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Original

# Effect of different concentrations of bovine serum albumin on the freezing of goat semen

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## ABSTRACT

**Objective**. Evaluate the effect of Bovine Serum Albumin (BSA) concentration on the freezability of goat semen in a Tris-based lyophilized preservative-diluter, without performing seminal washing, compared with a control preservative-diluter of lactose-skimmed milk (DC), removing the seminal plasma by centrifugation. **Materials and methods.** It will cover 90 ejaculates, volume, motility, concentration, viability, and sperm morphology. The fit ejaculates were mixed and the pool divided into five portions, each receiving one of 4 lyophilized combinations based on Tris-Glucose-Ac. Citrus and Glycerol with different concentrations of BSA (0.1%, 0.5%, 1% and 2%) or DC. It was frozen in 0.1 ml pellets in nitrogen vapors, and after 2 min., They were stored in liquid nitrogen until thawing 7 days later, the following were determined: motility percentage (30, 120 and 240 minutes), viability, damaged acrosomes and total anomalies (30 and 120 minutes) and were compared using a Binary Logistic Regression model. **Results**. The highest sperm motility and viability ($p<0.05$) in the three times was for 0.5%, 1% and 2% of BSA, which were higher than 0.1% of BSA and DC. Damages acrosome and total abnormalities at 30 and 120 minutes were lower ($p<0.05$) for 0.5%, 1% and 2% of BSA compared to 0.1% and DC. **Conclusions**. The cryopreservation of goat semen does not require seminal washing by centrifugation if 0.5-2% BSA is used as a membrane protector in a lyophilized dilute-conservative based on Tris-Glucose-Citric Acid and Glycerol.

**Keywords**: Seminal extender; semen preservation; seminal plasma (*Source: CAB*).

## RESUMEN

**Objetivo**. Evaluar en un diluyo-conservador liofilizado a base de Tris, el efecto de la concentración de Sero Albúmina Bovina (BSA) en la congelabilidad del semen caprino, sin realizar el lavado seminal, comparado con un diluyo-conservador control de lactosa-leche descremada (DC), eliminando el plasma seminal por centrifugación. **Materiales y métodos.** Se determinó en 90 eyaculados, volumen, motilidad, concentración, viabilidad y morfología espermática. Los eyaculados aptos,

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fueron mezclados y el pool dividido en cinco porciones, cada una recibió una de 4 combinaciones liofilizados a base de Tris-Glucosa-Ac. Cítrico y Glicerol, con diferentes concentraciones de BSA (0.1, 0.5%, 1 y 2%) o DC. Se congeló en pastillas de 0.1 ml en vapores de nitrógeno, y transcurridos 2 min., fueron almacenadas en nitrógeno líquido hasta su descongelación, 7 días después, se determinaron: porcentaje de motilidad (30, 120 y 240 minutos), viabilidad, acrosomas dañados y anomalías totales (30 y 120 minutos) y se compararon mediante un modelo de Regresión Logística Binaria. **Resultados.** La mayor motilidad espermática y viabilidad ($p<0.05$) en los tres tiempos fue para 0.5%, 1% y 2 % de BSA, que fueron superiores al 0.1% de BSA y DC. Los acrosomas dañados y las anomalías totales a los 30 y 120 minutos fueron menores ($p<0.05$) para 0.5%, 1% y 2 % de BSA en comparación con el 0.1% y DC. **Conclusiones.** La crioconservación del semen caprino, no requiere lavado seminal por centrifugación si se utiliza como protector de membrana BSA al 0.5-2% en un diluyo-conservador liofilizado a base de Tris-Glucosa-Ácido Cítrico y Glicerol.

**Palabras clave**: Diluyente de semen; preservación de semen; plasma seminal (*Fuente: CAB*).

## INTRODUCTION

Freezing makes it possible to preserve spermatozoa indefinitely; however, to achieve success in this process, it is necessary to know the particularities of the species whose ejaculates are to be cryopreserved (1).

Goat seminal plasma, unlike that of other species, has a high content of phospholipase A, an enzyme that reacts with egg yolk (YH) and skim milk (LD), elements required in the composition of the diluent-preservative as protectors membrane against cold shock (2), which occurs during the equilibrium period, when cooling from 37°C to 5°C, after dilution, and before freezing. This enzymatic reaction between phospholipase A and YH lecithin or LD triglycerides produces fatty acids and lysolecithins, which are toxic substances that affect sperm populations and reduce freezing capacity (3).

Various methods have been used to minimize or nullify phospholipase A's effect on goat semen; the conventional procedure has been the removal of seminal plasma by washing associated with centrifugation, which results in the loss of proteins, sugars, and lipids (4) necessary to maintain sperm's vital functions.

Some authors propose using low percentages of egg yolk in preservative diluents to minimize this effect (5), whereas others recommend preservative diluents that do not contain animal protein (6). Recent studies indicate that BSA protects the sperm membrane during cooling shock (7), and in other investigations, it has been used as a substitute for YH (8). The use of this protein as a membrane protector against cold shock in the composition of the preservative diluent could make it possible to eliminate the step of washing by centrifugation from the goat semen freezing process.

In this sense, the objective of this research was to evaluate, in a Tris-based lyophilized preservative diluent, the effect of BSA concentration on goat semen's freezability, without performing seminal washing, compared with a compound control preservative diluent by lactose-skimmed milk (DC), where the seminal plasma was removed by centrifugation.

## MATERIALS AND METHODS

**Study site**. This research was carried out in Havana, Cuba, at the Semen Technology Laboratory of the Research Center for Animal Improvement of Tropical Livestock (CIMAGT), located at 21º 31' 18.3'' N 77º 46.87' W, with an average temperature of 25 ºC and a relative humidity of 80%.

**Animals**. Two male goats of the Saanen breed, three of the Alpine breed, and six of the Boer breed that were 3 to 5 years old, housed individually, and fed in a controlled and stable manner, were used indistinctly as semen donors base of concentrates (1 kg/animal daily), fodder (5 kg/animal twice daily), mineral salts, and water ad libitum.

**Semen collection and evaluation**. A total of 90 ejaculates were processed in 12 replications. Semen collection was carried out using artificial vaginas twice a week, always in the presence of a female in heat who was tied to a trap in the mating room. Immediately afterward, the semen's volume (ml) was assessed using a graduated collector; the motility percentage was

assessed using microscopic evaluation at 40x magnification; the sperm concentration (× 106/ml) was assessed with counting in a Neubauer chamber; the percentage of live spermatozoa (viability) was assessed using eosin–nigrosin staining; and the percentage of total sperm abnormalities (morphology) was assessed using a phase-contrast microscope. The ejaculates classified as suitable for processing (volume ≥ 0.5 ml; motility ≥ 80%; sperm concentration ≥ 3,000 × 106/ml; viability ≥ 80%, and total sperm abnormalities ≤ 20%) were mixed, and the pool was divided into five portions.

**Dilute-preservatives**. A dilute-conservative composed of Tris (4.4 g), citric acid (2.3 g), and glucose (0.74 g) in 100 ml of bidistilled water with four levels of BSA (D1:0.1%; D2:0.5%; D3: 1%; and D4:2%) and the dilute-conservative control (DC) composed of lactose (4 g) and skim milk (10 g) in 100 ml of bidistilled water were used in all the combinations, penicillin with streptomycin (1000 IU/ml and 1 mg/ml, respectively) was used as antimicrobial agent, and the five formulations were lyophilized. Prior to the semen's dilution, each lyophilized medium's volume was restored with bidistilled water at 37°C, and the cryoprotectant—dimethyl sulfoxide (DMSO) at 0.2% (v/v) and glycerol at 6% (v /v) for the control and glycerol at 6% (v/v) for the rest of the combinations—was added.

**Semen dilution**. Each portion of ejaculate from the pool received the corresponding dilution-conservative according to the calculations made for the dilution and the freezing method to be used (i.e., whether it would be washed by centrifugation). When the lyophilized diluent-conservative based on Tris–glucose–citric acid and BSA in its four concentrations was used, the semen was diluted immediately after collection. This dilution occurred in one step, at a temperature of 37°C, according to the dilution calculations, without performing seminal washing. When the control lyophilized dilute-conservative (DC) was used, after the ejaculate's evaluation, the seminal plasma was removed by washing by centrifugation (2500 rpm for 15 minutes) for which the semen was diluted in ODT solution at a volume ratio of 1:9 (v/v; semen:ODT). Subsequently, the supernatant was completely discarded using a Pasteur pipette, and the dilution-preservative was added at 37°C in a single step, according to the calculations made for the dilution. The sperm concentration in all samples was 1.5 × 109/ml. After dilution, all samples were microscopically evaluated for motility before being subjected to the equilibration period.

**Balance period.** Immediately after dilution, each sample was placed in a container with water at 37°C and transferred to a refrigerator at 5°C for 2 hours to achieve a gradual decrease in temperature.

**Freezing of semen**. The semen was frozen in 0.1-ml pellets in nitrogen vapor (−75°C), by dripping it onto an acrylic plate located 4 cm from its surface. After 2 minutes, the frozen semen pellets were placed in vials previously identified plastics (stallion code, freezing date, species, freezing medium, and number of doses) and deposited in a thermos with liquid nitrogen (−196°C), where they remained until thawing.

**Thawing**. A water bath with temperature control was used. A tablet of each frozen sample was placed in a test tube with 1 ml of freezing solution (1.94% sodium citrate and 0.66% potassium bromide) at 37°C and stirred until it defrosted.

**Evaluation of thawed semen (incubation test)**. After thawing, the samples remained in a bain-marie at 37°C, where the percentage of motility was determined at 30, 120, and 240 minutes, as well as the percentage of viability (alive), the percentage of damaged acrosomes (broken or nonexistent), the percentage of abnormalities, and total sperm (at 30 and 120 minutes). Each sample was evaluated in duplicate by an experienced technician without prior knowledge of the samples' identification.

To determine post-thaw motility, 5 µL of each thawed semen sample was taken and placed on a tempered slide (37°C), and a tempered coverslip was placed on the drop, which was observed under a microscope with a thermal stage at 100× magnification, and was rated on a scale of 0–100.

The viability of the spermatozoa was evaluated using the eosin–nigrosin staining method. Four hundred spermatozoa were counted under a microscope with a heatable stage at 1,000× magnification. Sperm showing partial or complete purple staining were considered nonviable. Only sperm showing strict exclusion of staining were counted as viable.

To determine the percentages of damaged acrosomes and total sperm abnormalities, 3 drops of glutaraldehyde solution (2.9% sodium citrate and 2 mL glutaraldehyde) and 1 drop of semen from each thawed semen sample were placed on a slide heated to 37°C, homogenized and finely extended on a degreased slide, and dried and observed under a phase contrast microscope, counting 400 spermatozoa (1000× magnification, in immersion oil).

**Statistical analysis**. The variables motility, viability (alive), acrosome abnormalities, and total abnormalities were analyzed using a binary logistic regression model (9), adjusting a model with two categorical predictors—the diluent and the elapsed time—and, as a response variable, the probability that the sperm present motility, viability (alive), damaged acrosomes, and total abnormalities.

The probability of occurrence of these events was determined using the following model:

$$P(answer) = \exp(Y') / (1 + \exp(Y'))$$

The answer was whether the semen demonstrated motility, viability (alive), or abnormalities.

Thus, to prove that there is motility, the model was as follows:

$$P(if) = \exp(Y') / (1 + \exp(Y'))$$

where Y' = -0.6941 + 0.0 (30 min) − 0.1554 (120 min) − 0.4232 (240 min) + 0.0797 (0.1% BSA) + 0.4374 (0.5% BSA) + 0.4783 (1% BSA) + 0.4725 (2% BSA) + 0.1251 (control)

To determine the probability of survival (alive), the model was as follows:

$$P(if) = \exp(Y') / (1 + \exp(Y'))$$

where Y' = −0.6384 + 0.0602 (0.1% BSA) + 0.3240 (0.5% BSA) + 0.3707 (1% BSA) + 0.3274 (2% BSA) + 0.0935 (control) + 0.0 (30 min) − 0.1801 (120 min).

To determine the probability of total anomalies, the model was as follows:

$$P(if) = \exp(Y') / (1 + \exp(Y'))$$

where Y' = −0.6268−0.1249 (0.1% BSA)−0.8338 (0.5% BSA)−0.8618 (1% BSA) −0.8237(2% BSA)−0.6418 (control)+ 0.000000 (30 min) + 0.2426 (60 min).

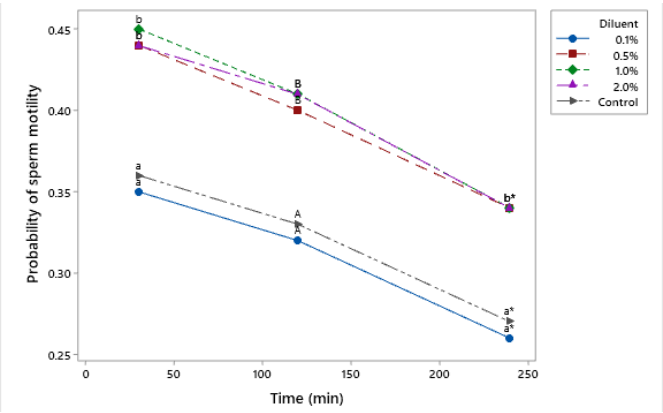
To establish the differences among the diluents and the times with respect to the predictions for each of the models, 95% confidence intervals were used.

The average percentages for each diluent concentration level at the various times tested were plotted, which added to the graph the regression trend that best fits the behavior of the response variable in each case.

**Compliance with ethical and animal welfare standards.** Articles 47–52 of Chapter VIII of Decree Law No. 31 on Animal Welfare of the Republic of Cuba were fully complied with.

## RESULTS

Figure 1 shows that the probability of sperm motility was higher ($p<0.05$) at all times for BSA concentrations of 0.5, 1.0, and 2.0%, than for the concentration of 0.1% BSA and the control. There was no significant difference ($p>0.05$) among the concentrations of BSA at 0.5, 1.0, and 2.0% at any of the three times. In the same way, the 0.1% concentration of BSA and the control demonstrated the same results ($p>0.05$) at all three times. In all treatments, the probability of sperm motility follows a negative linear trend in relation to the elapsed time.




Different letters within each time frame indicate a significant difference ($p < 0.05$).

**Figure 1.** Effect of BSA concentration and control on the probability of sperm motility during incubation.

BSA concentration's effect on the motility percentage at 30, 120, and 240 minutes of incubation is shown in figures 2, 3, and 4, respectively. These figures show that at all times, the BSA concentration with respect to motility percentage follows a quadratic trend. For times of 30, 120, and 240 minutes, the 0.1% BSA concentration demonstrated motility percentages of 34.2%, 32.5%, and 26.2%, respectively; the 0.5% BSA concentration demonstrated percentages of 40.8%, 39.2%, and 37.1%, respectively; the 1.0% BSA concentration demonstrated percentages of 41.7%, 40.0%, and 38.3%, respectively; and the 2.0% BSA concentration demonstrated percentages of 41.2%, 40.4%, and 37.9%, respectively. However, the motility percentages for the 0.5%, 1.0%, and 2.0% BSA concentrations were statistically similar ($p>0.05$) at all three incubation times.

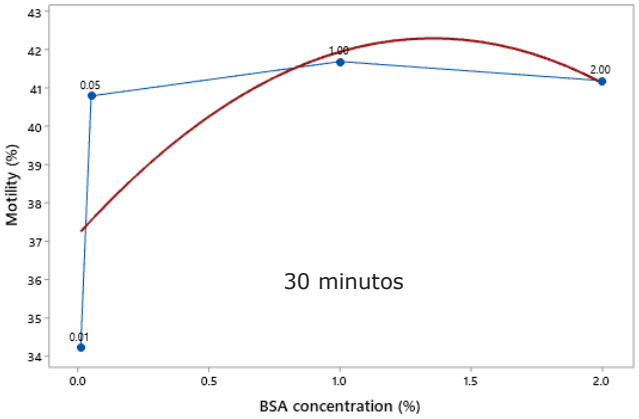



**Figure 2.** BSA concentration's effect on sperm motility percentage in frozen–thawed goat semen after 30 minutes of incubation

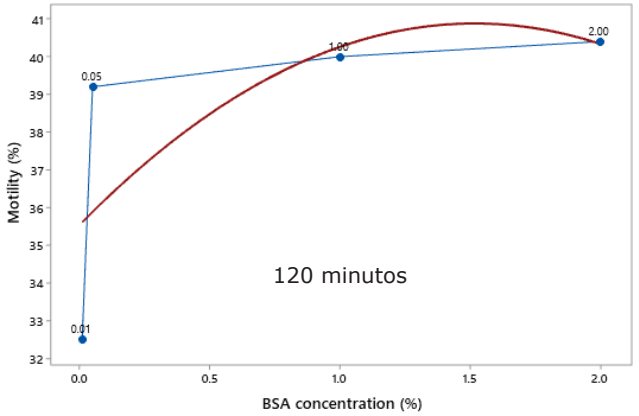



**Figure 3.** BSA concentration's effect on sperm motility percentage in frozen–thawed goat semen after 120 minutes of incubation.

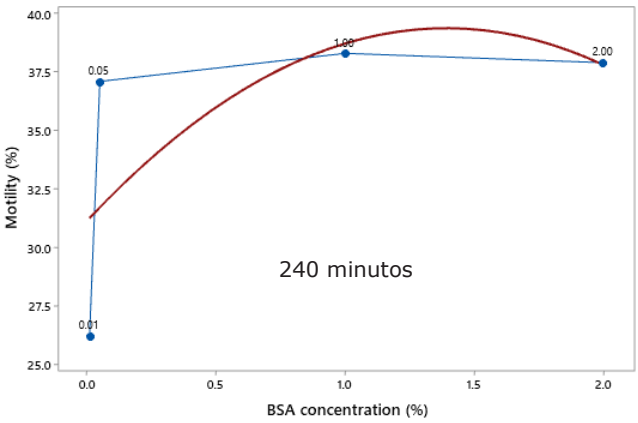
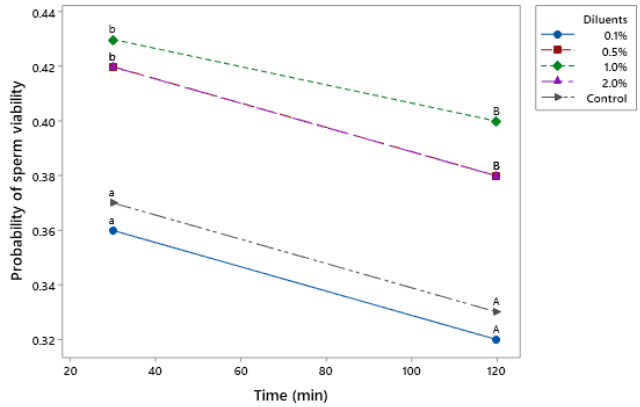



**Figure 4.** BSA concentration's effect on sperm motility percentage in frozen–thawed goat semen after 240 minutes of incubation.

The probability of post-thaw sperm viability between the BSA concentrations and the control during the incubation times (30 and 120 minutes) is presented in figure 5. The sperm viability for the 0.5%, 1.0%, and 2.0% BSA concentrations were the same ($p>0.05$) within each time and were higher ($p<0.05$) than the viabilities observed for the 0.1% BSA concentration and the control, which were equal to each other ($p>0.05$) at each time. The probability of sperm viability for all treatments across the two incubation times followed a negative linear trend.




Different letters within each time indicate a significant difference ($p<0.05$).

**Figure 5.** Effect of BSA and DC concentrations on probability of sperm viability during incubation.

Figures 6 and 7 show BSA concentration's effect on the percentage of sperm viability during the incubation test at 30 and 120 minutes, respectively. The lowest BSA concentration (0.1%) registered the lowest percentage of motility (35.91%); however, by increasing the BSA concentration, the highest percentages of sperm viability were reached (41.33%, 42.25%, and 41.83% for D2, D3, and D4, respectively). This behavior was maintained at 120 minutes (31.91%, 38.75%, 40.08%, and 38.41% for D1, D2, D3, and D4, respectively).

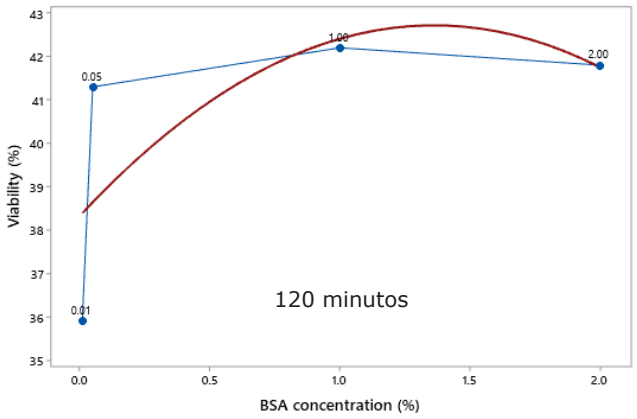



**Figure 6.** BSA concentration's effect on sperm viability percentage in frozen–thawed goat semen after 30 minutes of incubation.

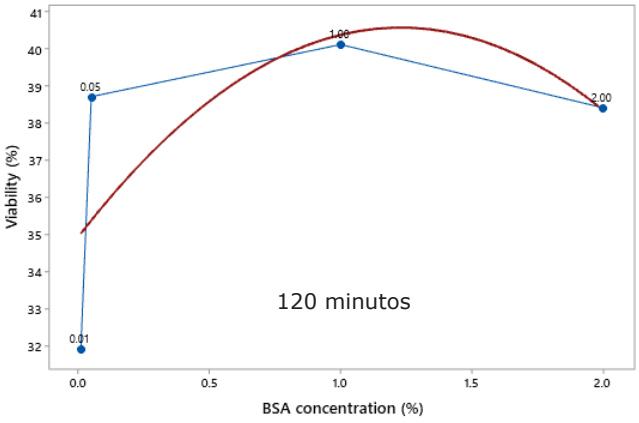
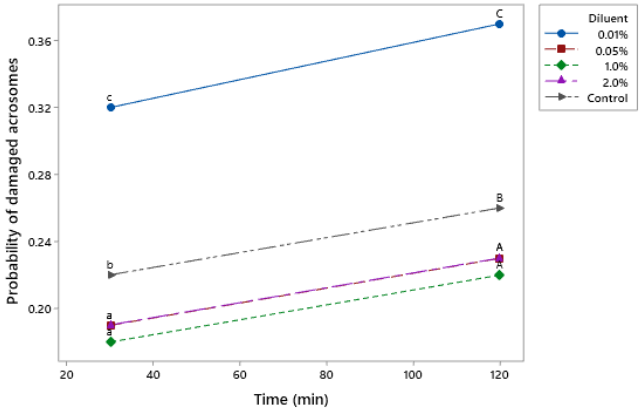



**Figure 7.** BSA concentration's effect on the sperm viability percentage in frozen–thawed goat semen after 120 minutes of incubation.

The probability of damaged acrosomes after thawing among the BSA-based diluent in its four different concentrations and the DC during the incubation test is presented in Figure 8. At 30 minutes, the lowest probability of damaged acrosomes corresponds to the BSA concentrations of 0.5%, 1.0%, and 2.0%. There was no difference among them ($p>0.05$). The control treatment had a higher probability of damaged acrosomes ($p<0.05$), but the probability was lower than that presented by the 0.1% BSA concentration. This effect of the BSA concentrations at 30 minutes was repeated at 120 minutes.




Different letters within each time indicate a significant difference ($p<0.05$).

**Figure 8.** BSA and DC concentrations' effects on the probability of damaged acrosomes during incubation

Figures 9 and 10 show the BSA concentrations' effects on the percentages of damaged acrosomes during the incubation test at 30 minutes and 120 minutes, respectively. At 30 minutes, D1 (0.1% BSA) presented the highest percentage of damaged acrosomes (32.42%), whereas the 0.5%, 1%, and 2.0% BSA concentrations demonstrated lower percentages of damaged acrosomes (19.05%, 18.92%, and 19.67%, respectively). This behavior continued at 120 minutes (37.17%, 22.17%, 21.83%, and 22.33% for 0.1%, 0.5%, 1.0%, and 2.0% BSA, respectively).

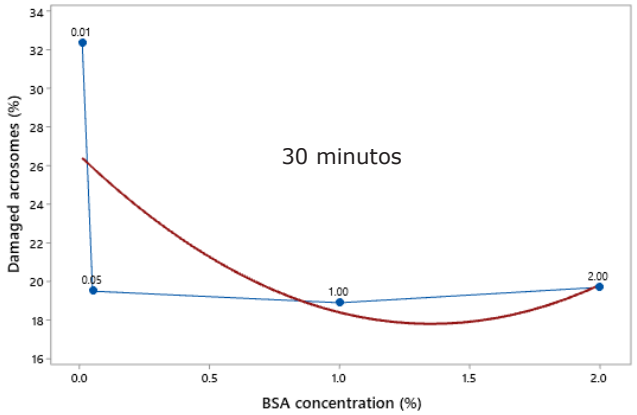



**Figure 9.** BSA concentration's effect on the percentage of damaged acrosomes in frozen–thawed goat semen after 30 minutes of incubation

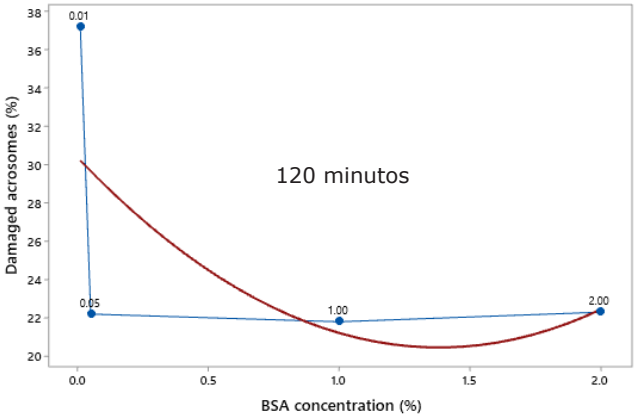
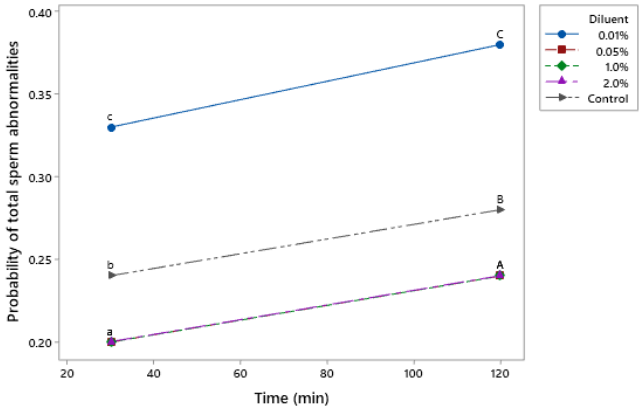



**Figure 10.** BSA concentration's effect on the percentage of damaged acrosomes in frozen–thawed goat semen after 120 minutes of incubation.

The probability of total post-thaw sperm abnormalities for the BSA-based extenders of four different concentrations and the DC during the incubation test is presented in Figure 11. At 30 minutes, the lowest probability of total sperm abnormalities corresponds to the BSA concentrations of 0.5%, 1.0%, and 2.0%, among which there was no difference ($p>0.05$). The control treatment had a higher probability of total anomalies ($p<0.05$), but it was lower than that presented by the 0.1% BSA concentration. This effect of BSA concentrations at 30 minutes was repeated at 120 minutes.




Different letters within each time indicate a significant difference ($p < 0.05$).

**Figure 11.** BSA and DC concentrations' effects on the probability of total sperm abnormalities during the incubation test.

Figures 12 and 13 show BSA concentration's effect on the percentage of total sperm abnormalities during the incubation test at 30 and 120 minutes, respectively. At 30 minutes, D1 (0.1% BSA) presented the highest percentage of total sperm abnormalities (33.33%), whereas BSA concentrations of 0.5%, 1%, and 2.0% demonstrated lower percentages of total anomalies (20.33%, 20.0%, and 20.83%, respectively). This behavior was maintained at 120 minutes (37.87%, 23.5%, 23.33%, and 23.92% for 0.1%, 0.5%, 1.0%, and 2.0% BSA, respectively).

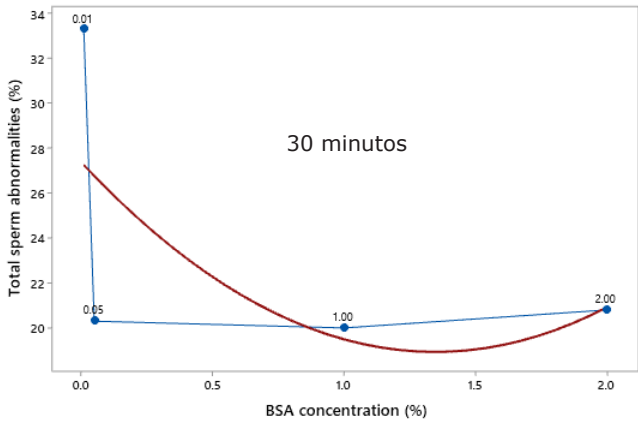



**Figure 12.** BSA concentration's effect on the percentage of total sperm abnormalities in frozen–thawed goat semen after 30 minutes of incubation.

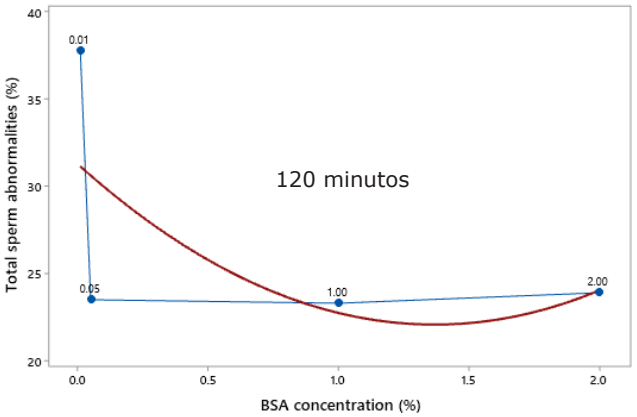



**Figure 13.** BSA concentration's effect on the percentage of total sperm abnormalities in frozen-thawed goat semen after 120 minutes of incubation

Observing the behavior of motility, viability, damaged acrosomes, and post-thaw total sperm abnormalities among the diluted-conservatives with BSA in four different concentrations—0.1% (D1), 0.5% (D2), 1% (D3), and 2% (D4)—we can confirm that the lowest probabilities ($p \leq 0.05$) of sperm motility and viability that coincide with the highest probabilities of damaged acrosomes and total anomalies ($p \leq 0.05$) are observed with D1, which corresponds to the lowest BSA concentration (0.1%). Apparently, this level is insufficient to protect the sperm membrane against cold shock, freezing, and thawing (10). However, as the concentration increased to 0.5%, 1%, and 2% (D2, D3, and D4, respectively), greater probabilities of motility and viability and a lower probability of damaged acrosomes and total anomalies were observed. These results were maintained throughout the incubation test.

The DC, composed of skimmed milk, lactose, DMSO, and glycerol, where seminal washing was performed by centrifugation, was statistically equal to D1, showing the lowest probabilities of sperm motility and viability as well as the highest probabilities of damaged acrosomes and total anomalies ($p<0.05$).

## DISCUSSION

Similar results to those found regarding BSA's effects on sperm motility and viability were reported by other authors (7), who have suggested the use of BSA as a component of the diluent-preservative because of its amino acid profile and protective functions. Like us, other authors have observed the best results for sperm motility, viability, and membrane integrity with a BSA concentration of 0.5%. These values decrease when lower concentrations (0.25%) are used. According to these authors (7), an indicator of BSA's protective effect against cold shock, freezing, and thawing is the analysis of sperm quality, determined according to sperm motility and viability, during the incubation test.

Other studies (9) using different concentrations of BSA (0%, 0.1%, 0.4%, 0.8%, 1.2%, and 1.6%) in the diluted-conservative have revealed that a 0.8% concentration is superior to the others used. These authors have suggested that very low BSA levels are insufficient and result in early depletion of the energy source available for sperm cell metabolism and that very high BSA levels tend to create an extreme hyperosmotic condition, which puts pressure on the spermatozoa membrane, possibly leading to fractures or ruptures and thus cell death.

Higher BSA concentrations have been used by other authors (11), who tested BSA concentrations of 5%, 7.5%, and 10% in the freezing of goat semen and observed the lowest results for sperm motility, to the extent that the concentrations were higher (35.0%, 30.8%, and 28.8%, respectively). A similar result was observed for membrane integrity (38.8%, 34.6%, and 33.4%), with significant differences among the three treatments and in both parameters.

The combination of DC penetrating and non-penetrating cryoprotectants is suggested to achieve better freezability; glycerol and DMSO are low-molecular-weight penetrating cryoprotectants that induce dehydration (12).

Lactose is a high-molecular-weight sugar that cannot diffuse through the plasma membrane. It induces cellular dehydration and, therefore, in both cases, reduces the probability of intracellular ice formation, favoring sperm survival of cryopreservation (3,13).

Skim milk acts as a membrane protector against cold shock, forms a protective film on the sperm surface, and replaces sperm membrane phospholipids that are lost or damaged during the cryopreservation process (14) while maintaining viability and sperm motility not only during the cold shock of the equilibrium period but also during freezing and thawing (15). However, seminal washing by centrifugation

reduces sperm motility and increases reactive oxygen species (ROS) and oxidative stress as well as post-thaw morphological defects (6,16,17,18).

Some authors agree with our criteria of not eliminating seminal plasma. Its presence is beneficial if it is associated with the appropriate component and in the correct proportion (19, 20), and it contains many antioxidant enzymes (superoxide dismutase and catalase), which regulate the overproduction of ROS during temperature drops and freezing (21,22). Additionally, through its antioxidant enzymes, it protects the structure of the sperm cell and prevents the increase of anomalies derived from the cryopreservation process (23).

In this experiment, the results demonstrate the superiority of a formulation containing Tris–glucose–citric acid–glycerol and BSA, where each element fulfills a specific function. They also show that the diluent-preservative's composition is important and determines the semen's quality after thawing. Tris is the most widely used buffer component in caprine semen freezing (17,24). Combined with citric acid, Tris provides the best buffer system for extenders of this species (25); elevated progressive motility (44%) and percentage of live spermatozoa (49%) when using this buffer compared to when using other BES, TES, and MES buffers have been observed (3).

Sugars play a fundamental role in sperm respiration and provide osmotic balance and cryoprotection in extenders. Glucose is an excellent substrate in goat semen's metabolism and is essential in providing energy for sperm to function physiologically. Furthermore, because of its low molecular weight, glucose can cross sperm cells' plasma membranes (1) and acts as a penetrating cryoprotector, minimizing the formation of intracellular ice and thus contributing to viability after cryopreservation (26,27).

Glycerol is the most widely used penetrating cryoprotectant in goats (28). When it enters a sperm cell, it binds to the water molecule, causes a gel state, and lowers the freezing point, which prevents intracellular ice formation and favors the rearrangement of membrane lipids and proteins—which increases their fluidity, favors dehydration at lower temperatures, and enhances their ability to survive cryopreservation (29).

BSA is a water-soluble low-density lipoprotein that acts as a non-penetrating cryoprotectant. It adheres to and interacts with sperms' membranes to produce its effects (11). BSA protects sperm cells not only against cold shock—by maintaining their selective permeability and functional integrity—but also prevents cracks and breaks that may occur during freezing and thawing (10, 30). It also contains large amounts of fatty acids, which, through glycolysis, are used to produce more, longer-lasting energy than that obtained from glucose. Therefore, an exogenous supply of BSA improves sperm's survival rate after thawing, and one of BSA's most important functions is the elimination of ROS that are produced as a result of oxidative stress (11).

In this study, all the dilute-preservatives that contain BSA in its four different concentrations have in common the presence of seminal plasma and their composition (Tris, citric acid, glucose, and glycerol); therefore, the differences in percentages of motility, viability, damaged acrosomes, and total abnormalities observed in the thawed semen are determined by the concentration of BSA used.

In conclusion, in the process of goat semen cryopreservation, it is unnecessary to carry out seminal washing by centrifugation if 0.5% BSA is used as a membrane protector in a lyophilized diluent-conservative based on Tris–glucose–citric acid and glycerol.

## Conflict of interest

The authors declare no conflict of interest.

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