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

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## 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 (Estrumate; an analogue of prostaglandin $F_2\alpha$) 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, $PGF_2\alpha$

## 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 $PGF_2\alpha$ 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, 31º 46´ N latitude and 53º 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 $PGF_2\alpha$ 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, ICI 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 -20ºC until assayed for progesterone using a progesterone radioimmunoassay (RIA) kit (IM1180, IMMUNOTECK, France); the intra-and inter-assay CVs and the analytical sensitivity were $\leq 5.4\%$, $\leq 9.1\%$ 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 trans-abdominal 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 $\chi^2$-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 singltons, 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

**Table 1: Response of Nadooshani goats to estrous synchronization methods (mean ± SEM)**

| Response parameter | CIDR | FGA sponge | Cloprostenol | Overall |
|---|---|---|---|---|
| Estrous response, % (No. of does) | 94 (31) | 97 (31) | 97 (29) | 96 (91) |
| Onset of estrus (hr)[a] | 25.6 ± 0.5 (23-35) | 26.2 ± 0.5 (24-34) | 26.0 ± 0.4 (24-31) | 25.9 ± 0.3 (23-35) |
| Duration of estrus (hr)[a] | 21.7 ± 0.4 (15-27) | 21.9 ± 0.5 (15-24) | 22.0 ± 0.3 (17-25) | 22.4 ± 0.4 (15-27) |
| No. of does | 29 | 31 | 29 | 89 |
| Serum $P_4$ level [b] (ng/ml) | 5.2 ± 0.8 | 5.2 ± 0.8 | 3.9 ± 0.6 | 4.8 ± 0.4 |
| No. of does | 31 | 31 | 30 | 92 |
| Kidding rate [c] (%) | 55 | 60 | 57 | 57 |
| No. of does | 17 | 18 | 17 | 52 |
| Gestation length (days) | 149.2 ± 0.8 | 148.7 ± 0.7 | 150.3 ± 0.9 | 149.4 ± 0.5 |
| Fecundity and prolificacy [c] | 1.35 ± 0.2 | 1.35 ± 0.1 | 1.44 ± 0.2 | 1.38 ± 0.8 |

[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**

| | No. of does | Progesterone level (ng/ml) |
|---|---|---|
| Returned to estrus | 39 | $3.5 \pm 0.6^{a}$ |
| Not returned to estrus | 52 | $5.8 \pm 0.5^{b}$ |

[a,b] 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 prisca*) treated with FGA, $PGF_2\alpha$ and FGA/$PGF_2\alpha$ (overall 98%) and Lehloenya *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. Greyling 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 $kg^{-1}$ $W^{0.75}$) 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 Lehloenya *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 progestagen/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 youger (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.

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