

Reproductive Parameters in Local Goats After Estrus Synchronization and Artificial Insemination with Frozen Semen During Breeding Season (Case Report)

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Abstract. This study aimed to determine selected reproductive parameters, including estrus synchronization response, pregnancy rate and prolificacy in Bulgarian local goats subjected to estrus synchronization and artificial insemination (AI) with frozen semen during the breeding season. The investigation was carried out with 101 lactating Bulgarian local goats during the breeding season. Estrus synchronization (ES) was performed using of intravaginal sponges containing 60 mg medroxyprogesterone acetate for 14 days, followed by an intramuscular injection of 500 IU PMSG (pregnant mare serum gonadotropin) on the day of sponge removal. The response to the synchronization was assessed on changes in the vaginal appearance. A single artificial insemination with frozen semen was conducted 48–52 hours after sponge removal. Pregnancy diagnosis was performed on day 35 after AI. Based on ultrasound pregnancy diagnosis and kidding data, pregnancy rate (PR) and prolificacy were recorded.

The registered estrus synchronization response, pregnancy rate and prolificacy were 100%, 22.8% and 108.6%, respectively. In conclusion, the applied estrus synchronization protocol and artificial insemination with frozen semen during breeding season provided an acceptable pregnancy rate and prolificacy in Bulgarian local goats, particularly when the primary objective was acceleration of genetic progress in the flock.

Further investigations into factors affecting the success of these assisted reproductive technologies are necessary to improve their efficiency.

Introduction

The artificial insemination is an effective tool for accelerating genetic progress and ensuring high profitability and sustainable development of goat farms (Gibbons et al., 2011; 2013; Yeni and Gundogan, 2018). The success of AI in goats depends on multiple factors, including animal age, season, estrus detection methods and timing of semen deposition, insemination after spontaneous estrus or estrus synchronization, semen type and site of spermatozoa deposition (Arrébola et al., 2012, 2016; Tekin, 2019). The cervical insemination is a non-invasive and unexpensive technique; however, due to the anatomical characteristics of the cervix in small ruminants, it does not always provide satisfactory results (Leethongdee and Ponglowhapan, 2014; Daskin et al., 2016). Unlike in sheep, the goat cervix is approximately 2 cm shorter and consists of fewer cervical folds (3–6) arranged linearly, resulting in a relatively straight cervical lumen. These features allow deep intracervical or intrauterine semen deposition in goats, which leads to better results after artificial insemination with frozen semen, compared with sheep. In multiparous goats, the cervix is longer

(4.2 ± 0.2 cm) than in nulliparous goats (3.5 ± 0.2 cm), while the number of cervical rings remains similar. The longer cervix in multiparous goats allows deeper semen deposition, resulting in a higher pregnancy rate (Intrakamhaeng et al., 2011, Hyacinth et al., 2016). A lower pregnancy rate obtained after AI with frozen semen is mainly attributed to a negative effect of the freezing procedure, which reduces sperm motility by 30–40% (Niño-González, 2008).

Reported reproductive outcomes following AI with frozen semen in goats vary widely depending on breed, age, synchronization protocol, timing of insemination, and semen deposition technique, with pregnancy rates ranging from 15.79% to 70% (Gibbons, 2002; Gibbons et al., 2013; Arrébola et al., 2012; Yotov et al., 2016; Dehouegnon and Koluman, 2018; Susilowati et al., 2023). The information regarding AI with frozen semen in indigenous goat breeds remains limited.

Therefore, additional studies are required to develop suitable technologies for the effective use of frozen semen from genetically superior bucks. This study aimed to evaluate selected reproductive parameters in Bulgarian local goats subjected to estrus synchronization and artificial insemination with frozen semen during the breeding season.

Material and methods

The experiment was conducted during the breeding season (September) with 101 Bulgarian local goats, aged 3–6 years and weighing 50–60 kg. All animals were lactating with an average daily milk yield of 0.6 ± 0.2 kg. Milking was performed twice daily in a milking parlor. The animals were housed in a farm with access to pasture and received supplemental concentrate (0.5 kg per animal), containing a vitamin-mineral premix. Drinking water was *ad libitum*. Prior to the experiment, all animals underwent antiparasitic and immunoprophylactic treatments.

Estrus synchronization was performed using intravaginal sponges containing 60 mg of medroxyprogesterone acetate (Ovigest 60, Hipra, Spain) for 14 days. After the sponge removal, each goat received an intramuscular injection of 500 IU PMSG (Folligon, Intervet International B.V., Netherlands). The response to synchronization treatment (number of synchronized animals / number of animals showing typical estrous signs on the day of AI $\times 100$) was evaluated based on vaginal picture (vaginal hyperemia, redness and edema of the external part of the cervix and the presence of typical estrous discharge) observed by vaginoscope for small ruminants equipped with a cold light source (IMV Technologies, France).

Animals showing inadequate response or abnormal discharge were excluded from insemination. A single artificial insemination with frozen semen in straws 0.25 mL (25×10^6 motile spermatozoa after thawing) was performed 48–52 hours after sponge withdrawal. The semen was thawed at 37°C for 30 seconds and deposited deep cervically using a special AI gun for small ruminants (Fig. 1).

Pregnancy diagnosis was performed on day 35 after AI using an ultrasound scanner (Draminski iScan, Poland) with a 7.5 MHz endorectal probe. Pregnancy was confirmed by visualization of an embryo with detectable cardiac activity. Pregnancy rate (number of

pregnant goats / number of inseminated goats $\times 100$) and prolificacy (number of kids born / number of goats kidded $\times 100$) were calculated based on ultrasound and kidding records. All procedures complied with Bulgarian legislation regarding the protection and welfare of experimental animals (Ordinance No. 20/01.11.2012).

Results and discussion

Data on positive ultrasound pregnancy diagnosis and reproductive parameters are presented in Fig. 2 and Table 1.

The applied estrus synchronization protocol is appropriate for goats in the breeding season, evidenced by 100% estrus synchronization response. A similar good effect of sponges containing medroxyprogesterone acetate was determined by Motlomelo et al. (2002).

On day 35 of pregnancy, embryos appeared as echogenic structures within the uterine lumen filled with anechoic amniotic fluid, with clearly visible cardiac activity. The observed pregnancy rate (22.8%) was comparable with that reported by Leethongdee et al. (2013), who recorded pregnancy rates of 15.79% after single and 38.70% after double cervical insemination with frozen semen.

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In contrast, Dehouegnon and Koluman (2018) reported a pregnancy rate of 70% in Alpine goats following estrus synchronization and AI with frozen semen, likely due to differences in synchronization protocols and insemination during “standing estrus” detected by a teaser buck.

However, Yotov et al. (2016a) observed a higher pregnancy rate (58.3% vs. 45.2%) in single AI of goats by frozen semen after estrus synchronization, compared with AI of goats during the natural estrus.

The lower pregnancy rate in the present study may also be attributed to the lactation status. According to Yotov et al. (2016b), artificial insemination in lactating goats resulted in lower pregnancy, compared with those in dry animals (72% vs. 19%). These findings support the multifactorial nature of reproductive performance following AI with frozen semen. In this aspect, Tekin (2019) determined no significant differences in pregnancy rates, depending on animal age. In goats aged 7 months (never kidding) and 19 months (first kidding), pregnancy rates were 31.4% and 32.3%, respectively. The same study revealed that when insemination of goats was after preliminary testing by a teaser and detection of “the standing estrus”, pregnancy rates and prolificacy were 67.3% and 119%, respectively.



Fig. 1. Deep cervical deposition of frozen semen in goat

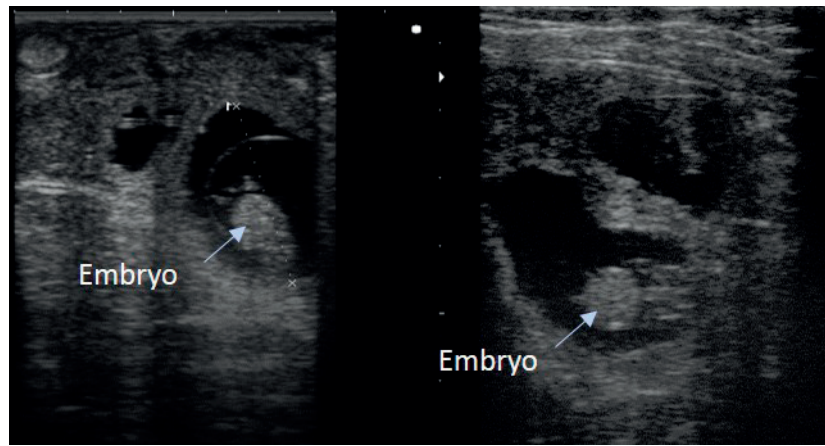


Fig. 2. Ultrasound pictures of pregnancy in goats on day 35 after AI

Table 1. Reproductive parameters in Bulgarian local goats after estrus synchronization and artificial insemination by frozen semen during breeding season

Goats under estrus synchronization (n)	101
Estrus synchronization response, % (n/n)	100 (101/101)
Pregnancy rate based on USE, % (n/n)	22.8 (23/101)
Kids born (n)	25
Prolificacy, % (n/n)	108.6 (25/23)

*USE – ultrasound examinations

The calculated prolificacy for Bulgarian local goats (108.6%) was close to the one obtained for Angora breed (124%) (Gibbons and Cueto, 2011) but differed considerably from the prolificacy registered for Majorera goat (160–167%) (Batista et al., 2009). It is known that the prolificacy is genetically determined and has a high positive correlation with the number of ovulated follicles. Nevertheless, the reproductive parameters also depend on the type of semen (fresh diluted, undiluted or frozen) and the place for sperm cells deposition (cervical or intrauterine) (Salvador et al., 2005; Paulenz et al., 2005; Arrébola et al., 2012). The abovementioned factors are very important for ensuring enough spermatozoa with preserved fertilizing capacity around the time of ovulation, which is crucial for increasing pregnancy and kidding rates. Paulenz et al. (2005) reported high pregnancy rates (87% and 78%) in AI of goats with fresh-diluted and undiluted semen, respectively. A high pregnancy rate (71%) was obtained after transcervical artificial insemination (Sohnrey and Holtz, 2005). A satisfactory pregnancy rate (66%) was achieved by Kulaksiz and Daskin (2012) using intrauterine deposition of frozen semen by laparoscopy.

However, laparoscopic insemination is not a routine method for insemination in goats, due to the high cost of the manipulation and the need for sedation and local anesthesia (Vrisman et al., 2014).

Final analysis of the current results and the information obtained from other authors confirmed that estrus synchronization protocols and artificial

insemination with frozen semen in fixed time could be used successfully in goats, but they have to be adapted to specific features of animals. The time for insemination and the semen deposition technique should also be taken into consideration for achievement of good reproductive performance.

On the one hand, the lower pregnancy results after AI with frozen semen have negative economic effects. On the other, wide application of AI by frozen semen from genetically valuable bucks allows fast production of animals with high genetic merit. The obtained information could be utilized for reproductive process optimization in goats.

In conclusion, the abovementioned estrus synchronization protocol and artificial insemination with frozen semen of Bulgarian local goat during breeding season provide acceptable pregnancy rates and prolificacy, when the main purpose is acceleration of genetic progress in the flock. Future detailed investigations in large numbers of goats are necessary to clarify some debatable questions and to make these assisted reproductive technologies more effective.

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Conflict of interest

The authors have not declared any conflict of interests.

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