Efficacy of CIDR, fluogestone acetate sponges and cloprostenol for estrous synchronization of Nadooshani goats during the breeding season

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(Received 14 Oct 2006; revised version 25 Jan 2007 accepted 13 Feb 2007)

Summary

The objective of this study was to evaluate three methods of estrous synchronization, viz. controlled internal drug releasing device (CIDR), intravaginal sponges impregnated with fluogestone acetate (FGA), and cloprostenol (Estramate; an analogue of prostaglandin F2α) in Nadooshani goats of Yazd province, Iran. The estrous synchronized does (n = 30 to 33 per treatment), after heat detection, were artificially inseminated (once) with diluted semen of fertile bucks. Pregnancy was determined by measuring blood serum progesterone levels on day 21 after insemination, followed by ultrasonography at mid-gestation. No significant difference was observed for the interval between the end of the synchronization protocol and the standing heat amongst treatments (range: 23 to 35 hrs). There was no significant difference in the interval between the time of standing heat and insemination among treatments (range: 15 to 27 hrs). Blood serum progesterone levels (overall mean: 4.80 ± 0.41 ng/ml; SEM), litter size (overall mean: 1.32 ± 0.05; SEM), non return rate to estrus and the kidding rate were not significantly affected by the synchronization methods. Serum progesterone levels were significantly lower (P<0.01) in does that returned to estrus after artificial insemination (AI). Prolificacy and fecundity were not significantly affected by the synchronization methods; however, cloprostenol method was found to be more convenient and economical under the conditions of this experiment.

Key words: Goat, Estrous synchronization, CIDR, Intravaginal sponge, PGF2α

Introduction

In herds of goats, artificial insemination (AI) could be used for increasing desirable production traits and number of offspring produced per sire per year, grading-up to a new strain or genotype, hastening genetic progress, increasing efficiency of breeding and controlling disease by using diluted or frozen semen of superior bucks (Evans and Maxwell, 1987). The use of AI is facilitated after estrous synchronization programs which induce tight estrus during a short period of time, and improve pregnancy and prolificacy rates.

During the breeding season, when goats are actively cycling, estrus can be synchronized with PGF2α or one of its analogues, such as cloprostenol (Gordon, 1997); however, the number of observations in different breeds is still insufficient for allowing firm conclusions. The most extensively researched method (Bearden and Fuquay, 2000) is the use of vaginal sponges impregnated with 40 to 50 mg of fluogestone acetate (FGA). Zarkawi et al. (1999) showed that induction of estrus in indigenous Damascus goats, outside the breeding season by using medroxyprogesterone acetate (MAP) plus injection of eCG (equine chorionic gonadotropin) at the time of sponge removal resulted in estrous response of 100%; conception rate and fecundity were 65.8 and 195.2%, respectively. Simmonetti et al. (2000) induced estrus in cycling Merino
ewes with sponges impregnated with different doses of MAP. There were no significant differences amongst groups for estrous incidence, interval to onset of standing estrus, and pregnancy rates. It was concluded that under similar conditions, a dose as low as 40 mg of MAP could be effectively used for estrous induction. Motlomelo et al. (2002) showed that several progestagen treatments, including MAP, FGA and controlled internal drug releasing device (CIDR) were equally efficient for estrous induction and synchronization of Boer and African indigenous goats; no significant differences were found in pregnancy rates 40 days after AI.

Fonseca et al. (2005) studied the effects of the duration of treatment with intravaginal sponges containing 60 mg MAP (6 and 9 days) for estrous synchronization in non-lactating Toggenburg goats. Both treatments were equally effective in inducing estrus (84 to 89%). Although the effects of treatments on fertility were not investigated in their study, they suggested, based on studies in ewes (Vinoles et al., 2001) and cattle (Diskin et al., 2002), that fertility should be greater in these goats as a result of the shorter-term progestagen treatments.

There are about 25 million goats in Iran, and goat production has a significant economical role for Iranian farmers. Flocks have been established aimed at preserving and breeding Iranian native goats. Nadooshani goats have been included in this program. Information on reproductive performance of Iranian goats are very scarce (Emady et al., 2006; Zamiri and Heidari, 2006). Nadooshani goats, a small native breed, are raised mostly for milk (with the milk yield of about 45 kg in 180 days of lactation) and fiber in Nadooshan (3266 meters above the sea level, 31° 46’ N latitude and 53° 47’ E longitude, and 140 mm annual rainfall) which is located in southwest of Yazd province, central Iran. The goat herds are raised on natural vegetations during most parts of the year, except for approximately 30 days during winter when they are stable-fed. Under local practices, the bucks are herded separately except during a short mating period that lasts from early October to early November. As a part of a nationwide program for preservation and genetic improvement of the native livestock, several Nadooshani goat herds have also been established which are bred by artificial insemination after estrous synchronization. Various methods of estrous synchronization are practiced in several goat flocks in Iran, but no research has been conducted concerning the efficacy of these methods, including in the Nadooshani goats. Therefore, the present investigation was conducted to compare the efficiency of CIDR, intravaginal sponges impregnated with FGA, and cloprostenol (an analogue of PGE2) for estrous synchronization during the breeding season of these goats.

Materials and Methods

Ninety-five female Nadooshani goats, aged 2 to 3 years, were randomly allotted to three groups and synchronized by either the CIDR (EAZI-BREED, New Zealand) containing 0.3 g of progesterone for 18 days (n = 33), intravaginal sponges impregnated with 30 mg of FGA (Cronolone, Intervet, The Netherlands) for 18 days (n = 32), or by two injections each of 1 ml (250 μg) cloprostenol (Estrumate, ICY Pharma, Canada) 12 days apart (n = 30). The goats in groups 1 and 2 were injected intramuscularly with 200 IU eCG (Intervet, The Netherlands) at the time of progestagen removal from the vagina on day 18. The second injection of cloprostenol in group 3 coincides with the injection of eCG at the time of CIDR and sponge removal in groups 1 and 2. The day after the end of synchronization, the goats were continuously observed for the standing heat by using a penile-deviated buck as an aid to heat detection.

To omit the confounding effect of male fertility on pregnancy rates, it is best to inseminate the females with mixed semen samples or use frozen semen of the same male for inseminating all females. Frozen semen was not available and it was not possible to mix the semen samples because these experimental goats belonged to a registered flock whereby the ancestry of the kids had to be known. Therefore, all does were intracervically (<1 cm deep into the cervical canal) inseminated once at a fixed
time (38 to 52 hrs after the end of synchronization) with fresh diluted semen (0.25 ml containing 300 to 400 million sperm diluted in homogenized-pasteurized skim milk) collected by an artificial vagina from either of three fertile bucks. Semen sample was immediately evaluated for pH, volume, color, sperm mass activity by monitoring the wave motion characteristics (Evans and Maxwell, 1987; Ax et al., 2000) and sperm concentration (hemocytometer determina-tion). Collected semen samples (1-2 ml in volume) containing between 2750 and 4000 million sperm per ml and with at least 90% motile sperm were used for artificially inseminating the does. Twenty-one days after insemination, blood samples were taken from jugular vein in 10-ml vacuum tubes (venoject) for pregnancy diagnosis. Serum was recovered by centrifugation (10 min at 3000 rpm) and stored at -20°C until assayed for progesterone using a progesterone radioimmunoassay (RIA) kit (IM1180, IMMUNOTECK, France); the intra-and inter-assay CVs and the analytical sensitivity were ≤ 5.4%, ≤ 9.1% and 0.03 ng/ml, respectively. Serum progesterone level of greater than 1.4 ng per ml was taken as an indication of pregnancy. Pregnancy was confirmed by ultrasonography at mid-gestation after AI, by using a 3.5 MHz transabdominal transducer (B mode, ULTRA-
SCAN 900, ALLIANCE MEDICAL INC., Canada). All the ultrasonographic observations were carried out by the same person. Two does from group 1 (CIDR) and two does from group 2 (FGA sponge) died before AI and parturition, respectively.

Statistical analysis

The effects of the treatments on the proportion of goats showing estrus and the proportion becoming pregnant were compared by the χ²-squared test, and other data were analyzed by the analysis of variance (ANOVA) using the SPSS (ver. 11.5) software.

Results

There was no significant effect of the synchronization method on estrous response, time of onset of estrus, estrous duration, serum progesterone concentration at 21 days after insemination, kidding rate, gestation length, fecundity and prolificacy (Table 1). Overall, 96% of the does were in estrus within 26 hrs, and 57% were pregnant following a single intracervical insemination of does at first detected estrus, as determined by ultrasonography on day 78, which kidded successfully. Of the 90 does that kidded, 65 had singletons, 25 had twins and two of them delivered triplets and at 21 days after AI, had a serum progesterone concentration (mean ± SEM) of 4.7 ± 0.5, 5.0 ± 0.9 and 6.8 ± 2.7 ng/ml, respectively. There was no significant difference in serum progesterone concentration among the four groups.

<table>
<thead>
<tr>
<th>Table 1: Response of Nadooshani goats to estrous synchronization methods (mean ± SEM)</th>
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<tr>
<td>Response parameter</td>
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<tr>
<td>Estrous response, % (No. of does)</td>
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<tr>
<td>Onset of estrus (hr)</td>
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<tr>
<td>Duration of estrus (hr)</td>
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<tr>
<td>No. of does</td>
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<td>Serum P₄ level (ng/ml)</td>
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<td>Kidding rate (%)</td>
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<td>Gestation length (days)</td>
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<td>Fecundity and prolificacy</td>
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a The range of values are shown in parentheses; b Serum progesterone level, determined on day 21 after AI; c All does becoming pregnant following insemination at the first synchronized estrus kidded successfully, therefore, the number of kids born per female kidded (fecundity) and the number of kids born per estrous female inseminated (prolificacy) were identical.
levels and gestation length with respect to the litter size (P>0.05). Serum progesterone concentration on day 21 was significantly lower in those goats that returned to estrus after AI (Table 2).

Table 2: Serum progesterone levels (Mean ± SEM) in does coming to estrus as compared to those not in estrus 21 days after AI

<table>
<thead>
<tr>
<th></th>
<th>No. of does</th>
<th>Progesterone level (ng/mL)</th>
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</thead>
<tbody>
<tr>
<td>Returned to estrus</td>
<td>39</td>
<td>3.5 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Not returned to estrus</td>
<td>52</td>
<td>5.8 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
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*<sup>a</sup>b</sup> significantly different (P<0.01)

**Discussion**

The three synchronization methods employed resulted in estrous response in 94 to 97% of the treated goats (Table 1). The results were comparable to the findings of Ak et al. (1998) in Angora goats treated with FGA/eCG (overall 100%), Amarantidis et al. (2004) in three groups of indigenous Greek goats (*Capra prisma*) treated with FGA, PGF<sub>2α</sub> and FGA/PGF<sub>2α</sub> (overall 98%) and Lehloenyaa et al. (2005) in Boer and Nguni goats treated with MAP/eCG (overall 95.5%), during the breeding season; and to the findings of Mavrogenis (1988) and Zarkawi et al. (1999) in Damascus goats treated with MAP/eCG (100%) and Blaszczyk et al. (2004) in Anglo-Nubian goats treated with FGA/eCG (100%), outside the breeding season. Intravaginal sponges containing 40 mg progestagen were effective in inducing estrus in 70% of the Sudanese Nubian goats (Ahmed et al., 1988). Intravaginal sponges containing FGA and CIDR devices were equally effective for the control of ovulation in Cashmere goats when combined with eCG injection (Ritar et al., 1990).

Researchers have also tried to determine the optimum dose of cloprostenol for estrous synchronization. Greying and Van der Westhuysen (1977) found that with 125 μg doses of cloprostenol, only 80% of their ewes came into estrus, as compared with 100% at the 250 μg dose level. On the other hand, doses of 125 μg were highly effective (100%) in inducing estrous synchrony in Sudanese Nubian goats (Ahmed et al., 1998). Cloprostenol seems to be very effective for rapid lysis of the caprine corpora lutea and subsequent falling of progesterone levels during the breeding season when does are cycling; however, progesterone can be used for estrous synchronization whether a corpus luteum is present on the ovaries or not. A good plane of nutrition is required for optimum response to synchronization methods. For instance, in an experiment to determine the effects of three dietary energy levels on estrous induction in Mashona goats treated with cloprostenol, Kusina et al. (2001) showed that the overt estrus occurred only in 60% of the does that were fed with a low energy diet (0.27 MJ ME kg<sup>-1</sup> W<sup>0.75</sup>) in comparison with 93 and 100% for the medium (0.53) and high (1.06) dietary energy levels, respectively.

Standing heat was observed between 23 and 35 hrs (mean of 26 hrs) after the end of synchronization protocol with no significant differences between the treatment groups. These are close to the values (28 to 33 hrs) for goats synchronized after progesterone treatment (Freitas et al., 1997; Motlomelo et al., 2002). These values are much smaller than the values for Sudanese Nubian (53 hrs; Ahmed et al., 1998), Angora (80 hrs; Doijode et al., 1992) and Black Bengal (95-137 hrs; Ishwar and Pandey, 1992) goats. Such variations could be due to breed differences and (or) nutrition both of which are known to affect the progesterone level (Lamond et al., 1972; Quirke and Gosling, 1976).

The duration of estrus was within the normal range of 18 to 36 hrs (Morrow, 1986), but it was less variable than that reported by Emady et al. (2006) for Abadeh goats (another Iranian indigenous goat) during the breeding season (SD of 12.3 vs. 2.4 hrs). Because of this very tight estrus, fixed-time AI is a possibility in Nadooshani goats. Mean duration of estrus was not significantly different amongst the treatment groups (overall mean = 22.4 hrs). Similarly, Ahmed et al. (1998) did not find any significant difference in mean estrous duration (52 hrs) for the Nubian goats treated with cloprostenol or intravaginal progesterone sponges followed by eCG injection.
It is believed that estrous synchronization using two prostaglandin
injections, 11 days apart, has no adverse effect on pregnancy rate in goats (Gordon,
1997). In the present study, kidding rate, fecundity and prolificacy were not
significantly affected by the synchronization methods.

Similarly, reproductive performance of Sudanese Nubian goats was not
significantly different for the does that were synchronized by intravaginal sponges
or cloprostenol (Ahmed et al., 1998). While some studies have reported that fertilization
and lambing rates were decreased in the ewes bred by artificial insemination at the
prostaglandin controlled estrus, others have not found an adverse effect of
prostaglandins on the ewe fertility (Gordon, 1997). The kidding rate of dairy goats
injected with 100 μg cloprostenol at an interval of 10 days, and inseminated with
frozen semen at a predetermined time after treatment, was reported to be 10, 44.7 and
21.4% for a single insemination at 60, 72 and 84 hrs after the second injection
(Simplicio and Machado, 1991). Results of the present study showed that the kidding
rate of Nadooshani goats, after a single insemination into the beginning of cervix,
was higher (57.1 vs. 42.0%) than for Angora goats (Evans and Maxwell, 1987).

The gestation length (144 to 155 days) was not significantly different between
treatment groups (Table 1) and was within the normal range of 140 to 155 days
(Jainudeen et al., 2000). In the present study and those of Zarkawi et al. (1999) and
Amarantidis et al. (2004), the gestation length was not affected by the litter size but
Lehloeny et al. (2005) reported a significantly shorter gestation length in does
with quadruplets. In our study, only two of the goat had triplets, and no quadruplet
gestations were recorded. The prolificacy rate of the does that became pregnant to AI
(average: 138.5) at the first estrus following synchronization was not significantly
different between groups, but it was 14 percent higher than the prolificacy rate of
the does that returned to estrus and were allowed to mate with the bucks at the second
estrus. The average litter size (mean ± SEM) of these does (1.38 ± 0.08 and 1.24 ± 0.07
for the former and latter groups, respectively) was not significantly different.

Serum progesterone levels were not affected by the litter size in Nadooshani goats,
however, Kanuya et al. (2000) reported that serum progesterone levels of the
cloprostenol-treated Small East African goats, measured at day 25 and 35 of
gestation, were significantly higher in twin-bearing as compared with single-bearing
does.

The fertility of goats after artificial insemination can be penalized by
 pseudopregnancy at the time of induction of estrus by prostegstagen/eCG or by other
means (Chemineau et al., 1996). Pseudopregnancy appeared in 3-4% of does,
and sometimes up to 20% in some breeds (Mialot et al., 1991; Hesselink, 1993).
Pseudopregnancy was lower in FGA/eCG treated (3.8%) than in naturally mated
(2.5%) goats (Mialot et al., 1991), in nulliparous (1%) than in parous (18%) does
(Hesselink, 1993), and in younger (10%) than in older (32%) goats (Hesselink, 1993). The
goats in our experiment were 2 to 3-year-old parous does, and only one of the 91 does
showed pseudopregnancy.

Reproductive performance of the Nadooshani goats was not significantly
affected by the estrous synchronization methods used in the present study, but
cloprostenol injection was found to be more convenient and economical under the
conditions of this experiment during the breeding season.

Acknowledgements

The authors would like to appreciate the sincere co-operation of the flock owners and
the staff of the Nadooshani Goat Breeding Station, Yazd province.

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