



Efficiency of different methods of estrus synchronization followed by fixed time artificial insemination in Persian downy does

Majid Hashemi^{1,2,3}, Mazaher Safdarian²

¹Razi Vaccine and Serum Research Institute, Shiraz Branch, Agricultural Research, Education and Extension Organization (AREEO), Shiraz, Iran.

²Animal Science Research Department, Fars Agricultural and Natural Resource Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Shiraz, Iran.

Abstract

For evaluating different methods of long term estrous synchronization followed by fixed time artificial insemination and to select the most efficient method, during the breeding season 160 Persian downy does were equally allocated to groups (n = 20/group). Estrus was synchronized using controlled internal drug release devices alone (CIDR) or with equine chorionic gonadotropin (CIDR-eCG), intravaginal sponge impregnated with 45 mg fluogestone acetate alone (Sponge) or with eCG (Sponge-eCG), subcutaneous auricular implant of 2 mg norgestomet alone (Implant) or with eCG (Implant-eCG) or two intramuscular injections of 10 mg prostaglandin F2 α 10 days apart alone (PGF) or accompany with eCG (PGF-eCG). The dose of eCG was 400 IU and injected intramuscularly at the end of treatments. Heat detection and fixed time artificial insemination were done 12 and 48 hours after the end of the treatments, respectively. The estrus was detected in 94.7-100% of the does who received different forms of progestagens and estrus response was not affected by eCG. Estrus response in PGF-based groups was significantly lower (P < 0.05) than progesterone-based groups. Injection of eCG did not affect fertility, fecundity and prolificacy rates in CIDR, Sponge and Implant groups. It also did not affect parturition rate in implant group. Finally, with respect to estrus response, fertility rate, costs and the simplicity of methods it can be concluded that the use of intravaginal CIDR for 16 days without administration of eCG may be a more efficient method for estrus synchronization in fixed-time artificial insemination programs in Persian downy does.

Keywords: AI, breeding season, estrus synchronization, goat, progesterone, prostaglandin,

Introduction

Goats exhibit seasonal cycles of reproduction and it can be controlled by manipulating their estrous cycle using different methods of estrus synchronization so that females express estrus at approximately the same and desirable time (Zhao *et al.*, 2010). Synchronization of estrus is a key component of artificial insemination that has provided a relatively simple and low cost method for dissemination of valuable genes in genetic improvement programs of goats worldwide. Moreover,

estrus synchronization can play an important role for managing production system, allowing the density of mating and kidding and production of meat and milk during specific times of the year for strategic marketing and other purposes (Baldassarre and Karatzas, 2004, Zhao *et al.*, 2010). In small ruminants, hormonal estrus synchronization is achieved either by reducing the length of the luteal phase of the estrous cycle with prostaglandin F2 α or by extending the cycle artificially with exogenous progesterone or more potent progestagens (Hashemi *et al.*, 2006, Abecia *et al.*, 2012). Progestogen administration is common and has been used with or without accompanying treatments such as gonadotropins or prostaglandin analogs. Accompanying treatments can make protocol more expensive for farmer (Whitley and Jackson, 2004). Estrus synchronization research is imperative to establish optimal doses and agents to use for favorable synchrony and fertility in each breed and introduce a simple, pragmatic and reliable protocol that can be adopted by farmers.

Iran is accounted for approximately 3% of the total number of goats in the world and is ranked in the seventh place. They produce 300 million liters of milk and 94 million Kg of red meat annually which play an important role in the economy of farmers in this region (Agriculture Statistics of Iran, 2013). Most of the goat's population in Iran (4.35 million heads) is raised in Fars province and Persian downy does is an indigenous goat in this region. In Iran, efficacy of reproductive performance of Nadooshani goat during the breeding season was investigated by using CIDR, fluogestone acetate sponges and cloprostenol (Bitaraf *et al.*, 2007), but there is no documented data on controlled breeding in Persian downy does. The aim of the present study was to evaluate different methods of long term estrous synchronization followed by fixed time artificial insemination and to select the most efficient method in Persian downy does.

Material and Methods

Location and experimental does

The experiment was carried out during the breeding season at the research farm of Jihad-Agriculture Organization in Abadde City (latitude 31° 9', longitude 52° 39', altitude 1288 m and annual rainfall of 140 mm) located in south of Iran. A total of 160

³Corresponding author: Mj.Hashemi@areeo.ac.ir

Phone: +98(71)36240021

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Persian downy does that had kidded at least once, ranging in age from 3 to 5 years and body weighting (BW) between 35 and 40 kg were used in the trial. Body condition scores (BSC) ranged from 2.5 to 3 (scale 1–5) and scored by palpation of lumbar region (Koyuncu and Altınçekiç, 2013). Experimental does were separated from bucks for at least two months before starting the trial. In addition, 5 Persian downy bucks of apparent breeding soundness and proven fertility and 10 bucks fitted with abdominal apron were used in this study. The females were allowed to graze on natural pastures of *Artemisia Siberi*, *Zygophyllum Atriplicoides*, *Stachys Inflata* and *Salsola Arbuscula* during the day (06.00–17.00 h) and were kept indoors at night to receive near 350 g barley per doe per night. Access to a mineral salts lick and water was ad libitum.

Synchronization of estrus

Does were allocated to eight treatments groups of 20 animals each on 15 of September. Animals were sorted by age, BW and BSC respectively and allocated to groups considering sorted list. Average of age was same in groups 1 to 6 (3.75 ± 0.79 years) and group 7 with group 8 (3.85 ± 0.81 years). Mean of BW and BSC were 36.92 ± 1.40 Kg and 2.88 ± 0.22 in group 1, 36.99 ± 1.38 Kg and 2.90 ± 0.21 in group 2, 37.11 ± 1.41 Kg and 2.80 ± 0.25 in group 3, 37.15 ± 1.43 Kg and 2.80 ± 0.25 in group 4, 37.22 ± 1.45 Kg and 2.83 ± 0.25 in group 5, 37.30 ± 1.46 Kg and 2.83 ± 0.25 in group 6, 36.95 ± 1.41 Kg and 2.85 ± 0.24 in group 7 and 37.13 ± 1.51 Kg and 2.80 ± 0.25 in group 8, respectively. Groups were identified by different colors. The estrous cycle of females in each group was synchronized with one of the following treatments. 1) CIDR, vaginal insertion of a controlled internal drug release device containing 0.3 g progesterone (CIDR®, inter Ag, New Zealand) for 16 days, 2) CIDR-eCG, CIDR treatment and intramuscular injection of 400 IU equine chorionic gonadotropin (eCG) (Folligon, Intervet, Holland) at CIDR removal, 3) Sponge: vaginal insertion of a polyurethane sponge impregnated with 45 mg fluorogestone acetate (Chronogest®, Intervet, Holland) for 16 days, 4) Sponge-eCG, Sponge treatment plus intramuscular injection of 400 IU eCG at sponge removal, 5) Implant, simultaneous insertion of a subcutaneous auricular implant of 2.0 mg norgestomet (Crestar®, Intervet, Holland) for 10 days and intramuscular injection of 2.5 mg estradiol valerate and 1.5 mg norgestomet, 6) Implant-eCG, Implant treatment plus intramuscular injection of 400 IU eCG at implant removal, 7) PGF, receipt of two intramuscular injections of 10 mg prostaglandin F_{2α} (Vetalutic®, Aboureihaan, Iran) 10 days apart, and 8) PGF-eCG, PGF treatment plus intramuscular injection of 400 IU eCG in tenth day.

Heat detection

Twelve hours after the end of the treatments, 10 bucks fitted with abdominal apron were introduced to does and the signs of estrus as tail wagging, bleating, searching of the male, frequent urination, hyperemia,

edema and contraction of the vulva, vaginal mucus discharge (Martemucci and D'Alessandro, 2011) were monitored twice daily for 1 h at 08.00 and 18.00 hours by visual observation during 4 days. Does were considered to be in estrus when standing to be mounted.

Fixed time artificial insemination

All treated goats were inseminated 48 hours after the end of the treatments by cervical route using fresh semen. Semen was collected with an artificial vagina from five proven fertile Persian downy bucks. Semen samples were evaluated for volume and motility and only ejaculates over 0.5 mL, mass activity ≥ 3 (0-5 scale) and progressive motility $\geq 70\%$ were pooled and extended in TRIS-based extender (Paulenz *et al.*, 2002). The temperature of extended semen was lowered slowly to 10 °C.

A breeding rack was used for cervical inseminations and the hindquarters of the goat lifted over the top rail while the front legs remained standing on the ground. A dose of semen containing at least 200×10^6 spermatozoa in 0.25 mL straw was deposited into the external os of the first cervical fold of each goat, using speculum fitted with an internal light source and insemination pipette (Vilarino *et al.*, 2011).

Variables and statistical analysis

The following parameters were calculated in each of the treatments:

Estrous response: number of females exhibiting overt estrus/number of treated females $\times 100$

Fertility rate: number of kidded females/number of females in estrus $\times 100$

Kidding rate: number of kidded females/number of treated females $\times 100$

Fecundity rate: number of kids at birth/ number of females in estrus $\times 100$

Prolificacy rate: number of kids at birth/ number of kidded females $\times 100$

These parameters were analyzed using the chi-square test. The statistical software program of SAS was used for the analyses.

Results

Three goats were excluded from the data due to loss of intravaginal pessaries (2 sponges and 1 CIDR). A mucosal discharge with an odor was observed only when sponges were withdrawn. The distribution of the onset of estrus following the end of treatments is illustrated in Fig. 1. Most of the females in various treatments, except in PGF group showed standing heat 36 hours after treatment termination. The estrus response and other reproductive parameters after using different methods of estrus synchronization are presented in Table 1. The estrus was detected in 94.7–100% of the does who received different forms of progestagens while injection of eCG did not affect this parameter. Estrus response in PGF-based groups was significantly lower ($P < 0.05$) than progesterone-based

groups and this parameter was increased significantly by eCG in does who were treated with prostaglandin F2 α . Injection of eCG did not affect fertility, fecundity

and prolificacy rates in CIDR, Sponge and Implant groups and the parturition rate in the implant group.

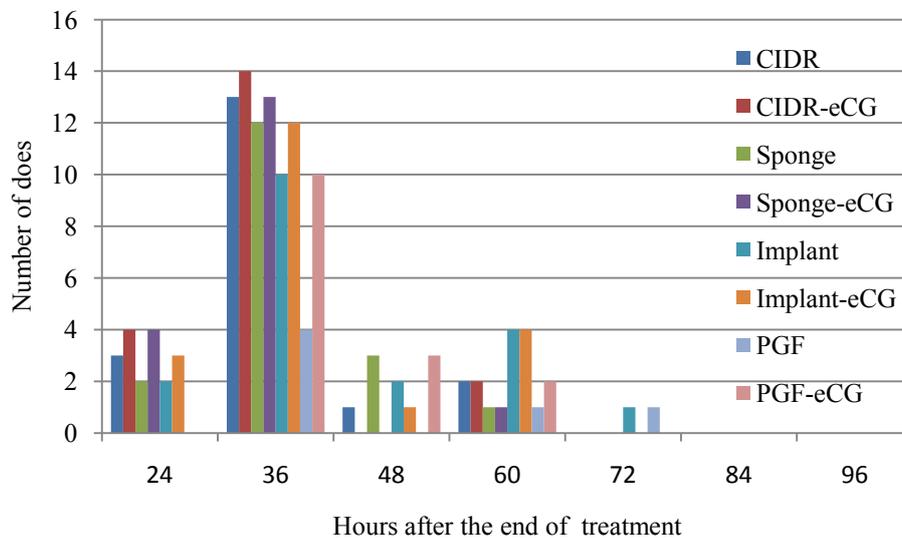


Fig.1 Distribution of the onset of estrus after treatments termination

Table 1. Reproductive parameters of Persian downy does in different estrus synchronization methods.

Treatments	n.	Estrous response (%)	Fertility rate (%)	Kidding rate (%)	Fecundity rate (%)	Prolificacy rate (%)
CIDR	19	100 ^a	84.2 ^a	84.2 ^a	89.7 ^{abc}	106.3 ^c
CIDR-eCG	20	100 ^a	70 ^{abc}	70 ^{bc}	75 ^{cd}	107.1 ^c
Sponge	19	94.7 ^a	66.7 ^{bcd}	63.2 ^{cd}	83.3 ^{bc}	125 ^a
Sponge-eCG	19	94.7 ^a	77.8 ^{ab}	73.6 ^b	94.4 ^{ab}	121.4 ^a
Implant	20	95 ^a	68.4 ^{abcd}	65 ^{cd}	84.2 ^{bc}	123.1 ^a
Implant-eCG	20	100 ^a	60 ^{cd}	60 ^d	75 ^{cd}	125 ^a
PGF	20	30 ^c	83.3 ^a	25 ^f	100 ^a	120 ^{ab}
PGF-eCG	20	75 ^b	53.3 ^d	40 ^e	60 ^d	112.5 ^{bc}

(a-f) Different superscripts in each column differ significantly ($P < 0.05$).

Discussion

All of the treatments (except PGF without eCG) were effective in synchronizing estrus in Iranian indigenous goats. In contrast to the observations of Romano (1996), Amarantidis et al (2004) and Zhao et al., (2010), the intravaginal pessaries fell off in some of does in our trial. Factors such as animal size, employed technique, texture and consistency of device could influence retention of device in the vagina (Alifakiotis et al., 1982, Romano, 1998). The percentage of goats exhibiting overt estrus in this trial was comparable to the findings of other researchers who reported 94.4-100% after using FGA and eCG (Motlomelo et al., 2002, Amarantidis et al., 2004, Dogan et al., 2005, Bitaraf et al., 2007, Bukar et al., 2012, Omontese et al., 2012). In the present study, estrus response with CIDR and eCG was higher than the estrus response of 84% observed in Sahel goats (Omontese et al., 2012) and 66.7% in Saanen dairy goats (Kajaysri and Thammakarn, 2012). However, with two injection of prostaglandin F2 α estrus response was lower than the 95% found in indigenous Greek goats (Amarantidis et al., 2004) and the 97% in Nadooshani goats (Bitaraf et al.,

2007). Results indicate that the use of CIDR, sponge or implant treatments alone or with eCG to be equally efficient in synchronizing estrus in goats. This shows that even though CIDR contains a less potent hormone (i.e. progesterone), its efficiency was comparable to that of progestagens. In contrast to this finding, some authors reported higher estrus response when progesterone or progestagens were used in association with gonadotrophins (Oliveira et al., 2001, Omontese et al., 2012). The result of present study is in agreement with Bukar et al., (2012) who found that the use of eCG concurrent with second injection of prostaglandin F2 α significantly increased estrus response. Although eCG enables the follicles to reach final maturation stage, it present some limitations like, high cost and formation of antibodies following repeated synchronization treatments in a fraction of the goat population (Roy et al., 1999). Moreover, timing of the fixed-time insemination was disorganized by the prolonged follicular phase of such females (Holtz et al., 2008). The onset and density of estrus are important as they could affect the efficacy of fixed-time artificial insemination program. In progesterone-based groups a higher proportion of does were detected in estrus up to 36 hours following eCG



administration at pessary removal. The use of eCG is known to reduce the time to onset of estrus (Omontese *et al.*, 2012). The difference in rate of absorption and metabolize of each hormones can affect the onset of estrus (Romano, 1996). The fertility, kidding and prolificacy rates in the present study were lower than that observed by Olivera *et al.*, (2001) who used subcutaneous auricular implant or CIDR with or without administration of eCG in Saanen goats. A single injection of prostaglandin also was used at the time of pessary removal and it may be the causes of this difference. Differences in breed, nutrition, and management also could account for the differences in estrus response and reproductive performance between the current study and other reports (Whitley and Jackson, 2004). In contrast to fecundity and prolificacy rates, kidding rate in Nadooshani goats was lower than our results. Kidding rate was reported 55%, 60% and 75% using CIDR, Sponge and prostaglandin followed with eCG treatments, respectively (Bitaraf *et al.*, 2007). It should be pointed out that in various studies, reproductive parameters may be calculated differently which should be taken into consideration when results are compared. For example, the number of kids born per female kidded and the number of kids born per female in estrus were accounted for calculating fecundity and prolificacy, respectively (Bitaraf *et al.*, 2007). Finally, with respect to estrus response, fertility rate, costs and the simplicity of methods it can be concluded that the use of CIDR for 16 days without administration of eCG may be a more efficient method for estrus synchronization in fixed-time artificial insemination programs in Persian downy does.

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