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The use of progesterone-supplemented Co-synch and Ovsynch for estrus synchronization and fixed-time insemination in nulliparous Saanen goat

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Abstract: The effect of Chronogest-supplemented Ovsynch and Co-synch protocols on estrus synchronization, follicular development, and fertility after fixed-time insemination was investigated in goats during the breeding season. Co-synch (n = 24) and Ovsynch (n = 25) protocols were applied to nulliparous does with a body weight of more than 30 kg. The onset and duration of estrus were determined with teaser bucks. The does were laparoscopically inseminated with 11.5×10^6 motile spermatozoa/straw at the second gonadotropin-releasing hormone (GnRH) injection for Co-synch and 8 h after the second GnRH injection for Ovsynch. The rates of estrus exhibited in the does were 92% and 84% and the times from sponge removal to the onset of estrus were 31.1 h and 30.9 h; the duration times were 34.4 h and 29.4 h for Co-synch and Ovsynch, respectively ($P > 0.05$). The follicle diameters at the second GnRH injection were 0.72 cm and 0.68 cm, and the number of ovulations was 2.6 and 2.8 for Co-synch and Ovsynch, respectively. There were no significant differences between the Co-synch and Ovsynch groups for the nonreturn rate within 30 days (NRR_{30}) (62% and 40%) and the pregnancy rates (38% and 24%) determined on day 30. The kidding and prolificacy rates were 38% and 1.4 for the Co-synch-treated goats and 24% and 1.2 for the Ovsynch-treated goats ($P > 0.05$). The Co-synch protocol could yield better results compared to the Ovsynch protocol, which was related to the variation in ovulation time during the onset of puberty and the breeding season. Further studies should be done to determine the best time for fixed-time insemination and the acceptable insemination dose to improve the fertility results after timed artificial insemination in does.

Key words: Goat semen, timed artificial insemination, Ovsynch, laparoscopy, progesterone

1. Introduction

Goat artificial insemination (AI) is an alternative for the maximum utilization of valuable breeding males that are genetically superior (1). Goat semen can be used for AI in fresh, cooled, or frozen form (1). Fresh extended semen has a short fertile lifespan, whereas acceptable fertility with cryopreserved goat semen is controversial, which limits the widespread use of AI in goat husbandry (2).

Estrus synchronization facilitates reproductive management and AI in dairy goats. Successful fertilization during induced estrus periods depends on the time of AI or mating relative to ovulation (3). Several hormonal protocols have been employed (1,2,4) for goat estrus synchronization. Progesterone, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and equine chorionic gonadotropin (eCG)-based synchronization methods result in good conception rates with either natural service or double insemination, but not with single insemination after estrus detection (5).

Fixed-time AI is helpful in settings where estrus detection is not very efficient or when there is no time to carefully detect it (4). The unpredictability in the timing of ovulation in does means that at least 2 inseminations have to be performed in order to obtain acceptable conception rates. In addition, the does with a prolonged follicular phase do not fit with the timing of the fixed-time AI in the breeding schedule (4). Fixed-time AI can be achieved by knowing the time from the onset of estrus to ovulation in natural estrus and in hormone-induced estrus (6). During the last decades, there has been considerable interest in the development of methods for the ovulation synchronization in goats (3–7).

The gonadotropin-releasing hormone (GnRH)–$PGF_{2\alpha}$–GnRH (Ovsynch) protocol was used with the aim of synchronizing cows to eliminate estrus detection and to achieve timed AI (8). Ovsynch involves the administration of GnRH, followed by $PGF_{2\alpha}$ 7 days

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later and a second treatment of GnRH given at 36 to 48 h after the administration of $PGF_{2\alpha}$. In the Ovsynch protocol, cows and heifers are not observed for estrus but are inseminated at a specific time (12–20 h) following the second GnRH administration (9,10). The first administration of GnRH is given at a random stage of the estrus cycle, causing either luteinization or ovulation of the largest follicle in cycling or anestrus cows (8,9,11,12). The administration of $PGF_{2\alpha}$ causes a regression of the corpus luteum (CL) or the luteinized follicle induced by the GnRH. A new dominant follicle forms and is available for ovulation by the time of the second GnRH administration, which is given at 36 to 48 h after the $PGF_{2\alpha}$ treatment (8,9). The Ovsynch protocol synchronizes the ovulation and may be a useful alternative to the sponge-eCG in goats during the breeding season (4).

There are many reports that deal with progesterone-supplemented Ovsynch protocols that show consistent increases in the proportion of cows with subluteal progesterone concentrations, the diameter of the largest follicle, and the ovulatory response to the first administration of GnRH on day 0 (13). Comparisons of Ovsynch and Ovsynch plus controlled internal drug release (CIDR) treatments for lactating dairy cows have indicated that the addition of progesterone to the Ovsynch protocol improved pregnancy rates (13). Treatment with progesterone for 7 days before $PGF_{2\alpha}$ improves the synchronization and detection of estrus and conception rates. Moreover, progesterone treatment induces a final luteinizing hormone (LH) surge and a highly synchronous time to estrus, which allows for the use of timed AI without estrus detection (14). Martínez et al. (15) reported that progesterone-supplemented Ovsynch protocols reduce the incidence of premature estrus and improve the pregnancy rates in heifers.

The AI technique in small ruminants is based on depositing the semen in the entrance of or within the cervix, or by passing the catheter from the cervix into the uterine cavity directly. This is sometimes rarely possible, especially in young animals. Laparoscopic insemination makes it possible to deposit semen into the uterus near the oviduct shortly before the expected ovulation time (1). The laparoscopic insemination procedure could be used as an alternative technique in young animals for improving the progression of genetic merit.

This study attempted to monitor the efficiency of the Ovsynch and Co-synch protocols (combined with the classical Chronogest containing a vaginal sponge) on estrus synchronization and follicular development, and to generate fixed-time insemination in nulliparous Saanen goats during the breeding season.

2. Materials and methods

The experiment was carried out in Bursa (40.19°N, 29.06°E; altitude 155 m), in western Turkey, during November (the natural breeding season) under natural lighting. A total of 49 nulliparous Saanen does, ranging in age from 9 to 13 months and weighing over 30 kg, were used. The does were group-housed in an open barn and fed a daily ration of 400–700 g of alfalfa pellets. In addition, the animals had free access to water.

On day 0, the does received an intramuscular injection of 0.004 mg of the GnRH analog Buserelin (Receptal[å], Intervet International B.V., Netherlands) and intravaginal polyurethane sponges impregnated with 45 mg of fluorogestone acetate (Chronogest, Intervet, France), followed 7 days later by sponge withdrawal and the injection of 250 µg/mL of $PGF_{2\alpha}$ analog cloprostenol (1 mL Juramate, Jurox Pty Ltd., Australia). On day 9, all of the does received a second injection of 0.004 mg of Buserelin. The treated does were randomly allotted to 1 of the 2 groups according to insemination time. The animals in the Co-synch group were inseminated laparoscopically at the time of the second GnRH injection (n = 24) and the animals in the Ovsynch group were inseminated laparoscopically at 8 h after the second GnRH injection (n = 25).

Estrus was detected with the aid of teaser bucks every 6 h, from 12 to 72 h following progestagen withdrawal and injection of $PGF_{2\alpha}$. Estrus behavior was evidenced by the immobility reflex (the classical indicator of estrus) (4). Estrus onset was defined as the time elapsed between the sponge removal and the first accepted mount of the teaser buck. The estrus duration was defined as the time elapsed between the first and last accepted mount within the same estrus period. Estrus cessation was defined as the time elapsed between sponge removal and last accepted mount within the same estrus period.

The does were fasted for 24 h and received an intravenous injection of diazepam (Diazem Ampul, DEVA İlaç San. ve Tic. A.Ş., Turkey) at a dose of 0.33 mg/kg of body weight for sedation before being subjected to intrauterine insemination with frozen semen (11.5×10^6 motile spermatozoa/straw) by laparoscopy. The laparoscopic equipment consisted of a rigid laparoscope with a 10-mm external diameter and a 90° viewing angle in combination with a cold light source and air filtration pump (Karlz Storz, Germany). As operative instruments, we used only 2 trocars: the first with a 10-mm external diameter for the laparoscope and the other with a 4-mm external diameter for the insemination needle. Following sedation, the does were placed in a dorsal position on a surgery table, leaning back at the angle of 45°. The semen was deposited directly within the uterus using an insemination pistol covered with aspic. Half of the insemination dose was injected into each uterine horn (1).

Ovarian status in terms of the CL and follicle were examined by transvaginal ultrasonography (US) (Terason Portable Ultrasonography System, Teratech Corp., USA) equipped with a 4–8 MHz endocavity array transducer (Terason Vet-128, 8EC4). The size and the number of developed follicles at the time of the sponge insertion and removal, at the second GnRH injection, at the first estrus onset, and at the time of insemination were determined by transvaginal US. Two days after insemination, the does' ovaries were reevaluated for ovulation rates.

For the nonreturn rate within 30 days (NRR_{30}), estrus was detected with the aid of teaser bucks twice daily from 12 to 30 days following the intrauterine insemination. Pregnancy was diagnosed by transvaginal US at 30 days following artificial insemination. Five months later, the occurrences of the birth and the number of kids per birth were recorded.

The onset, duration, and cessation of the induced estrus periods were subjected to analyses of variance (one-way ANOVA) and the differences among the means were tested for significance by Fisher's protected least significant difference test. Estrus response, pregnancy and prolificacy rates, developed follicle count, and ovulation rate were analyzed using the chi-square test. For evaluation of follicle diameter, the repeated measures of ANOVA were used. A 95% significance level was noted. SPSS 10.0 was used for all of the statistical analyses.

3. Results

The results in terms of estrus response within 72 h; the interval between the sponge removal to the onset, duration, and cessation of induced estrus; follicular development; and fertility-related results are presented in the Table. Estrus was detected in 92% and 84% of the does for the Co-synch and Ovsynch groups, respectively. The onset and duration of estrus were 31.1 h and 30.9 h, and 34.4 h and 29.4 h, for the Co-synch and Ovsynch groups, respectively ($P > 0.05$).

The follicle diameters at sponge insertion were 0.66 cm and 0.67 cm, and at sponge withdrawal they were 0.64 cm and 0.68 cm for the Co-synch and Ovsynch groups, respectively ($P > 0.05$). The follicle diameters at the onset of estrus were 0.70 cm and 0.72 cm, and at the second GnRH injection, they were 0.83 cm and 0.81 cm for the Co-synch and Ovsynch groups, respectively ($P > 0.05$). The number of ovulations was 2.6 and 2.8 for the Co-synch and Ovsynch groups, respectively ($P > 0.05$).

There were no significant differences with respect to NRR_{30} (62% and 40%) and pregnancy rates (38% and 24%) determined on day 30 for the Co-synch and Ovsynch groups, respectively ($P > 0.05$). The does that did not get pregnant were detected for estrus for up to 2 cycles, and then resynchronized and naturally mated.

The kidding and prolificacy rates were 38% and 24% and 1.4 and 1.2 for the Co-synch-treated and Ovsynch-treated goats, respectively ($P > 0.05$).

Table. Estrus response, follicle development, and kidding rates in does treated with Ovsynch or Co-synch combined with an intravaginal fluorogestone acetate sponge.

Observed parameters		Co-synch + sponge n = 24	Ovsynch + sponge n = 25
Estrus response (%)		92	84
Estrus onset (h)		31.1	30.9
Estrus duration (h)		34.4	29.4
Dominant follicle diameter (cm)	Sponge insertion	0.66	0.67
	Sponge withdrawal	0.64	0.68
	Estrus onset	0.70	0.72
	Second GnRH injection	0.83	0.81
Ovulation number		2.6	2.8
NRR_{30} (%)		62	40
Pregnancy rate (%)		38	24
Kidding rate (%)		38	24
Prolificacy rate		1.4	1.2

4. Discussion

In small ruminants, estrus was induced by the insertion of a progesterone-impregnated intravaginal device combined with eCG (14). Due to the lack of synchrony of ovulation, the progesterone-based synchronization regimes required the detection of estrus (4). During the past decades, considerable efforts were made to develop methods for estrus and ovulation synchronization in small ruminants (16). The GnRH–PGF2α–GnRH (Ovsynch)-based synchronization programs synchronize the preovulatory gonadotropin surge and result in relatively synchronous ovulation in cows (17). Holtz et al. (4) reported that the Ovsynch method could be used for timed AI in dairy goats.

Treatment with progesterone for 7 days before $PGF_{2\alpha}$ improves the synchronization and detection of estrus and conception rates (14). In the present study, estrus behavior, as evidenced by the immobility reflex, was detected in 92% and 84% of the does for the Co-synch and Ovsynch groups, respectively ($P > 0.05$). Nak et al. (18) used the progesterone-supplemented Ovsynch protocol and reported a 91% immobility reflex in lactating does. Holtz et al. (4) reported that the immobility reflex was detected in 63% of Ovsynch-treated does. Our results are in agreement with those of Martínez et al. (15), who reported that the progesterone-supplemented Ovsynch protocol would reduce the incidence of premature estrus in heifers. Titi et al. (16) reported that there was a trend for improved fecundity in the progesterone-supplemented Ovsynch group after natural mating in Damascus does. Progesterone treatment results in an induced final LH surge and a highly synchronous time to estrus, which allows for the use of timed AI without estrus detection (14). The onset of estrus to the LH surge, or in other words the ovulation interval, could be influenced by the protocol of estrus synchronization (19). Holtz et al. (4) reported that the onset and duration of estrus in lactating does treated with Ovsynch were 44.8 h and 40.4 h, respectively. In the present study, the onset and duration of estrus were 31.1 h and 30.9 h, and 34.4 h and 29.4 h, for the Co-synch and Ovsynch groups, respectively ($P > 0.05$). It was observed that the administration of exogenous progesterone during the interval of the first GnRH to $PGF_{2\alpha}$ for 7 days reduced the interval sponge removal to estrus onset and estrus duration compared to the findings of Holtz et al. (4). Similar results for estrus onset (33.3 h) and duration (30.9 h) were reported by Nak et al. (18) in lactating does synchronized with Ovsynch supplemented with progesterone.

Progesterone prevents ovulation and delays the maturation of LH-dependent follicles (14). There were no differences between the follicle diameters at sponge insertion (0.66 cm and 0.67 cm) and withdrawal (0.64 cm and 0.68 cm) for the Co-synch and Ovsynch groups, respectively ($P > 0.05$). It can be concluded that progesterone administration results in delayed follicular development. The decrease of progesterone level restores the LH pulse frequency and amplitude. This allows the subsequent development of dominant follicle and leads to estrus and ovulation (14,20). The follicle diameters at estrus onset were 0.70 cm and 0.72, and at the second GnRH injection they were 0.83 cm and 0.81 cm for the Co-synch and Ovsynch groups, respectively ($P > 0.05$). These results showed that termination of the progesterone results in subsequent follicle development.

The pregnancy rates after laparoscopic insemination with different motile spermatozoa vary from 33% (3) to 52% (21) after different synchronization programs. Fertility rates of up to 60% in multiparous goats were observed after timed AI (22,23). Nulliparous goats had lower ovulation rates and had poorer fertility than multiparous goats after fixed-time AI (6). The kidding and prolificacy rates were 38% and 24%, and 1.4 and 1.2, for the Co-synch- and Ovsynch-treated goats, respectively ($P > 0.05$). The fertility rate in the present study was low for the Co-synch and Ovsynch groups compared to the findings of Holtz et al. (4), who used 200×10^6 spermatozoa. Our does' poor fertility can be explained by lower motile spermatozoa in the insemination dose (11.5×10^6 motile spermatozoa/straw) and the use of nulliparous goats.

The success of fertilization depends on the semen quality and proper timing between insemination and ovulation. Good conception rates were obtained when either natural service or insemination with fresh semen after estrus induction was used compared to frozen semen insemination (3,14). Watson (23) reported that the survivability of cryopreserved spermatozoa in the female tract was shorter than that of fresh spermatozoa. For an acceptable level of fertility after AI, there needs to be a sufficient number of fully competent spermatozoa capable of achieving fertilization over the period when ovulation is likely to occur (23). However, the insemination time employed in the present study had no statistically significant effect on the pregnancy and NRR_{30} rates; higher pregnancy and NRR_{30} rates were encountered with the Co-synch protocol. Insufficient synchronization may cause variable time intervals between AI and ovulation, which can explain variations in the fertility rates. The variability of ovulation seems to be a limiting factor for the efficiency of estrus synchronization and AI (24).

Sexual development is influenced by both genetic and environmental factors. In does and bucks, the age at puberty ranges from 6 to 12 months of age and the weight ranges from 30 to 45 kg, depending on nutrition, location, and the season of birth. In addition, the onset of puberty is influenced by the transition into and out of the breeding season (25,26). In this study, nulliparous Saanen does

ranging in age from 9 to 13 months and weighing over 30 kg were used. The difference between the overall NRR_{30} results and the final kidding rates was about 40%. The does that did not get pregnant were detected for estrus for up to 2 cycles. Most of these does did not exhibit estrus behavior. These does were resynchronized after 2 months and mated naturally. For these reasons, these does were considered as immature and the 30 kg of body weight was too small for the synchronization of nulliparous goats.

The ovulation rate is a major determinant of the prolificacy of animals. Simões et al. (6) reported that there were significant differences in the number and time of ovulations between nulliparous and multiparous goats. The ovulation rate was lower in nulliparous Serana goats than in multiparous Serana goats, both in induced estrus (1.2 and 2.0, respectively) and in natural estrus (1.2 and 1.8, respectively) (6). The ovulation and prolificacy rates of the present experiment were 2.8 and 2.8, and 1.4 and 1.2, for the Co-synch and Ovsynch groups, respectively. These findings are in accordance with those of Holtz et al. (4), who reported that the ovulation and prolificacy rates were 2.9 and 3.3, and 1.86 and 1.83, in lactating Saanen goat breeds for Ovsynch and sponge groups, respectively.

It was reported that timed AI should be performed earlier in nulliparous goats than in multiparous goats, in order to improve the fertility rate (6). During the breeding season, the Co-synch protocol could yield better results compared to the Ovsynch protocol because of the earlier ovulation time in nulliparous goat. Moreover, the Co-synch protocol requires lower labor compared to the Ovsynch protocol. Further studies should be done to determine the best time for fixed-time insemination and the acceptable insemination dose to improve fertility results.

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