



## Recent advances in goat artificial insemination in Brazil

*Recentes avanços da inseminação artificial de caprinos no Brasil*

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### Resumo

Esta revisão reporta os recentes avanços da Inseminação Artificial em caprinos (IA) no Brasil nas últimas duas décadas. Estes avanços foram pautados primariamente no estudo e conhecimento dos eventos reprodutivos de cabras submetidas a diferentes formas de controle do ciclo estral e ovulação. Estas abordam principalmente o desenvolvimento e caracterização precisa de protocolos de indução e sincronização de estro em cabras durante a estação de anestro fisiológico e de acasalamento natural. Paralelamente, foi desenvolvida a Técnica Embrapa de IA cervical em caprinos por meio da imobilização cervical. Associadamente, os estudos caracterizaram a relação do muco cervical e o momento ideal para execução da IA com diferentes tipos de sêmen. Protótipos e um Kit de IA foram desenvolvidos e testados em condições de laboratório e de campo no âmbito do programa nacional de melhoramento genético de caprinos leiteiros – CapraGene<sup>®</sup>. Fatores que interferem na taxa de gestação após a IA foram identificados e classificados. Estudos com base em ultrassonografia transretal revelaram a prevalência de desordens reprodutivas caracterizando os principais problemas associados à subfertilidade ou infertilidade na cabra. Todos estes estudos permitiram consolidar uma das mais produtivas e aplicadas coleções de conhecimento sobre IA em caprinos dos últimos 20 anos no Brasil.

**Palavras-chave:** caprinos, controle do ciclo estral, inseminação artificial.

### Abstract

*This review reports the advances of goat Artificial Insemination (AI) in Brazil in the last two decades. These advances were based primarily on the study and knowledge of the reproductive events of goats subjected to different methods of control of the estrous cycle and ovulation. These approaches are mainly related to the development and precise characterization of protocols of estrus induction and synchronization in goats during either the natural breeding or nonbreeding season. In parallel, the Embrapa Technique of cervical AI through cervical immobilization in goats was developed. In association, the studies characterized the cervical mucus and the ideal moment for AI execution with different types of semen. Prototypes and an AI kit were developed and tested at both laboratory and field conditions in the national dairy goat breeding program - CapraGene<sup>®</sup>. Factors affecting the pregnancy rate after AI were identified and classified. Studies based on transrectal ultrasonography have revealed the prevalence of reproductive disorders characterizing the major problems associated with subfertility or infertility in the goat. All these studies allowed to consolidate one of the most productive and applied collections of AI knowledge in goats of the last 20 years in Brazil.*

**Keywords:** artificial insemination, estrous cycle control, goat.

### Introduction

Considered the second generation of reproductive biotechnologies, the Artificial Insemination (AI) had its first reports in Brazil possibly in the 1940s and 1950s (Mies Filho, 1955). It is a tool for rapid and safe introduction of genetics in herds of different species. In goats, the knowledge used to control the estrous cycle, as well as its application and AI technique used in Brazil and the world were shaped by studies carried out in Europe and Oceania. The application of this technological package did not reach satisfactory rates in both intrauterine semen deposition by the cervical route and pregnancy. Protocols with a long duration (12 days or longer) of progesterone or progestogen-containing devices and relatively high doses (400 to 500 IU per animal) of equine chorionic gonadotrophin (eCG) were widely used to support AI programs. In the Brazilian tropical conditions, several synchronization protocols (Fonseca, 2002) and synchronous estrus induction protocols were tested (Fonseca et al., 2002; Fonseca et al., 2005; Fonseca et al., 2008). Some of them, reached excellent results when associated with AI in goats during the breeding (Maia et al., 2017) or nonbreeding season (Fonseca et al., 2017a) and routinely used to support Brazilian breeding plan for dairy goats and progeny test – CapraGene<sup>®</sup> (Facó et al., 2011).

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The success rate of intrauterine semen deposition was a major impediment to a successful pregnancy. The further away from the fertilization site the semen is deposited, the lower the resulting pregnancy rate. Previous studies using the traditional AI technique with an animal contained in anterior bipedal support resulted in relatively high time for technical execution and relatively high deposition of intracervical semen, which has contributed to the AI failure in goats. Therefore, the Embrapa AI Technique was developed (Fonseca et al., 2011a). In this technique, goats are contained in quadrupedal support, cervical immobilization is performed, AI is rapidly performed and more than 90% of deposition of intrauterine semen is reported, resulting in high pregnancy rates (Fonseca et al., 2017a).

The use of real-time transrectal ultrasonography allowed the development of efficient protocols for both synchronization (Maia et al., 2017) and estrus induction (Fonseca et al., 2017a,b). By the ultrasound, it was possible to determine the time of ovulation, the establishment and maintenance of pregnancy (Fonseca, 2002; Fonseca et al., 2012) as well as reproductive disorders (Maia et al., 2018c), that may affect AI efficiency.

This review reports the recent advances of goat AI in Brazil in the last two decades.

### **The recent history of goat Artificial Insemination in Brazil**

As the proposal of this review was to address the recent advances of AI in goats in Brazil, a brief compilation of the projects and main results obtained from the course of their development will be presented. Phase 1 included projects supported by Embrapa (02.03.003.11.00.00) and CNPQ from 2006 to 2010 (490488/2008-0 and 559151/2010-1), where we studied alternatives to control the estrous cycle (Fonseca et al., 2011b, Oliveira, 2010) and ovulation (Esteves et al., 2013) and the development of a new AI technique in goats (Fonseca et al., 2011b) with fresh (Zambrini, 2006), cooled (Siqueira, 2006; Siqueira et al., 2009; Pinto, 2011; Pinto et al., 2014) or frozen semen (Fonseca et al., 2017a,b). Phase 2 was funded by Embrapa (02.08.02.005.00.04), CNPQ (310166 / 2012-8) and Fapemig (CVZ-PPM 00042-14) from 2010 to 2014 and was based on the improvement of AI technique, by different strategies, such as: the development of a prototype "cervical immobilization forceps", protocols of estrus induction and synchronization, cervical mucus characterization and its relation to the ovulation time, the clinical and therapeutic characterization of the main reproductive disorders, among others. Phase 3 already has support from Fapemig (CVZ 00201-17), CNPQ (314952/2018-7), and it is based on the conclusion of the "AI forceps" prototype and on new challenges that can now be safely tested by the knowledge and results of Phases 1 and 2. More specifically, Phase 3 is focused on the refinement of estrous cycle control with new alternatives based on protocol simplification and the decreased use of hormones. Phase 3 involves the control ovulation, semen dosage, embryo transfer and technology transfer to the technical/productive sector by training human resources.

### **Factors affecting the efficiency of Artificial Insemination in Goats**

The efficiency of AI can ultimately be measured by the number of lives offspring born after its implantation. Even before estrus and AI, several factors can positively or negatively impact this efficiency. The main ones are listed below, highlighting our main recommendations:

#### *Female selection*

This is the initial step that can ensure animals with a greater possibility of conceiving after AI. In this respect, the animal category requires special attention. They may be nulliparous (never kidded), primiparous (kidded once) or pluriparous (several births). In the case of nulliparous, it is important to know whether they meet the minimum weight and age. It is essential to take care of large, heavy and relatively aged animals. It may be a typical picture of "beautiful goat's syndrome". For some reason, these animals do not conceive by continuous attempts, and they remain, usually due to their appearance, in the establishment. One of the most common causes of this problem is hydrosalpinx (Maia et al., 2018a), which when diagnosed is indicative of animal discard for productive function. AI in nulliparous animals should be viewed with precaution and performed only by technicians with previous experience in pluriparous (Fonseca and Alvim, 2018). As the primiparous and pluriparous goats are usually more affected by hydrometra (Maia et al., 2018a,c), it is recommended to perform previous transrectal ultrasonography to diagnose any possible reproductive disorders (Maia et al., 2018c). It is recommended not to subject these animals to be AI.

Another important aspect is the body condition score (BCS). Ranging from 1 (very lean) to 5 (obese) the desirable score is between 2.50 and 3.75 and 80% of the herd should be in this condition. The non-introduction of animals with BCS outside this range is desirable (Fonseca and Alvim, 2018). Sometimes BCS variation occurs depending on the lactation stage, and this should also be considered.

Vaccinations and worming programs have restrictive schedules during and after preparation for mating, whether natural or artificial. We should be aware of these restrictions. Nutritional alterations should also be made with caution and technical guidance (Fonseca et al., 2011b).



### *Female preparation*

Once selected the females, the second step is their preparation. In this case, estrous cycle control tools should be thoroughly evaluated and chosen due to their technical and economic feasibility and also sanitary surveillance considering there are drugs that require a period for the animal consumption (as progestogen). Therefore, the use of prostaglandins for estrus synchronization during the natural breeding season is recommended. For the same reasons, estrus induction with hormone cocktails based on progesterone-containing devices is preferable for lactating goats while those containing progestágeno, because of the necessity for milk discard, should be restricted for non-lactating animals (Fonseca et al., 2011b). Recently, the use of light induction program associated with prostaglandin for estrus synchronization was tested and the results were promising (Barreto et al., 2017). Regardless of the type and protocol, it is important to know/predict the animal response in order to establish the AI calendar, such as the number of animals in estrus and the average interval from the last intervention to estrous onset (Fonseca and Alvim, 2018).

### *Semen preparation*

Both maintenance and preparation of semen for AI should follow several technical criteria. Distraction in this step may compromise any technique regardless of pre or post-AI factors. Before acquiring semen, make sure that both pre and post-freeze analysis present the minimum requirements (CBRA, 2013, Fonseca and Alvim, 2018), and assistance of a veterinarian can be crucial. Proper semen thawing training and applicator assembly followed by immediate packaging on adequate thermal condition up to AI are indispensable.

### *Artificial Insemination Technique*

Although worldwide used, the traditional AI technique with anterior bipedal support has limitations. Among them, there are the relatively low rate of semen deposition in the uterus, the possibility of carrying contamination from the vagina to the uterus, progressive discomfort of the animal and helper – due to the duration of the procedure; among others. Embrapa technique of transcervical AI by cervical immobilization was developed considering these factors (Fonseca et al., 2011a). This technique yields a high rate of intrauterine semen deposition, takes less than 60 seconds, is of easy contention for the goat and helper, and achieve high pregnancy rate (Fonseca et al., 2017a, Maia et al., 2017).

### *Time of Artificial Insemination*

The ideal time to perform AI should consider the estimated mean time between the last hormonal intervention and estrous onset and the time between the latter and ovulation, due to their high variation (Fonseca and Alvim, 2018). Besides, the type of mucus observed is also an index of the most appropriate moment (Fonseca et al., 2017b). One of the earliest ultrasound-assisted ovulation studies conducted every 4 h identified that AI performed after ovulation resulted in high pregnancy rate when early diagnosed (18 days of estrous cycle), but pregnancies may be lost in the following weeks (Fonseca et al., 2012). The basic premise is to inseminate before ovulation and to give enough time for the sperm to be fully capacitated for fertilization at the most appropriate time for the female gamete.

### *Cervical mucus*

Cervical mucus observed in goats presenting estrus may range from crystalline to caseous (1 to 5). Crystalline mucus is the first marker of estrus, appearing before the first acceptance of the mounting. Later, cervical mucus undergoes changes from the onset of estrus to the end of the estrous and ovulation (compatible with caseous mucus). Following the previous orientation, the mucus suitable for AI execution is striated and striated/caseous when using frozen-thawed semen (Fonseca et al., 2017b). When using semen of high economic and zootechnical value, it is recommended first to evaluate the female to see if the mucus has the adequate condition before semen thawing.

### *Hormonal strategic use*

Three great peculiar problems of goats end up decreasing the pregnancy rate either after AI or natural mating. The first one is related to ovulatory asynchrony, corpora lutea of a short life (early regression), and low progesterone production that alone or together can decrease pregnancy rate. The application of gonadorelin (Bartz et al., 2007) and hCG (Dias et al., 2018) at the time of insemination has presented exciting results. Similarly, the possibility of inducing accessory corpora lutea to enhance plasma concentrations of progesterone was already confirmed in sheep (Fonseca et al., 2018) and apparently increases pregnancy rate in goats too (Cortes et al., 2018).



### Recording – notes

There are several forms for recording events that precede and occur during or even after AI. It is suggested to the breeder/inseminator to routinely fill out forms with the maximum information that can be used to later infer the AI success or failure (Fonseca and Alvim, 2018). For instance, the mucous type associated with the end of protocol and/or the interval from the estrus onset to AI may be important indexes. The BCS recorded at the time of AI and at the time of definitive pregnancy diagnosis after 60 days may reveal significant alterations that may have occurred after AI (Fonseca and Alvim, 2018).

### Pregnancy diagnosis

The most viable and efficient way to diagnose pregnancy in goats is by transrectal ultrasonography. The use of appropriate equipment including the physical dimension of the transducer, its preparation and the use of disposable condom-like cover are essential. Considering the potential occurrence of early embryonic loss (Fonseca et al., 2012) and that embryonic loss may evolve into hydrometra (Maia et al., 2017), early pregnancy diagnosis should be avoided or at least later confirmed.

### Impact of AI on the production and productivity in dairy goats

Although AI can be used for different purposes, probably it is in the dairy goat production system that this tool achieves its most significant impact. After collection, cryopreservation and storage of large numbers of male semen, AI allows the introduction, widespread and testing these males under different conditions of productive management. Males selected according to their zootechnical characteristics for AI can be introduced and tested in different ways, such as qualitative and quantitative analysis of milk and conformation of their progenies. Based on this, male genetic evaluation is ranked according to the different characteristics tested. Some published information about CapraGene® AI results were recorded (Table 1). These results together to field results constitute the basis of Brazilian dairy goat production evolution. Brazil currently has two genetic evaluation programs for Saanen bucks (Facó et al., 2014, Lôbo et al., 2017). Data produced by the CapraGene® program indicate that currently the dairy goat herds raised in Brazil Southeastern region are among the best ones in the world when taking into consideration their qualitative and quantitative parameters of milk (Lôbo et al., 2017).

Table 1. Scientific published results from official Brazilian artificial insemination program supported by Breeding plan for commercial dairy goat production systems in Brazil (CapraGene®)

Breed	Animals (n)	Pregnancy (%)	Reference
Anglo Nubian	48	45.8	Fonseca et al. (2009)
Dairy goats	23	60.8	Esteves et al. (2013)
Saanen	14	64.3	Pietroski et al. (2013)
Saanen	60	50.0	Fonseca et al. (2014)
Alpine and Saanen	79	54.4	Oliveira et al. (2015)
Saanen	32	62.5	Fonseca et al. (2017a)
Alpine and Saanen	22	50.0	Fonseca et al. (2017b)
Alpine and Saanen	58	89.7	Maia et al. (2017)
Alpine and Saanen	290	73.8	Dias et al. (2018)
Alpine and Saanen	30	70.0	Bonato et al. (2018)

### Final considerations

Artificial Insemination is one of the reproductive biotechnologies with a wider diversity of use. Its impact on production systems is immediate, progressive and positive if technically supported and zootechnical databases. For their perfect performance and efficiency females should be carefully selected and prepared according to technical recommendations. All animal preparation, considering nutritional, management and/or pharmacological treatment (protocols of synchronization or induction of estrus), the AI technique, the characteristics associated to the estrous cycle and the ideal time for AI (cervical mucus) should be thoroughly known. Taken together, these factors may allow the attainment of pregnancy rates similar to or even higher than those observed after natural mating. As a final product, the progenies can significantly increase the desired productive indexes such as milk production. All the knowledge generated in the last two decades within these projects has allowed having the Brazilian goat herd among the best and most productive in the world.

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