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Cryopreservation and diffusion of goat genetic material in the Argentine Patagonia

Criopreservação e difusão de material genético de cabra na Patagônia Argentina

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Abstract

The high demand from goat Argentinian breeders for improving their Angora herds determined the need of implementing a genetic breeding program based on the use of artificial insemination (AI) with frozen semen. The field work included the phenotypic selection, estrus synchronization, heat detection and artificial insemination of the superior females of each Angora breeder. The criteria for the acceptance of seminal doses, the suitability in synchronization and detection of estrus and precautions during AI, determined the overall reproductive efficiency of the Angora genetic improvement program. While AI only allows male genetic diffusion, embryo transfer (ET) enables the dissemination of all the genetic characteristics of a breed. The feasibility of its implementation, considering the probable number of offspring obtained per donor and the economical profit that would justify its execution, should be evaluated. The cryoconservation of embryos has become an essential component of assisted reproductive technologies. Due to the limited bibliography regarding, in particular, embryo vitrification in small ruminants, the aim of our last research was to evaluate pregnancy success of vitrified goat embryos, using a simple cryopreservation method, utilizing plastic micropipette.

Keywords: artificial insemination, embryo transfer, oestrus synchronization, vitrification.

Resumo

A alta demanda de criadores de cabra argentinos para melhorar seus rebanhos Angorá determinou a necessidade de implementar um programa de criação genético baseado no uso de inseminação artificial (IA) com sêmen congelado. O trabalho de campo incluiu a seleção fenotípica, sincronização do estro, detecção de cio e inseminação artificial de fêmeas superiores de cada criador da raça Angorá. O critério para a aceitação de doses seminais, a compatibilidade na sincronização e a detecção de estro e precauções durante a IA determinaram a eficiência geral da reprodução no programa de melhoria genética da raça Angorá. Enquanto que a IA permite apenas a difusão genética do macho, a transferência do embrião (TE) permite a disseminação de todas as características genéticas de uma raça. A viabilidade desta implementação, considerando o número provável de prole obtido por doador e o ganho econômico que justificaria sua execução deve ser avaliada. Devido à bibliografia limitada, particularmente com relação a vitrificação de embrião em pequenos ruminantes, a meta da nossa última pesquisa foi avaliar o sucesso de gestação em embriões de cabra vitrificados, usando um método simples de criopreservação, utilizando micropipeta plástico.

***Palavras-chave**: inseminação artificial, transferência de embrião, sincronização de copulação, vitrificação.*

Introduction

In northern Patagonia (Argentina), approximately 550.000 Angora goats (which produce hair mohair), are raised in an extensive production system with low inputs. Nearly 6000 goat breeders base their subsistence economy on these animals. The genetic improvement of Angora goats in Argentina was organized according to a classic genetic pyramid diagram.

At first, our genetic nucleus (INTA Angora goats) was the only genetic center for the improvement of the Angora breed in northern Patagonia and the only supplier of genetic superior males. Due to an insufficient availability of males for satisfying the goat breeders demand, different restrictions had to be surpassed before implementing a genetic improvement program. Our principal limitation was the difficulty of using the genetically superior males (13 national and imported young bucks) for natural service and even for AI with fresh semen, considering that this breed has a very short breeding season in Patagonia (three months), low semen production and ejaculatory frequency when compared with other breeds (Ritar et al., 1992).

The Angora males in Patagonia have a restricted seasonal reproductive activity in response to photoperiod at high latitudes, this induces changes in the concentration level of gonadotropic hormones, androgens, testicular size and semen production, determining a short year time for the utilization of Angora bucks in breeding programs. Another restriction in males sexual performance is the beginning of puberty,

because it is conditioned by time of birth (spring) and nutritional level, both leading to a late commencement of sexual activity at 18 months of age (average live weight 34 kg). At this age, bucks have not yet completed sexual development and rearing often extends to 30 months, before their inclusion in intensive genetic improvement programs.

The high demand from goat breeders for improving their Angora herds, added to the above mentioned high Angora male seasonality, determined the need of implementing a genetic breeding program based on the use of artificial insemination with frozen semen in the 8 "top" regional goat breeders, denominated "New Genetic Nucleus" of the Angora breed (700 females). Furthermore, in this way, both male sanitary risks and transport through long distances between breeders were avoided. Besides, unlimited conservation of germoplasm would prevent loss of genes in case of accidental buck death.

Breeding program and artificial insemination

The first step in the breeding program was to select genetically superior males as semen donors, taking into consideration aplomb problems, reproductive tract abnormalities and body development delay. In a second step, at the beginning of their first breeding season, the selection was made based on sexual male behavior, semen quality and semen resistance to freezing (12 collections, 3 per week). An important feature to consider was the variability between males regarding their semen resistance to freezing processes, as reported previously (Watson, 1995). An early selection of males based on this feature increased the overall efficiency of the freezing semen program, performed in accordance with the general methodology described by Corteel (1974), and extensively analyzed in the review of Lebouef et al. (2000). The main causes of male elimination were the incapacity to ejaculate in the artificial vagina (10%), low volume and sperm concentration per ejaculation (15%) and post-thawing low quality semen (25%; Gibbons, 2003).

The field work included the phenotypic selection, estrus synchronization, heat detection and artificial insemination of the superior females of each Angora breeder included in the "New Genetic Nucleus".

The most used method for artificial insemination with frozen semen in female goats is via the vagina and is generally performed by cervical artificial insemination (CAI), or in some cases, by semen deposition in the uterine body, depending on the breed and corresponding possibility to pass through the cervix rings. Average conception rates of 60–65% were reported by French researchers (Leboeuf, 1992; Leboeuf et al., 1998), although many other sources admit less encouraging conception rates (Holtz, 2005). Perhaps differences between breeds and/or production systems could explain such differences in CAI efficiency.

Our first work using CAI with frozen semen in the Angora breed in Patagonia, was performed with a dose of 200 million spermatozoa and yielded a 55% of kids born/goat inseminated (Gibbons et al., 1992). However, average reference values of reference for different nutritional status of Angora herds in Patagonia, vary between 45 to 50% of kids born/goat (883 goats inseminated; Arrigo, personal communication). On the other hand, laparoscopic artificial insemination (LAI) with frozen semen yields higher pregnancy rates and allows a reduction in the concentration of the seminal dose (200 vs. 100 million spermatozoa/inseminated goats with the CAI and LAI techniques, respectively). Under optimal conditions in our Angora herd, we obtained a 69% pregnancy rate by LAI with 100 million frozen spermatozoa/insemination dose (Gibbons et al., 1997). Similarly, other authors with different breeds (Cashmere: Ritar et al., 1990; dairy goats: Vallet et al., 1992) reported a 20% increase in pregnancy rates when applying LAI in relation to average values of CAI. However, it must be remembered that laparoscopic insemination entails elaborate equipment and special skills.

Analysis of these data, provided general support for choosing the laparoscopic AI methodology for superior Angora females. The application of this technique allowed us to achieve higher pregnancy rates and to attain a more rational use of frozen semen. Even when we reduced sperm concentration per insemination dose (50 million sperm/goat), pregnancy rate was not significantly affected (unpublished). The hormonal treatment for estrus synchronization in Angora goats consisted of intravaginal sponges with 60 mg of medroxyprogesterone acetate (MAP) for 18 days and combined with the application of 100 IU eCG im at the time of sponge removal. The onset of estrus was detected with the aid of an adult teaser buck at 24, 36, 48 and 60 hours after sponge withdrawal. In brief, the LAI was carried out at 48 hours in goats in estrus at 24 and 36 hours, and at 60 hours in females that showed estrus at 48 and 60 hours, after sponge withdrawal. That meant the possibility of inseminating all goats of a nucleus farm in only one day, reducing the labour time of the insemination program.

Overall pregnancy rate, when insemination was carried out in the 8 multiplication centers of the "New Genetic Nucleus" during three years, was lower than that obtained in our Angora herd (54 vs 69%), although reproductive efficiency (kids born/goat inseminated) of 67% was acceptable for the genetic program (Table 1).

Table 1. Reproductive efficiency values for artificial insemination by laparoscopy using frozen semen during three years in the genetic improvement scheme for Angora goats in the north of Patagonia.

Goats under estrus synchronization treatment (n)	1964
Synchronized on estrus (n)	1690
Rate of estrus synchronization (%)	86
Goats inseminated by laparoscopy (n)	1252
Goat kidding (n)	677
Pregnancy rate (%)	54
Kids born (n)	842
Kids born/does inseminated (%)	67
Prolificacy (%)	124

In the last step of the breeding program, superior Angora bucks were selected by a progeny test, considering productive characteristics of their progeny born, from artificial insemination, in the farms of the 8 goat breeders. The most important achievement of the Angora genetic improvement program was the fact that breeders of the "New Genetic Nucleus" became suppliers of genetically superior bucks to commercial goat breeders.

In conclusion, several factors are involved in obtaining a favorable result from the implementation of AI in a genetic breeding program. The bucks must be evaluated and selected based on their individual production characteristics and that of their progeny, as well as on their suitability for being incorporated in a seminal freezing schedule. Nutritional management and herd health must be prioritized. With regard to processes of collection, dilution, cooling, equilibration and freezing of semen, they should be accomplished meticulously, in order to avoid human failures, wrongly attributed to animal factors. The criteria for the acceptance of seminal doses, the suitability in synchronization and detection of estrus and precautions during AI, will determine the overall reproductive efficiency.

Embryo transfer

The accuracy of genetic evaluation in a male goat is much greater than for a female goat because the number of offspring is limited by sex. Another assisted reproductive technology is embryo transfer (ET), that may increase the number of offspring from a genetically superior female and yield an average of 4-5 kids born/female donor, each time a donor is treated to induce multiple ovulations (MO). Recent advances in the reproductive efficiency of MOET, have increased the possibility of its use in breeding programs through the dissemination of female genetic material of high productive value. The AI only allows male genetic diffusion, while ET enables the dissemination of all the genetic characteristics of a breed. Additionally, it contributes to improve genetic testing by increasing the number of kids produced. In turn, the use of young donor females can also reduce the generation interval, which allows a higher rate of genetic improvement.

The most important use of MOET is for the multiplication of genotypes. The embryos can come from females of high genetic value, selected from a large population or from a breed of high production and/or that have special characteristics. This would the application of MOET in local breeds, or alternatively, the use of imported frozen embryos (Mueller, 1993). Besides, it is important to consider the role of embryo transfer in the conservation of genotypes at risk of extinction. Faced with the possibility of using this biotechnology, the feasibility of its implementation, considering the probable number of offspring obtained per donor and the economical profit that would justify its execution, should be evaluated.

In agreement with Holtz (2005), the variability in the number of ovulations per donor and in the yield of viable embryos, remain the main limitations for MOET implementation. A number of factors such as breed, environment, breeding season, nutrition, health, source and regime of superovulation drugs and fertilization technique will determine its reproductive efficiency. There are numerous MO treatments in use, but none of them fulfill expectations concerning predictability and reliability of ovarian response. Based on this information, and although we are still investigating this point, at present we are using the following hormonal regime in MOET programs in Angora and Criollo breeds. The estrous cycles of does are synchronized for their use as embryo donors and embryo recipients, by the insertion of intravaginal progestagen sponges containing 60 mg MAP (Progespon®, Syntex, Argentina), for a period of 18 days (day 0, sponge insertion). At sponge removal, embryo recipients receive 200 IU eCG im (Novormon®, Syntex, Argentina). The embryo donors are superovulated using a protocol with a total dose of 80 mg pFSH (Folltropin V®, Bioniche, Canada) im – every 12 hours in 6 decreasing doses (18, 18, 14, 14, 8, 8 mg), during the last 3 days of the intravaginal progestagen

treatment (days 16-18). The onset of estrus is detected with the aid of an adult teaser buck (beginning 24 hours after sponge removal), and donors are inseminated using laparoscopy, with frozen/thawed semen (200 x 10^6 sperm per doe), 12-14 hours after the onset of the induced estrus. It is very important to consider the possibility that repeated porcine FSH treatments produce anti-FSH antibodies (Remmy et al., 1991) and a decrease in ovulatory response (40 to 50% in the 3rd treatment, up to 70 to 80% in the 4th or 5th treatment). This problem could be avoided by using ovine or caprine FSH (Baril et al., 1992).

In superovulatory treatments, fertilization rates of 32% (cervical AI) and 65% (laparoscopic AI) using fresh semen were obtained (Moore and Eppleston, 1979), while 70-75% fertility was achieved with frozen semen by laparoscopy (Fieni et al., 1990). Our mean fertility values in the Angora goats are 60% with frozen semen and 68% by natural service (unpublished). Currently, we are studying the stimulation by male effect as a tool for increasing the fertility rate at the beginning of the breeding season. It is very important to take into consideration that fecundation rates are dependant on ovulatory response. In Alpine and Saanen goats, fertility diminishes when ovulatory response is high (>15 corpora lutea, 49% vs. <15 corpora lutea, 66%; Baril et al., 1989).

In brief, results obtained in INTA Bariloche for Angora and Criollo goats are the following: Average number of corpora lutea: Angora goats: 8.6 - Criollo: 16. Surgically recovered embryos: 45 to 60%. Average of recovered embryos: Angora: 5.2 - Criollo: 7.2. Pregnancy rate by immediate semi-surgical ET technique: 57% (Gibbons and Cueto, 2010).

Nowadays, in response to the demand from the Angora Goat Producers Association to multiply and store genetic material of the "elite" goats, and to introduce new germoplasm by the importation of frozen embryos, it was decided to develop previously, a simple and efficient method of cryopreservation.

The cryoconservation of embryos has become an essential component of assisted reproductive technologies, allowing the storage of high value total germoplasm. It provides a method for the exchange of genetic material with low sanitary risk and allows embryo preservation from exotic or endangered species (Moore and Quadros Bonilla, 2006). Embryo cryopreservation also provides an option when superovulation yields are greater than availability of recipients.

The most common technique used for cryopreservation of goat embryos is slow freezing, which requires an expensive biological freezer and intensive processing. In general, when transferring cryopreserved blastocysts under favorable conditions, pregnancy rates between 45 and more than 80% may be expected, partially depending on the number of embryos transferred per animal (Holtz et al., 2000).

The vitrification of small ruminant embryos, by direct plunging into liquid nitrogen, has emanated following several cryobiological investigations (see review, Holtz, 2005). The technique has the advantage of being rapid without requiring special freezing equipment. The first kids born following embryo transfer of vitrified goat embryos in 1990, were reported by Yuswiati and Holtz (1990). Several years later, Traldi et al. (1999) reported more acceptable results with the vitrification of in vivo and in vitro-derived caprine embryos. Even though there are practical benefits and economical advantages in the cryopreservation of embryos, acceptable results, to date, have been limited. Specifically, embryo vitrification procedures have not been extensively used, as no standard protocol exists for specific species. Due to the limited bibliography regarding, in particular, embryo vitrification in small ruminants, the aim of our last research was to evaluate pregnancy success of vitrified goat embryos, using a simple cryopreservation method, utilizing plastic micropipette tips (Traldi et al., 2009; Gibbons et al., 2011).

Our results showed a high rate of pregnancy (64%) and embryo survival (64%) in does receiving blastocysts. However, unlike sheep, no pregnancies were recorded in the recipient goats receiving vitrified/thawed morulae (Gibbons et al., 2011). Current data clearly indicate that the tip vitrification method is very effective for preserving goat blastocysts, but inadequate for goat morulae. These observations are in agreement with Yacoub et al. (2010), who pointed out that caprine blastocysts could be preserved by means of the vitrification procedure, but this did not apply to caprine morulae. Although these preliminary results are promising, a larger number of vitrified embryo transfers using this technique, need to be performed.

Future research will be required for reducing costs and increasing the number of offspring per donor. This will facilitate its commercial application, as has already been accomplished in bovine species. There is no doubt at present that ET is the safest method, for sanitary reasons, when importing different high production biotypes. The increase in international commerce of genetic material through the use of cryopreserved embryos, has proved the importance of this technique in providing sanitary guarantees against exotic diseases and, furthermore, as a tool for the improvement of international goat production.

Finally, we believe that reproductive technologies are effective tools for the genetic improvement in goat production. The application of AI and/or ET should be conditioned to the actual needs of each production system. It is also necessary to reach a good understanding of the reproductive aspects of each particular breed and to make an accurate estimate of costs and benefits. Any progress towards the exhaustive comprehension of these techniques, will support basic information for the development of new biotechnologies (such as cloning and transgenesis), which will benefit animal production and provide a welcome contribution to human society.

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