorded a value of 92.3% estrus induction rate (40 mg FGA sponge for 5 days+100µg PGF2α at the sponge insertion+200IU eCG at sponge removal) during breeding season (Martemucci and D'Alessandro, 2010b). The higher response rate in our study might be due to higher dose of eCG that enhanced follicular stimulation after sponge removal. Martinez and co-workers reported lower estrus induction rate (77.3%) using CIDR for 7 days (1.3g progesterone+GnRH at

sponge insertion+125µg PGF2α and 400IU eCG at sponge removal) in anestrus ewes (Martinez et al., 2015).

Progesterone has a “priming” effect on the brain, which enhance the response to gonadotropins administered after the end of progesterone treatment (Niasari-Naslaji et al., 2001). The synchrony of estrus exhibition observed in SeCG group is advantageous as timed artificial insemination could be applied following this protocol thus reducing the need for heat detection aids. The second highest estrus response rate (90%) was recorded in PG-PG program, this result is similar to the previously reported in Barki ewes (Abdalla et al., 2014), while in Awassi ewes the response was 85% (Alnimer et al., 2005). In goats, double PGF2α injection protocol produced 100% estrus induction rate in both farm and field conditions (Andrabi et al., 2015). Unlike progesterone-based protocols, regimens that use PGF2α are not suitable during non-breeding season because of absence of functional CL. However, Almadaly and co-workers reported about 30% estrus induction rate using a PGF2α program outside the breeding season (Almadaly et al., 2016). The authors explained that this result confirms the all year cyclicity of some ewes in the Mediterranean climate of northern Egypt. In the present study, estrus was spontaneously exhibited in 60% of ewes in control group, while Ovsynch protocol “GPG” produced about 50% estrus response rate. Other workers reported lower values;

Martemucci and D'Alessandro obtained 33.3% response rate in Awassi ewes (Martemucci and D'Alessandro, 2010a), Lone et al obtained a value of 28.75% in infertile ewes (Lone et al., 2016). Interestingly, GPG program failed to induce estrus in Rahmani ewes out of breeding season (Almadaly et al., 2016).

Fertility parameters represented by pregnancy and lambing rate did not significantly differ between groups; in GPG group all ewes conceived and lambed (100%) but litter size was low as compared to other groups. In SeCG group pregnancy and lambing rates were 90% and 88.9% respectively (one ewe was aborted); Martemucci and D'Alessandro recorded similar pregnancy rate of about 92.3% (Martemucci and D'Alessandro, 2010b). On the other hand, pregnancy rate obtained in the current study was higher than values reported (75%) after using 4-days MAP sponge (Husein and Kridli, 2003), and also higher than values reported (66.6%) after using 6-days PRID program (Almadaly et al., 2016). In our study, pregnancy rate in PG-PG group was 88.9% (100% lambing rate); comparable value of about 83.3% was achieved in goats raised in field conditions (Andrabi et al., 2015). Abdalla and others recorded higher values of pregnancy rate (95%) using the same protocol (Abdalla et al., 2014). Much lower rates were recorded in Awassi ewes: 55% and 40% as pregnancy and lambing rate respectively (Alnimer et al., 2005). The observed variability in estrus response rate and fertility parameters among studies could be attributed to different breeds of ewes, climatic conditions, and different dosage and combination of treatments

## 5. CONCLUSION

From results we recommend using intravaginal progesterone sponge for 7 days+500 IU eCG at sponge removal (SeCG protocol) for estrus synchronization of ewes raised in field conditions during breeding season. SeCG program achieved high estrus response rate and good fertility potential.

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