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Original article

Reproductive performance with short-time controlled internal drug release (CIDR)-based synchronization protocol for fixed-time artificial insemination in nulliparous and primiparous Saanen goats

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Abstract

In this study, it was aimed to evaluate the reproductive performance of primiparous and nulliparous Saanen goats following the insemination made cervically via frozen commercial buck semen with short-time controlled internal drug release (CIDR, Eazi-Breed, Pfizer)-based synchronization. In the study, a total of 50 Saanen goats, 26 of which were aged 9 months and 24 of which were aged 2 years, were used. The CIDRs including 0.33 g of progesterone were applied to all the goats for 7 days and while the CIDRs were taken out of the vagina, the 500 IU equine chorionic gonadotropin (eCG) was injected intramuscularly. The first dose was administered in a fixed-time manner with mini straws of 0.25 ml including $300x10^6$ motile frozen spermatozoa 24 hours after the injection without making estrus detection, and the second dose insemination was applied 24 hours later. In the study, although the pregnancy rate, fecundity, prolificacy and multiple birth rate values (83%, 1.25, 1.50 and 60%) obtained in the primiparous goats were found to be higher than those found in the nulliparous age group (80%, 0.84, 1.05 and 38%), they were not statistically significant. It was concluded that the effects of the CIDR-based double-dose insemination applications in the primiparous and nulliparous Saanen breed goats in breeding season for 7 days on fertility were similar and the cervical insemination method, a quick and more practical option compared to the intrauterine insemination, could be used successfully in both age groups.

Key words: goat, fixed time artificial insemination, CIDR, nulliparous, primiparous

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Introduction

A successful artificial insemination (AI) program requires the use of protocols providing an acceptable pregnancy rate. An effective pregnancy rate is closely related to the synchronization of ovulation in females. In the estrus application with any source of progesterone, eCG is generally applied in different doses during the removal of apparatuses (CIDR/vaginal sponge) from the vagina. This hormone is commonly used in mares to get a high LH- and FSH-like activity released by trophoblastic cells during pregnancy and stimulate estrus and ovulation in goats. A long-term application of intravaginal progestogen source (10-18 days) was associated with low fertility values (Viñoles et al. 2001). Menchaca et al. (2007) reported that an intravaginal apparatus including 0.3 g of progesterone induced high serum progesterone concentrations preventing follicular dominance in most of the goats and encouraged follicular cycle together with the emergence of a new follicular wave within 3-4 days. According to Rubianes and Menchaca (2003), since the growth stage of each follicular wave generally lasts about 5 days in goats, when intravaginal apparatuses are removed 5-7 days later, new follicles reach ovulation about 60 hours later. If a fixed-time artificial insemination (FTAI) is realized about 54-56 hours after the removal of intravaginal apparatuses, this might result in acceptable pregnancy rates (Vilariño et al. 2011). Hence, new trends in the reproduction physiology of sheep and goats require the revision of some traditional concepts related especially to long-term progesterone exposure and the use of eCG. Shortening of protocol times via using progestogens does not only minimize vaginal secretion and infection but it also keeps up fertility (Souza et al. 2011).

Artificial insemination is a worldwide effective technique speeding up genetic improvement especially applied with frozen-thawed sperma (Leboeuf et al. 2000). FTAI is an application used to increase the productivity of animal breeding programs in farms. After the estruses in goats are synchronized for FTAI generally with a progestogen application, inseminations can be made without needing to determine estrus (Holtz et al. 2008). Hence, the synchronization of estrus in goats also facilitates the continuous availability of products such as milk and meat by allowing for an AI application at any time of the year. In small ruminants, depending on the releasing place of sperma, there are few types of AI techniques, namely vaginal, cervical and laparoscopic (Leboeuf et al. 2000). With these methods, acceptable pregnancy rates (50-70%) can be obtained. Unsatisfactory pregnancy rates were attributed to such differences as racial differences in initial semen quality and freezing conditions, AI method and insemination skill, insemination time and season. However, in order to obtain acceptable pregnancy rates via using frozen sperma, cervical or laparoscopic insemination techniques are the only way (Kumar and Naqvi 2014). Deep intrauterine insemination is applied successfully in sheep and goats especially when the number of sperms is limited or the sperm quality is insufficient. Generally, when insufficient number of sperms are used in AI application, pregnancy rates decreases. For frozen goat spermatozoa, various insemination doses ($11{,}5 \times 10^6$, 20×10^6, 40×10^6, 60×10^6, 200×10^6, 300×10^6 sperm) were reported (Nogueira et al. 2011, Nur et al. 2013, Leil et al. 2020). It is seen that the most appropriate method is laparoscopic AI, especially for low dose of sperma, and intrauterine AI, and it guarantees high pregnancy rates with frozen-thawed sperma in $10\text{-}20 \times 10^6$ doses (Kulaksız and Arı 2016). Hence, both dose and method affect the success of pregnancy rates greatly. Despite high pregnancy rates obtained with the laparoscopic method, this insemination method is rather expensive and requires equipment and specialized workforce (Tekin 2019). Rubianes and Menchaca (2003) found the pregnancy rate as 76% after the first insemination in the goats in the anestrus period via short-time vaginal progestogen protocols. Kulaksız and Daşkın (2012) determined the pregnancy rate as 66% in primiparous Saanen goats as a result of the cervical insemination made following the fluorogestone acetate-based (FGA) synchronization. Nur et al. (2013) found the pregnancy rate as 38% in nulliparous goats following the progesterone-based laparoscopic insemination. Due to the anatomic structure of the cervix in goats, more sperm cells are needed in order to obtain a reasonable pregnancy rate via cervical insemination. The sperma doses suggested for obtaining successful results in cervical and vaginal artificial inseminations in goats are reported to be 200×10^6 and over (Kulaksız and Arı 2016).

In this study, it was aimed to compare the effects of the fixed-time cervical artificial insemination application made in primiparous and nulliparous goats by using frozen sperma together with CIDR-based synchronization on fertility parameters.

Materials and Methods

Animal material

In this study, 50 Saanen goats having a live weight of 32-44 kg and raised in the Research and Application Farm of the Faculty of Agriculture of Bursa Uludag University were used. The study was carried out with the approval of Uludag University Local Ethics Board for Animal Experiments (No: 2013/12-04).

Table 1. Reproductive responses of primiparous and nulliparous Saanen goats.

Parameters		Nulliparous	Primiparous	Total	p-value	χ^2
Pregnancy rate (%)		80 (21/26)	83 (20/24)	82 (41/50)	0.657	0.197
Fertility rate (%)		80 (21/26)	83 (20/24)	82 (41/50)	0.657	0.197
Fecundity		0.84 (22/26)	1.25 (30/24)	1.04 (52/50)	0.317	1.0
Profilicacy		1.05 (22/21)	1.5 (30/20)	1.3 (52/41)	0.379	0.773
Multiple rate (%)		38 (8/21)	60 (12/20)	48 (20/41)	0.383	0.760
Survival rate (%)		90.9 (20/22)	100 (30/30)	96 (50/52)	0.757	0.096
Sex of kid	Male	11	14	25	0.713	0.135
	Female	11	16	27		

Estrus synchronization treatments and artificial insemination

The goats were divided into two groups according to their ages, primiparous group (2 years old) (n=24) and nulliparous group (9 months old) (n=26). In all the goats, with the aim of achieving estrus synchronization in the mating season, the CIDR's including 300 mg of progesterone were inserted into the vagina, and remained in the vagina for 7 days. On the day when the CIDRs were removed, the 500 IU eCG (equine chorionic gonadotropin) injection was applied intramuscularly. Twenty four hours after the injection, without making estrus detection, the goats in both groups were administered the first dose insemination in a fixed-time manner cervically with mini straws (IMV type) of 0.25 ml including $300x10^6$ motile frozen spermatozoa via using vaginal speculum and the second dose insemination was applied 24 hours later. The insemination procedure was carried out with the help of a specially prepared apparatus by keeping the hind legs of the goats up. After the vulva area of the goat was sterilized, the cervix was lighted with the help of a torch and the sperm was released slowly by maneuvering into the depths of the cervix as much as possible with the help of vaginal speculum. After the insemination procedure, the goats were kept motionless in the apparatus for two-three minutes and the movement of the sperm in the female genital system was made easy.

Experimental conditions

In the period when the study was carried out (September), the goats were kept in the rangeland during the daytime and, when they returned from the rangeland, they were fed manually in order to get better performance in the insemination period. Within this scope, at night in the pen, hay (1 kg/goat) was given as roughage and a mixture (300 g/goat) including wheat (65%), corn (10%), sunflower meal (23%), limestone (1.2%) and mineral-vitamin premix (0.7% and 0.1%) was given as dense forage. The goats were able to meet their needs for clean water and mineral freely.

Reproductive parameters

The ear numbers and the birthdates of the goats giving birth, and the information about the newborn yearlings (sex, birth type, birth weight, aftermath) was recorded. By benefiting from the obtained data, the fertility characteristics of the herd were calculated. During the study, by benefiting from the birth data, such characteristics as pregnancy rate, multiple rate, fecundity, prolificacy and survival rate were examined in the goats (Kaymakçı 2006).

Pregnancy rate: Number of does pregnant/number of total does x 100

Kidding rate: Number of does kidded/number of does pregnant x100

Fertility rate: Number of does kidding/number of does inseminated x100

Multiple rate: Number of does having multiple kids/number of does kidded x 100

Fecundity: Number of kids born per doe of the inseminated

Prolificacy: Number of kids born per doe kidded

Survival rate: Number of living kids/number of kids born x100

Statistical analysis

The data related to pregnancy rate, multiple rate, fecundity, prolificacy, fertility, and gender of yearlings were analyzed by using chi-square test. All the results were analyzed at a significance level of α=0.05 by using Minitab 19 statistical software (Minitab 19).

Results

When the nulliparous and primiparous Saanen goats were cervically inseminated in the reproduction season in a fixed-time manner following the CIDR+500IU eCG application, they became pregnant at a high rate and gave birth healthily (Table 1). Although the pregnancy rate, fecundity, prolificacy, multiple rate values found in the goats included in the primiparous group were numerically higher than those found in the goats included in the nulliparous group, they were not statistically significant. In both groups, viability was found generally high; only 2 yearlings died immediately after birth in the nulliparous group.

Discussion

It is known that sexual maturity age in female goats differs according to the genetic structure of animals, birth season, body energy reserves, feeding and management system. In a study made on goats, the first successful mating age and live weight were reported to be 7.4 months and 17 kg, respectively (Abebe 2008). In Turkey, the average sexual maturity age in Saanen goats ranges from 6 to 12 months (İnce 2010). Although Rischen and Riese (1982) stated that reproduction in goats need to be postponed until they reach 60-75% of the adult body weight (32-42 kg), Freitas et al. (2004) reported that 92.9% of the Saanen females showed estrus by reaching puberty at the age of 147.8 days and at the weight of 22.5 kg. In order to get maximum fertility in farm animals, they are aimed to become pregnant when they reach puberty. For this reason, it is necessary to prepare a breeding program by planning the birth time in a way that kids can reach puberty at the beginning of reproduction season. The success of synchronization and then the outcome of pregnancy are affected by many factors including the reproductive functions of females. In this study, 5 goats from the nulliparous group and 4 goats from the primiparous group did not become pregnant. Although the obtained pregnancy rates were rather high, a few animals' having failed to become pregnant might be attributed to the ovulation distribution's not being wide following the estrus synchronization in the goat. Vinoles et al. (2001) observed that the follicle growth process in the sheep administered the long-term progesterone synchronization completed at the end of progestogen application and ovulation occurred within the following 48 hours, but in the animals administered the short-term progesterone protocols, this process was already continuing. Moreover, possible negative effects of the short-term progesterone application on follicle development are eliminated via eCG application (Doğruer et al. 2019). In this study, the goats were inseminated twice, 24 and 48 hours after the 500 IU eCG injection made when CIDR was removed, without taking into account if they were in the estrus period or not. In previous study, goats were inseminated 12-24 hours after the onset of estrus or 48-60 hours after the removal of the source of progestogen (Doğruer et al. 2019). Romano (2004) reported that the type of progestogen affected the onset time of estrus and, hence, it would affect the fixed-time artificial insemination. The goats, whose estrus were synchronized with CIDR, showed estrus between 36-44 hours following the removal of the apparatus. Hence, the duration chosen for the FTAI seem to be appropriate for the insemination protocol.

Lebouf et al. (2000) reported that with the use of frozen-thawed or fresh sperma, the pregnancy rates were 61% and 64% in the goats, whose estruses were synchronized. Baki Acar et al. (2013) determined that different synchronization protocols applied for 11 days in the 7-9-month-old nulliparous Saanen goats had an effect on reproductive parameters. Although some protocols led to higher super ovulation rates in young females, other methods like male goat effect were not effective in young animals following sexual maturity. In this study, the pregnancy rate obtained in the 9-month-old Saanen goats (80%) was rather high. On the contrary, the pregnancy rate of 40.7% found by Ritar et al. (1990) in young Kashmir goats, that of 38% reported by Nur et al. (2013) in the nulliparous goats (9-13 months) and those of 31.4% and 32.3%, respectively, reported by Tekin (2019) in the 7-and 19-month-old Saanen goats are rather lower than that found in this study. Such a pregnancy was obtained in the nulliparous goats as high as the one obtained in the primiparous goats in this study might underpin the fact that young goats have quality oocytes at an early age.

Moreover, while the pregnancy rate obtained in this study in the primiparous Saanen goats (83%) shows similarity to the pregnancy rates of 79.1% and 83.3% which Doğruer et al. (2019) obtained in Damascus goats, it is higher than the value of 63% determined by Ritar et al. (1990) in the 18-month-old Kashmir goats, the value of 71.43% found by Bhattacharyya et al. (2012) in the Kashmir goats, the value of 66% determined by Kulaksız and Daşkın (2012) in the Saanen goats, the values of 24%-38% obtained by Nur et al. (2013) in the Saanen goats, the value of 53.57% found by Ciptadi et al. (2014) in the Boer goats, the values of 50% and 58% determined by Holtz et al. (2008) in the Boer goats and the values of 66.7% and 77.8% reported by Erarslan and Karaca (2017) in the Hair goats. It is considered that this might have resulted from the lack of experience which the goat doing her first birth had about how to care and feed her kid. However, in the other group, all the yearlings reached weaning age healthily. The possibility that breed, different hormones and methods used for synchronization, the sperma concentration and the insemination method might have an effect on the difference appearing in the pregnancy rates in the mentioned studies should be taken into consideration. Although AI programs are used for both primiparous and multiparous goats, it is reported that the fertility rates in primiparous females are lower than the ones in multiparous females. Similarly, Romano et al. (2000) reported that the fertility of the multiparous goats was the highest (59.5%) and, they were followed by nulliparous goats (58.3%) and the primiparous goats (55.6%), respectively. However, in this study, the fertility rates obtained in both primiparous (83%) and nulliparous (80%) groups are very close to each other. This result is in line with that reported by Nogueira et al. (2011) (82.2%). On the other hand, it is higher than the values reported in the Nubian (63.0%), Alpin and Saanen (64% and 16%), Saanen (59.5%), Damascus (54.2%-75%) breeds (Goonewardene et al. 1997, Romano 2004, Kulaksız and Daşkın 2012, Doğruer et al. 2019). However, it is lower than the values obtained by Erarslan and Karaca (2017) in the Hair goats (92.9% and 100%). This difference between the fertility rates might be resulting essentially from the initial semen quality, the existence of sperma having more volume and motility after higher thawing and the method of insemination.

In the present study, the multiple birth values (38% and 60%) determined in the goats inseminated via frozen commercial sperma took place between the values of 41.7%-50% reported by Eraslan and Karaca (2017) and that of 54.6% reported by Tekin (2019). However, they were lower than the value of 83% reported by Kulaksız and Daşkın (2012). These differences might be attributed to such sperm quality factors as liveliness, mobility and longevity. Ovulation rate is an important determinant of prolificacy in animals. Simões et al. (2008) reported that there were important differences between the nulliparous and the multiparous goats in terms of ovulation number and ovulation time. The ovulation rates found in the nulliparous Serana goats were lower than those found in the multiparous goats in both induced estrus (1.2 and 2.0, respectively) and normal estrus (1.2 and 1.8, respectively). On the contrary, Tekin (2019) reported that the prolificacy value (1.45) found in the 7-month-old Saanen goats was higher than that (0.9) found in the 19-month-old goats. However, in this study, although the fecundity and the prolificacy values obtained in the primiparous (1.25-1.50) and the nulliparous (0.84-1.05) groups were found numerically higher in the primiparous group, the difference was not statistically significant. The values obtained in the present study show similarity to the fecundity value of 1.27 reported by Bhattacharyya et al. (2012), the values of 1.2 and 1.4 obtained by Nur et al. (2013), and the values of 1.0 and 1.33 determined by Andrabi et al. (2015). On the other hand, they are lower than the values of 1.75-1.84 found by Ritar et al. (1990), the value of 1.64 determined by Kharche et al. (2013), the values of 1.33-1.86 obtained by Goonewardene et al. (1997) and the value of 1.9 reported by Nogueira et al. (2011). In the literature, it is reported that prolificacy shows differences between the goat breeds. Moreover, in this study, in the nulliparous group, the male and female rates amounted to 50%-50% and, in the primiparous group, they amounted to 53.3%-46.6%. Similarly, Ciptadi et al. (2014) determined the general gender rate of the kids obtained from AI (57.85%) within the normal acceptable limits.

Conclusion

This study provides new information about the FTAI application made via frozen commercial sperma by using the short-term CIDR protocol in goats. It is considered that the high reproductive performance results obtained in both primiparous and nulliparous Saanen goats after the AI resulted from various factors. These are the factors: using CIDR instead of vaginal sponge for estrus synchronization, application process of spermatozoa, insemination method, and finally using smaller volume mini pipettes. As a result, it is concluded that CIDR-based cervical AI application can be applied as successfully in nulliparous goats as in primiparous goats during the mating season.

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