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GOAT SEMEN FREEZING: THE TWO FACES OF THE COIN

(*Congelação de sêmen caprino: as duas faces da moeda*)

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ABSTRACT

Although the niche market for goat breeding products is on the rise, this activity is still carried out in family and disorganized systems. Thus, technification of this sector is necessary, including the introduction of reproduction biotechnology, such as semen cryopreservation and artificial insemination (AI). Semen freezing enables the breeder to improve the genetic quality of their herds at a lower cost, increasing the male potential without limit of time or space, and facilitating breeding management when in association with AI. As a consequence, the productivity and profitability of this sector is elevated, which could favor the transition from a livestock subsistence system to an industrial one. Nevertheless, the use of frozen semen of goats still presents some limitations, since the extender and procedures used for this biotechnology subject the gametes to numerous structural and functional injuries, both lethal and sublethal. These factors are reflected in the fertility rates, which are reduced after AI with frozen goat semen. In this perspective, the objective of the current review work is to report the positive and negative aspects of semen freezing biotechnology, with emphasis on the goat species.

Key words: Cryopreservation, sperm, fertility, injury, animal production.

RESUMO

O nicho consumidor de produtos da caprinocultura se encontra em plena ascensão. Apesar disso, tal atividade pecuária ainda é realizada em sistema familiar e de forma desorganizada. Deste modo, a tecnificação do setor é necessária, inclusive pela introdução de biotécnicas da reprodução, como a criopreservação de sêmen e a inseminação artificial (IA). O uso do sêmen congelado possibilita ao produtor melhorar a qualidade genética de seu rebanho a um menor custo, ampliar o potencial do reprodutor sem limite de tempo ou espaço e facilitar o manejo das criações, quando em associação a IA. Como consequência, são elevadas a produtividade e lucratividade do setor, o que inclusive favorece a transição de um sistema pecuário de subsistência para o industrial. Contudo, a congelação do sêmen caprino e o seu uso ainda apresenta certas limitações, visto que os diluidores e procedimentos empregados para esta biotécnica submetem os gametas à inúmeras injúrias estruturais e funcionais, letais e subletais. Tais fatos se refletem nas taxas de fertilidades, as quais se mostram reduzidas após a IA com sêmen congelado caprino. Diante dessa perspectiva, foi objetivado com este trabalho de revisão expor os pontos positivos e negativos da biotécnica da congelação de sêmen, com ênfase para a espécie caprina.

Palavras-chave: Criopreservação, espermatozoides, fertilidade, injúrias, produção animal.

INTRODUCTION

Goat farming plays an important socioeconomic and cultural role in countless rural regions of the world (CASTEL *et al*., 2010; BETTENCOURT *et al*., 2015; RAWASH *et al*., 2018; BERIHULAY *et al*., 2019), such as the Brazilian Northeast (AQUINO *et al*., 2016). The State of Pernambuco is included in this scenario, with emphasis on the semi-arid region (MOREIRA and GUIMARÃES FILHO, 2011; AQUINO *et al*., 2016). This is a consequence of the fact that these ruminants have high productive performance, even in places which are inhospitable for the agricultural exploitation or breeding of other animal species. Added to this

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are the low food consumption and shorter reproductive cycle, in relation to bovines (CORREIA *et al*., 2001), which results in a quick economic return (LUO *et al*., 2019).

Due to their high rusticity and adaptability, low nutritional demand (ALJUMAAH *et al*., 2019; BERIHULAY *et al*., 2019), productive capacity of foods with high nutritional value (AZIZ, 2010; CASTEL *et al*., 2010; ALJUMAAH *et al*., 2019), and easy adaptation to intensive production systems, the number of goats has increased, massively, all around the world (ALEXANDER *et al*., 2010; AZIZ, 2010; CASTEL *et al*., 2010; ŽUJOVIĆ *et al*., 2011). With the rapid expansion of the world goat industry (MILLER e LU, 2019), animal selection programs have intensified; although still generally restricted to developed countries (AZIZ, 2010). This is the result of the activity having been carried out previously in a predominantly subsistence manner, in a disorganized and unstructured way in many places, including the Northeast of Brazil (NUNES, 2010; AQUINO *et al*., 2016).

Considering the above, the need and importance of implementing technological innovation methodologies are clear (TEIXEIRA *et al*., 2013; LUO *et al*., 2019), with emphasis on assisted reproduction, which plays a valuable and growing role in animal production and management (ALEXANDER *et al*., 2010; LUO *et al*., 2019). Reproduction biotechnics generate significant benefits for the livestock sector (JADOUN *et al*., 2012; RAWASH *et al*., 2018), supporting animal breeding programs and making it possible to increase the reproductive and, consequently, productive potential of the herds (NUNES, 2010; PARAMINO and IZQUIERDO, 2014). Among the biotechniques of reproduction used in goat farming, artificial insemination (AI) stands out, due to its simplicity, relatively low cost (BALDASSARRE e KARATZAS, 2004; NUNES, 2010), and high efficiency (LEBOEUF *et al*., 2000).

Through AI, male reproductive potential is maximized (NUNES, 2010; OLIVEIRA *et al*., 2013), especially when associated with the use of frozen semen (BALDASSARRE and KARATZAS, 2004). Sperm freezing enables the germplasm to be transported and stored indefinitely, extending the breeder lifespan and enabling the use of the material regardless of the breeding season (BALDASSARRE and KARATZAS, 2004). Despite the advantages, the technique can generate sperm injuries, with loss of structural and functional integrity (YIMER *et al*., 2014; YODMINGKWANA *et al*., 2016), which results in the impairment of fertility after AI (AGOSSOU and KOLUMAN, 2018). Thus, the aim of the current review is to report the positive and negative points of semen freezing biotechnology, with emphasis on the goat species.

DEVELOPMENT

Semen freezing

Cold is the most efficient promoter of the anabiosis state and the Russian veterinarian IVANOV was the first to evidence its action in semen preservation (MIES FILHO, 1982). However, research on semen freezing, of different species, only intensified after the discovery of glycerol as a cryoprotective agent (PESCH and BERGMANN, 2006; HEZAVEHEI *et al*., 2018). Through this advance, it became possible to store semen indefinitely and its successful use for AI (HOLT, 2000), since the glycerol better preserves the gamete fertility (HEZAVEHEI *et al*., 2018).

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Semen cryopreservation is a reproductive biotechnology of great importance (SALAMON and MAXWELL, 1995; BALDASSARRE and KARATZAS, 2004). In goat farming, it stands out for enabling advances in terms of genetic improvement and increased productivity of herds, when in association with other management practices (NUNES, 2010). By freezing semen, the genetic material of animals with high zootechnical value can be stored indefinitely, used on a large scale and easily commercialized, regardless of the breeding season (BALDASSARRE and KARATZAS, 2004; RAHMAN *et al*., 2008). Thus, the incorporation of this technique into the sexed semen and artificial insemination industry offers profound commercial advantages (JOHNSON, 2000; BALDASSARRE and KARATZAS, 2004; HOSSEPIAN de LIMA *et al*., 2015).

Despite the above, goat semen cryopreservation is a complex process and depends on the balance of numerous factors for success (GANGWAR *et al*., 2016). Particularly during freezing and thawing, the sperm cell is frequently compromised, with damage to its structural and functional integrity (YIMER *et al*., 2014; ANAND and YADAV, 2016; NARWADE *et al*., 2017). In addition, the gamete structure undergoes variations between species, with emphasis on the levels of cholesterol and saturation in the membranes, which determines its susceptibility to thermal shock (NUNES, 2010; OLIVEIRA *et al*., 2013). Therefore, cryopreservation protocols optimized for sperm from one species may not be compatible with those from another species (RAHMAN *et al*., 2008; CARDOSO *et al*., 2020).

In goats, the sperm cryopreservation process is a challenge (PURDY, 2006), requiring improvements that maximize the viability and fertility of the gametes after thawing (GANGWAR *et al*., 2016; YODMINGKWANA *et al*., 2016). To date, no significant results have been obtained with AI, using frozen semen of this species (LEBOEUF *et al*., 2000; AGOSSOU and KOLUMAN, 2018). Contributing to this situation, goat breeders present the enzyme phospholipase A in their seminal plasma, which is secreted by the bulbourethral glands, and interacts with the phospholipids and triglycerides, present in seminal extenders based on milk or egg yolk (LEBOEUF *et al*., 2000; OLIVEIRA *et al*., 2013).

From the lecithin and triglyceride hydrolysis, catalyzed by phospholipase A, harmful components to sperm are generated, which are represented by lysolecithin and toxic fatty acids, respectively (LEBOEUF *et al*., 2000). As a result, for goat semen cryopreservation in milk and egg yolk extenders it is necessary to remove the seminal plasma, by centrifugation, before semen dilution is performed (NUNES, 2010; OLIVEIRA *et al*., 2013). However, it is known that centrifugation can damage the gametes, increasing the production of reactive oxygen species (ROS) and the oxidative stress (AGARWAL *et al*., 2005; DARAMOLA, 2017).

Except for the need to remove the seminal plasma, the goat semen freezing process follows basic principles, common to other species. The semen should be diluted in an appropriate medium, which offers a favorable environment for its longevity and fertility (RAHEJA *et al*., 2018). Therefore, the seminal extender should provide a buffering, nutritional (MIES FILHO, 1982; PURDY, 2006), and protective role against cryoinjury (SALAMON e MAXWELL, 2000; PURDY, 2006). This is because the insults faced by the gametes during cryopreservation result from osmotic changes, pH fluctuations, energy depletion during metabolism, thermal shock and cryodamage, changes in membrane composition, and ROS generation (RAHEJA *et al*., 2018).

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Accordingly, the following need to be present in the seminal extender: carbohydrates as an energy source, non-penetrating cryoprotectants for sperm nutrition and protection throughout refrigeration, and penetrating cryoprotectants to protect the gametes during freezing. Moreover, buffers are essential to avoid sudden changes in pH and osmolarity (HAFEZ and HAFEZ, 2004; PURDY, 2006), as well as antibiotics to prevent damage from microbial contamination (MAZUROVA *et al*., 2015; GACZARZEWICZ *et al*., 2016; MOHAMED, 2017; AHMED *et al*., 2017). Special attention has also been directed to the importance of the presence of antioxidants in these media (SILVA *et al*., 2019), since the damage caused to cells during the cold storage is largely attributed to oxidative stress (CASTRO *et al*., 2016; PERIS-FRAU *et al*., 2020).

The extenders commonly used for freezing goat sperm are based on milk or egg yolk, which consist of non-penetrating cryoprotectants (GARCÍA *et al*., 2017; SIEME *et al*., 2015). However, these methods can be harmful to the gametes (GANGWAR *et al*., 2016), a fact that results from the variability in biological constituents, the presence of detrimental agents to sperm (AURICH *et al*. 2007), and potential health risk (GIL *et al*., 2000; REHMAN *et al*., 2013; SWELUM *et al*., 2019). Added to this is the need to remove seminal plasma for the caprine species, due to Phospholipase A (OLIVEIRA *et al*., 2013), as previously described.

Studies have been developed to establish formulations free of animal products for sperm cryopreservation (GARCÍA *et al*., 2017). Among these, seminal extenders based on coconut water (ACP-101®) (OLIVEIRA *et al*., 2011; DARAMOLA *et al*., 2017) and soy lecithin (VIDAL *et al*., 2013; FATHI *et al*., 2019) have been used for goat sperm cryopreservation. These can better ensure biosecurity and lower the risk of disease transmission across borders (REHMANA *et al*., 2013; ANZAR *et al*., 2019), which meets international regulations for semen import and export (ANZAR *et al*., 2019), making possible the unrestricted commercialization of genetic material (MANJUNATH, 2012). Moreover, alternative formulations may eliminate the need for centrifugation to remove the goat seminal plasma.

For the semen freezing, it is essential to have a penetrating cryoprotectant in the extender, and glycerol is used universally for this purpose (HAFEZ and HAFEZ, 2004; SIEME *et al*., 2015). Penetrating cryoprotectants are characterized by their low molecular weight and consequent permeability to cell membranes, acting intra and extracellularly to preserve sperm survival and fertility during freezing (PURDY, 2006; PRIEN and IACOVIDES, 2016). The protective effect of these agents is the result of the colligative properties, which determine the eutectic point reduction, cellular dehydration, and subsequent reduced formation of intracellular ice (ÁVILA-PORTILLO *et al*., 2006). However, they may have a toxic effect on gametes and, should be used with caution (SALAMON and MAXWELL, 2000; SILVA *et al*., 2012).

The properly processed and diluted sperm are subjected to refrigeration and freezing curves, which should be performed gradually to avoid cellular damage (MIES FILHO, 1982; DOLEŽALOVÁ *et al*., 2016). Fast curves are responsible for injuries, arising from changes in the physical properties of the cell membranes (OLIVEIRA *et al*., 2013), due to cold shock, the formation of ice crystals (PESCH and BERGMAN, 2006) and osmotic imbalance evidenced during thawing (MORRIS *et al*., 2012). On the other hand, very slow curves are also harmful, because they subject the spermatozoa to the solution effect, characterized by excessive cellular dehydration and consequent increase in the solute concentrations in the intracellular medium

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(PESCH and BERGMAN, 2006; OLIVEIRA, 2013). Thus, an ideal cooling rate should avoid the adverse effects of these two extremes (HOLT, 2000).

The thawing process is the step that precedes the use of the cryopreserved semen. In this event, intracellular water and cell volume are restored (HOLT *et al*., 1992), representing a critical point, during which remarkable sperm damage can be generated (AL-BADRY, 2012). Much of this arises from osmotic imbalance, as the cells are exposed to a hyperosmotic environment (SIEME *et al*., 2015). In addition, during thawing the gamete presents high oxygen consumption, which results in increased ROS production and oxidative damage (GUERRA *et al*., 2004). Therefore, the adopted thawing protocol is a decisive factor to conserve the sperm viability (AL-BADRY, 2012).

Based on the above, it is clear that, despite its importance for the animal production industry (SALAMON and MAXWELL, 1995; BALDASSARRE and KARATZAS, 2004), the cryopreservation process, as a whole, represents an atypical and stressful event for sperm (STORNELLI *et al*., 2005). This is evidenced by the ultrastructural, biochemical, and functional damage generated (MORRIS *et al*., 2012; YODMINGKWANA *et al*., 2016; ANAND and YADAV, 2016; NARWADE *et al*., 2017). In this sense, injuries to the plasma membrane, acrosome (NARWADE *et al*., 2017), and mitochondrial potential (CÂMARA and GUERRA, 2008), and reduction in motility (YIMER *et al*., 2014; ANAND and YADAV, 2016; NARWADE *et al*., 2017), lipid peroxidation (YENI *et al*., 2010), induction of capacitation (MORTIMER and MAXWELL, 2004), and apoptosis (FERRUSOLA *et al*., 2010) are worth mentioning.

The dannification suffered by sperm subjected to the freeze-thaw process, is translated into unsatisfactory fertility rates after AI (LEBOEUF *et al*., 2000; AGOSSOU and KOLUMAN, 2018). Because of this, investigations are necessary to improve sperm cryopreservation biotechnology, with the objective of achieving fertility results close to those obtained with the use of fresh semen, after AI with frozen semen.

FINAL CONSIDERATIONS

Through the literary survey performed, it is noticeable that semen cryopreservation, with emphasis on freezing, is a biotechnology of paramount importance for animal production, including goat farming. This lowers breeding costs, avoiding the need to purchase and maintain breeders, and raises profits through genetic improvement using high quality germplasm, management facilitation, or even by selling semen. Despite this, the fertility rate after AI with frozen goat semen is lower than desired, emphasizing the need to ameliorate the sperm cryopreservation biotechnology.

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