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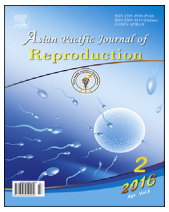

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Pregnancy rate in Bulgarian White milk goats with natural and synchronized estrus after artificial insemination by frozen semen during breeding season

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ABSTRACT

Objective: To determine the pregnancy rate (PR) in goats with natural and synchronized estrus after artificial insemination (AI) by frozen semen during breeding season.
Methods: The experiment was carried out with 89 Bulgarian White milk goats divided as follows: group I (n = 54) animals with natural estrus (NE), allocated in two subgroups treated with or not with GnRH after the last insemination and group II (n = 35) goats with synchronized estrus by intravaginal sponges and injection of PMSG after the sponges withdrawal. Both groups were also divided according to number AI – single or double. All animals in group I were inseminated by frozen semen after proved standing estrus as a part of goats received second insemination 8 h later. Single insemination in synchronized goats was done 48 and double at 48 and 56 h after the sponge removal. Ultrasound pregnancy diagnosis was performed on day 30 post insemination.
Results: The pregnant animals in both subgroups with natural estrus and single AI (33.3% and 45.2%) were less than those (58.3%) after estrus synchronization (ES). The pregnancy rate in synchronized goats with double insemination was 63.6%, whereas in animals with natural estrus varied from 40 to 50%. Overall pregnancy rate in group I (60%) was significantly ($P < 0.05$) higher than (37%) in goats without GnRH treatment.
Conclusion: The goats with natural estrus and GnRH treatment tend to enhance pregnancy rate after double artificial insemination 8 h apart. The insemination number has no significant impact on pregnancy rate in synchronized goats as the overall pregnancy rate is better than in animals with natural estrus without GnRH administration.

1. Introduction

The optimal reproductive performance has an important role in intensive goat breeding [1,2]. The improvement of the productive characteristics in goats descending from different regions and the accelerate production of genetically valuable offspring require artificial insemination with semen from elite sires [3–6]. Recently, artificial insemination of synchronized animals by frozen semen has been reported as one of the most used option for this purpose [7–10]. According to Simões [11] the use of P_4 or progestagens remains crucial for synchronization of fertile ovulation if we want to maximize the fertility during the anoestrous season. During the breeding season different estrus induction or synchronization schedules (P_4-PMSG; P_4-PMSG-$PGF_2\alpha$; GnRH-$PGF_2\alpha$-GnRH) have been utilized [12,13]. Nevertheless, the question about achievement of satisfactory pregnancy rate after ES and AI of goats with frozen semen is still open.

Effective pregnancy rate from 63.6 to 66% in goats [14,15] and 75–83% in sheep [16] after laparoscopic insemination has been achieved. In contrast to sheep, in the goats there are specific features (highly susceptible to pain, sedation and local anesthesia are required, high price) that limit routine utilization of the laparoscopy [17]. Registered pregnancy rate in goats after cervical or transcervical insemination with frozen semen is rather variable – from 15.8% [18], 38.5–48.6% [7], 57% [19], 60–65% [8,20] up to 71% [21]. In most of the studies, season [22], breed and age of goats [15,23], estrus synchronization protocol [24], time and number of AI [2] body condition score

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and the breeding technology [6,25] have been shown as a reason for different results. The above mentioned data presumes an accomplishment of investigations for adjustment of the reproductive programs to the breed of the goats, the breeding technology or the specific climatic condition in the different regions.

The aim of this study was to determine the pregnancy rate in local goats with natural and synchronized estrus after artificial insemination by frozen semen during breeding season.

2. Material and methods

The experiment was conducted on 89 Bulgarian White milk goats, 2–4 year of age and weighing 45–50 kg, housed in the same technology, located at latitude 42.25° and longitude 25°37′. The experiment was done during the breeding season (October–November).

All animals were arranged in groups according to the farm breeding management. Group I (n = 54) included animals with a natural estrus, allocated in two subgroups, that received or not received 25 μg GnRH (Ovarelin, Ceva Animal Health, France) immediately after the last insemination. Group II (n = 35) was consisted of goats with synchronized estrus by intravaginal sponges, containing 30 mg FGA (Syncro-part, Ceva Animal Health, France) for 12 days and injection of 500 IU (Syncro-part PMSG, Ceva Animal Health, France) at day of sponges withdrawal. Both groups were also divided according to number of AI – single or double. The artificial insemination was deep cervically with 0.25 mL frozen semen in straw (Sersia, Rennes Cedex 7, France), one straw per goat. Each goat with a natural estrus was inseminated after proved standing estrus by buck teaser and these with double AI 8 h later. The synchronized animals were inseminated at a fixed time – 48 h and 48 and 56 h following sponge withdrawal in single and double insemination, respectively. All goats were tested for pregnancy 30 days post AI by ultrasound scanner A5 Vet SonoScape (SonoScape, Co. LTD, Shenzhen, China) with a linear probe 5–12 MHz. Pregnancy rate in different groups and subgroups was determined on base ultrasound diagnoses.

Statistical analysis was performed with Stat-Soft 1984–2000 Inc. statistical software (Copyright©1990–1995 Microsoft Corp.) by means of non-parametric analysis for comparison of two proportions, using Student's t-criterion. Differences were considered significant in P – values < 0.05.

3. Results

The positive pregnancy diagnosis on day 30 post insemination was connected with visualization of increased uterine lumen, fulfilled with anehogenic fluid and echogenic embryo. In some of cases a corpus luteum graviditas into one of the ovaries was also observed (Figure 1).

The percentages of pregnant goats (33.3% and 45.2%) with natural estrus and single AI without or with GnRH treatment, respectively, were less than the obtained value (58.3%) in the group submitted to estrus synchronization and AI in fixed time (Table 1). The pregnancy rate (45.2%) in the non-synchronized subgroup II (AI plus GnRH administration) tended to increase compared to non-treated one (33.3%), but significant difference was not detected (P = 0.27). The results after double AI showed 40% pregnancy rate in subgroup I and 50% in subgroup II. These values were also less than the percentage (63.6%) in goats with estrus synchronization and AI in fixed time. The comparative analysis of the results according to number of insemination into the groups did not determine significant effect of this parameter on the pregnancy rate (P > 0.05). However, there was an impression the high difference (13.3%) between the pregnancy rates after single and double AI in goats with natural estrus, but non-treated by GnRH.

Statistically difference between overall pregnancy rate (37.0% and 48.2%) for both subgroups with natural estrus was not observed (P = 0.2), regardless of the increased percentage of pregnant animals after GnRH administration. The highest overall pregnancy rate (60.0%) was accounted in goats with synchronized estrus, as the showed value was statistically

Table 1
Pregnancy rate in Bulgarian White milk goats according to type of estrus and number of inseminations.

No. of inseminations	Type of estrus		
	Natural estrus (group I)		Synchronized estrus (group II)
	GnRH (–)	GnRH (+)	
Single	33.3 (4/12)	45.2 (6/13)	58.3 (14/24)
Double	40 (6/15)	50 (7/14)	63.6 (7/11)
Overall	37 (10/27)[a]	48.2 (13/27)[ab]	60 (21/35)[b]

Values in the same row marked with different letters differ significantly at P < 0.05.

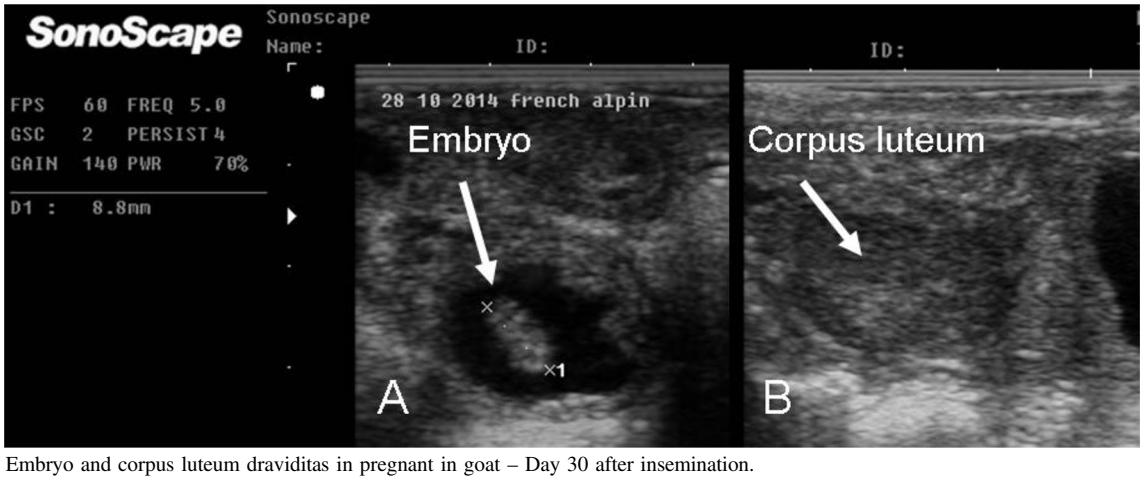

Figure 1. Embryo and corpus luteum draviditas in pregnant in goat – Day 30 after insemination.

($P < 0.05$) greater than the estimated 37% in animals with natural estrus and AI without GnRH treatment.

4. Discussion

The accelerate introduction of newly reproductive biotechnologies in goats on a world scale have been connected with effective reproduction achievement [11,13]. The most important indicators for successful reproductive performance were pregnancy rate and number of newly born kids [1,26]. This study represents a data about pregnancy rate in local breed goats after using of two biotechnologies under field condition – estrus synchronization and artificial insemination by frozen semen.

In agreement with other research [27], our study also indicated that the pregnancy rate after artificial insemination by frozen semen is multi-factorial determined. The lowest pregnancy rate (33.3%) obtained in animals with natural estrus without GnRH treatment is close to the reported 38.7% by Leethongdee *et al.* [18]. A reason for this unsatisfactory value can be a discrepancy between time of insemination and ovulation. The estrus duration in goats is rather variable from 24 to 48 h and ovulation could be occurred between 9 and 37 h after onset of estrus behavior [28]. It is known that artificial insemination at the start of the standing estrus results in decrease of a pregnancy rate and it can be explanation for the above mentioned result. This is in accordance with an increased percentage of pregnant goats after GnRH injection. Similar data was reported by Olfati and Moghaddam [29] after GnRH treatment during artificial insemination in sheep. The administration of a gonadotropin releasing hormone conducted to earlier induction of ovulation and enhancing the subsequent luteal phase [30,31]. According to Pierson *et al.* [32] GnRH application during the estrus synchronized more precisely ovulation and improves the success of AI in fixed time in does. The most important evidence for negative effect of the earlier insemination was the registered tendency to a pregnancy rate enhancement in all animals with natural estrus and twice artificial insemination. Other prerequisite for successful conceive after using of frozen semen is enough fertile spermatozoa to be deposited into the cervix immediately before the ovulation. In current study the double AI 8 h apart provided higher sperm cells and could also be a reason for better pregnancy results.

Pregnancy rate in goats with synchronized estrus was satisfied and closed to the reported 57% in Murciano-Granadina goats [19] and 57–61% in Saanen and Alpine goats [33]. It was indicator for acceptable reproductive response of Bulgarian White milk goats after applied estrus synchronization protocol. In synchronized goats the preliminary progesterone exposition modulates the pituitary LH secretion by a negative feedback, modifying the hypothalamic GnRH secretion. It led to synchronized LH release after sponge withdrawal and together with PMSG injection they are responsible for development of a large number of follicles to preovulatory stadium, followed by their ovulation [11,34]. The insignificant difference (5.3%) between percentages of pregnant animals after single and double AI showed that the insemination number has no considerable influence on pregnancy rate. Nordstoga *et al.* [8] also determined insignificant difference between pregnancy rates after single or twice insemination. On the other hand our result assumed that single AI from 48 to 56 h can ensure good fertility rate and can reduce the expenses for labor and frozen semen. This hypothesis is supported from the data of Pellicer-Rubio *et al.* [35] who performed single AI by frozen semen 52 h after the sponge removal and achieved 71–78% fertility rate.

The positive effect of the estrus synchronization on the success after AI with frozen semen was also demonstrated by significant ($P < 0.05$) higher overall pregnancy rate (60%) in synchronized goats than 37% in animals with natural estrus and no GnRH administration. The estrus synchronization led to simultaneously expression of estrus and ovulation in large number of animals, followed by expulsion of fertile ovum and AI in optimal time grantee successful fertilization [4,36]. In our experiment the enhancement of the pregnancy rate in the synchronized group was also conditioned by the better cervical relaxation that permitted deeper deposition of the spermatozoa. In this aspect, Paulenz *et al.* [37] reported significantly higher conception rate after cervically or intrauterine than vaginal deposition of frozen semen. Leethongdee and Ponglowhapan [10] suggested that deep application of frozen semen can increase pregnancy rate. Future detailed investigations in large number goats are necessary to clarify some discussed questions.

From this field study it may be concluded that pregnancy rate in Bulgarian White milk goats with natural estrus and GnRH treatment during the last insemination show tendency to enhancement after a double AI with frozen semen 8 h apart. The pregnancy rate after estrus synchronization no differs significantly according to insemination numbers. In addition, the overall pregnancy rate in synchronized animals is better ($P < 0.05$) than in goats with natural estrus and absence of GnRH administration. The obtained information could be utilized for reproductive process optimization in goats.

Conflict of interest statement

We declare that we have no conflict of interest.

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