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Evaluation of soybean lecithin-based extenders in canine semen cryopreservation

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ABSTRACT

This study aimed to evaluate the effectiveness of three concentrations of soybean lecithin-based extenders (SL) (0.5%, 0.1%, 0.05%) compared to native (homemade) egg yolk plasma (EYP) (40%) and a commercial egg yolk-based extender, 6% low-density lipoproteins (LDL) (CaniFreeze®, CF, IMV Technologies, L'Aigle, France) in the cryopreservation of canine spermatozoa. On the basis of the results of a preliminary study to optimize the preparation protocol for soybean lecithin-based media, these media underwent prolonged magnetic agitation at +4°C for up to 6 hours, followed by two cycles of microfiltration (0.22 µm). Twenty ejaculates, collected 48 hours apart from five healthy adult dogs with known fertility, were used in this study. Each ejaculate underwent an initial evaluation and was then divided among the five extenders studied (EYP 40%, CF, 0.5% SL, 0.1% SL, 0.05% SL). The diluted semen was frozen, thawed, and analyzed using the Hamilton Thorne HT-IVOS II CASA system and flow cytometer (Guava EasyCyte Plus, USA) to assess the kinetic characteristics and spermatozoa plasma membrane and acrosomal integrity. The data showed that the freezing medium containing 0.05% SL preserved post thaw total and progressive motility effectively, compared to the other SL-based extenders. However, the membrane and acrosomal integrity parameters remained lower compared to the control media. Therefore, SL-based extender can represent a good alternative to animal-derived membrane stabilizers if supplemented with other molecules acting synergically to protect canine spermatozoa functional integrity. SL offers the advantage of meeting biosafety requirements in the production and use of frozen canine semen. Nevertheless, further investigations are needed to optimize the formulation and to standardize the preparation process of this cryopreservation extender.

Key words: dog; spermatozoa; cryopreservation; soybean lecithin; extender

Introduction

Canine semen was successfully cryopreserved for the first time by SEAGER in 1969 (BENCHARIF and DORDAS-PERPINYA, 2020). This conservation process is of great interest in canine breeding programs. However, this process can result in considerable detrimental effects (BELALA et al., 2019). Cold-induced damage may

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sometimes be irreversible, affecting the spermatozoa's (spz) structural and functional integrity, and compromising the fertility of the frozen semen (ALÇAY et al., 2019). Cryopreservation extenders play a crucial role in protecting spermatozoa throughout this process. Their effectiveness depends on their composition, particularly the cryoprotectants (ŞENGÜL et al., 2024). Cryoprotectants can be categorized into agents that prevent ice crystal formation, i.e., cryoprotectants proper (primarily glycerol in dogs), and membrane stabilizers that protect membranes during cooling, traditionally represented by egg yolk (EY) in canine semen conservation (FARSTAD, 2009). Egg yolk is the premier protectant against spz cold shock, with its cryoprotective properties attributed to the low-density lipoproteins (LDL) it contains (MOUSSA et al., 2002). However, after prolonged use, various disadvantages emerge, including the risk of bacterial contamination due to its animal origin (DE LEEUW et al., 1993) and granules that inhibit sperm respiration (AMIRAT et al., 2004) and interfere with kinetic analysis techniques. For these reasons, replacing whole egg yolk has become necessary, leading researchers to explore several alternatives, such as LDL and native and freeze-dried egg yolk plasma (EYP), which are interesting sources of phospholipids, but unfortunately still of animal origin, presenting a potential biosafety risk. Soybean lecithin (SL) represents a plant-based source of phospholipids that perfectly meets the biosafety requirements in the formulation of canine semen cryopreservation media. Thus, several authors have investigated its benefits as a membrane stabilizer to replace EY in the composition of freezing extenders (SÁNCHEZ-CALABUIGA et al., 2017; DALMAZZO et al., 2019; ZAKOŠEK PIPAN et al., 2020). However, reported results seem inconsistent, requiring further research and exploration (HERMANSSON et al., 2021). Therefore, the objective of the present study was to evaluate the effectiveness of three concentrations of SL-based extenders (0.5%, 0.1%, and 0.05%) after two cycles of homogenization, compared to EYP-based media (EYP & CF) in the cryopreservation of canine spermatozoa.

Materials and methods

Study area and animals. This study was conducted at the level of the Biotechnologies Platform for Animal Medicine and Reproduction (BIOMERA), Saad Dahleb Blida University 1 (Blida, Algeria) during the period from November 25, 2022, to July 10, 2023.

Five dogs were sampled at 48-hour intervals, with four ejaculates per dog, resulting in a total of twenty ejaculates that met the inclusion criteria for the study (Table 1).

Table 1. List of dogs collected for the study

Name	Breed	Age (Year)
Zoli	Belgian Shepherd Malinois	7
Prince	Labrador	3
Floocky	Crossed	5
Chopper	Belgian Shepherd Malinois	6
Fidele	Belgian Shepherd Malinois	3

Semen collection and initial evaluation. In the current study, we collected the semen of each animal manually (Fig. 1), and fractionally on three conical tubes (pre-spermatic fraction, spermatic, and post-spermatic), according to the technique described previously (BELALA et al., 2019). The tubes were heated in the oven and maintained at a temperature of 37°C. All the fractions were evaluated, but only the sperm-rich fraction (the second) was used.

Fig. 1. Semen collection in dogs

Each ejaculate was initially analyzed under a conventional microscope (concentration, total and individual motility, and vitality) following collection, for its quality and suitability for freezing according to acceptance thresholds. Sperm concentration was assessed using a photometer calibrated for canine sperm (SDM Canine, Minitube, Germany) (Fig. 2) (no less than 200 million spermatozoa/ml, a total motility score of 3.5/5, 70% progressive spermatozoa, and 80% normal spermatozoa).

Fig. 2. Photometer SDM1, Minitube

Sperm analysis using CASA system. Computer analysis of the semen was carried out using a Hamilton Thorne IVOS II version 1.11.3 system (USA) belonging to the AMRBP, and a Léja® analysis slide (IMV Technologies, Aigle, France) with four chambers of 20µl depth (Fig. 3).

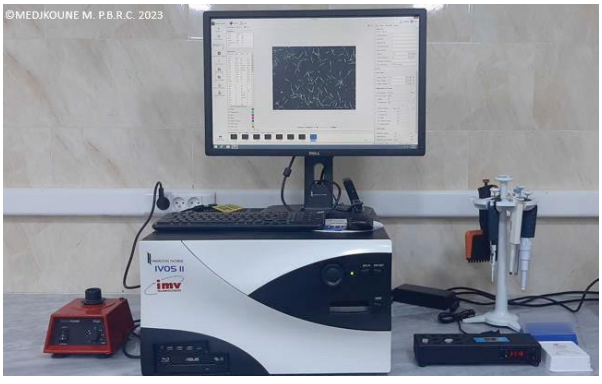


Fig. 3. Hamilton-Thorne IVOS II analyzer

The parameters generated by the HT IVOS II system utilized in our study were as follows:

Concentration (M/ml); Percentage of motile spermatozoa (MOT, %); Percentage of progressively motile spermatozoa (PROG, %); Kinetic Parameters: Curvilinear Velocity (VCL, µm/s), Amplitude of Lateral Head Displacement (ALH, µm), Straight Line Velocity (VSL, µm/s), Average Path Velocity (VAP, µm/s); Beat Cross Frequency (BCF, Hz); Straightness (STR, %); Linearity (LIN, %), as described previously (BELALA et al., 2024).

Flow cytometry (FCM) evaluation. Guava EasyCyte HT Plus cytometer (Millipore, USA) was used in our study to evaluate two sperm parameters: viability (cytoplasmic membrane integrity) coupled with acrosomal integrity. This assessment was performed using a specific rapid kit (EasyKit) designed to measure the percentage of living spermatozoa (with intact cytoplasmic membrane) and intact acrosome. After several steps of dilution and semen preparation, the sample was added to the fluorochromes, incubated at 37°C for 15 minutes, and then directly loaded and analyzed by the cytometer (Fig. 4). The kit enabled the evaluation of the percentage of living spz with intact acrosome, using the Fluorescein IsoThiocyanate-Pisum sativum Agglutinin (FITC/PNA/PI) (Viab-Acro) test.

Fig. 4. Guava EasyCyte HT Plus Cytometer (Millipore, USA)

Preparation of semen extenders

Extraction of egg yolk plasma. The fractionation of egg yolk into granules and plasma was performed using the technique described by MCBEE and COTTERILL (1979). It involved repeated centrifugation, following dilution with a saline solution in order to obtain a satisfactory degree of purity.

Soybean lecithin-based extenders. In this study, five cryopreservation media were used: one commercial cryopreservation medium based on EY,

6% low-density lipoproteins (LDL) (CaniFreeze®, CF, IMV Technologies, L'Aigle, France) and four media prepared from a common base (tris-buffered medium) supplemented with three decreasing concentrations of SL (0.5%, 0.1%, and 0.05%) and 40% native egg yolk plasma EYP.

The 6% LDL (CF) was used as a reference (control) extender because it has been previously shown to effectively preserve canine spermatozoa. The composition of CF and EYP is described previously (BELALA et al., 2016).

The standard protocol for preparing SL-based media, as widely reported in the literature, involves homogenization at room temperature for 15 minutes, followed by centrifugation at 2900×g for 15 minutes, and finally two filtrations at 11 µm and then 0.45 µm. A preliminary study was conducted to optimize the preparation protocol and improve lecithin solubilization. We compared several experimental protocols involving different homogenization durations at 4°C (15 minutes, 45 minutes, 90 minutes, 2 hours, 4 hours, 6 hours) combined with centrifugation at 2900×g for 10 minutes and double microfiltration at 0.22 µm. The effectiveness of the protocol in solubilizing lecithin was evaluated on the basis of microscopic appearance and the presence or absence of granules, the viscosity of the solution measured by capillary filling time (TR) in the analysis chamber (Leja® Slide, IMV Technologies, L'Aigle, France), and the spz kinetic parameters after dilution of fresh semen. On the basis of the results of this preliminary study, the optimal protocol selected for our further experimentation involved magnetic stirring at +4°C for 6 hours, followed by two cycles of microfiltration (0.22 µm). All freezing media were then aliquoted into equal-sized portions and stored at -20°C until use.

Cryopreservation steps

Semen dilution. Five cryotubes were placed in a water bath at 37°C and filled with 100 µl of each experimental diluent. From the sperm-rich fraction of each ejaculate (sample), a fixed volume of 100 µl was added to each of the five cryotubes (Survival Solution). On the basis of the concentration of the sample measured by spectrophotometry (Minitube SDM1), the volume of each diluent was adjusted to achieve a final concentration of 100 million spermatozoa/ml in each cryotube.

Equilibration. The five cryotubes were placed into 15 ml conical tubes containing water at the temperature of the water bath (37°C), and were then placed at +4°C for 1 hour to allow slow and gentle cooling of the diluted semen. After this equilibration period, the diluted semen was manually filled into pre-identified and pre-refrigerated 0.25 ml straws (IMV Technologies, L'Aigle, France). The latter were sealed with polyvinyl alcohol powder and placed horizontally on a pre-refrigerated metal rack maintained at +4°C for an additional 30 minutes.

Straw freezing. The straws were frozen using nitrogen vapor in a device consisting of a polystyrene box filled with liquid nitrogen. The straws were placed on a rack 4 cm above the liquid nitrogen level for 10 minutes. This conventional freezing technique, widely used, brings the temperature of the straws down to -140°C. The straws were then immediately immersed in nitrogen, which allows the temperature to drop from -140°C to -196°C. Subsequently, the straws were transferred to a storage container with controlled nitrogen levels for 24 to 48 hours.

Straw thawing. For thawing, the straws were directly immersed in a straw thawer set at 37°C for 30 seconds. Each group was analyzed 10 minutes afterward. Prior to analysis, the semen from each group was diluted to a quarter of its original concentration to achieve a final concentration of 25 million spermatozoa per ml in an EasyBuffer B® solution (IMV Technologies, L'Aigle, France). The semen analyses were performed at three different time points: after collection, after the equilibration period, and after thawing.

Ethical statement. All the animal studies were conducted with the utmost regard for animal welfare, and all animal rights issues were appropriately observed. No animal suffered during the course of the work. All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA. 14).

Statistical analysis. Statistical analysis of the raw data was carried out by IBM software - SPSS version 25, © 2017. After a descriptive analysis, a linear mixed effects model was used to analyze the effects of semen extender on each spz parameter. The results were presented as mean ± SD, and the significance level was set at 5%.

Results

Preliminary study data. The following Fig. (5) and Fig. (6) present the results of the preliminary study before and after double microfiltration. The first images show clearly the turbid microscopic appearance with abundant granules and high solution viscosity (FT=8s). The experimental protocol. including magnetic stirring at +4°C for 6 hours,

Time	15 min	45 min at 4°C	90 min at 4°C
FT	8s	8s	7s

Time	2H at 4°C	4H at 4°C	6H à 4°C
FT	8s	6s	4s

1. "Pure drops with agitation" microscopic images

Time	15 min	45 min at 4°C	90 min at 4°C
FT	6s	6s	5s

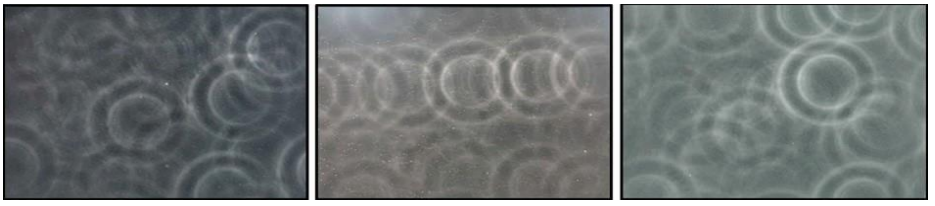
Temps	**2h à 4°C**	**4h à 4°C**	**6h à 4°C**
FT	**6s**	**4s**	**3s**

2. "15-10 min of centrifugation" microscopic images

Fig. 5. Preliminary study results before double microfiltration

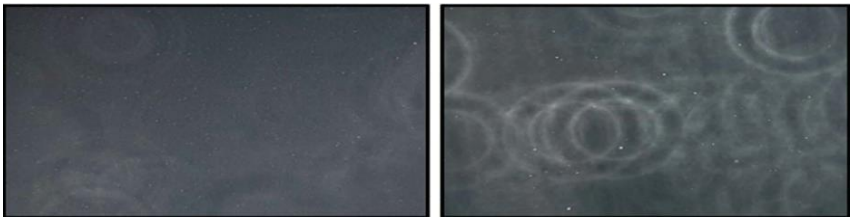
FT: filling time of the analysis chamber (Léja® Slide, IMV Technologies, L'Aigle, France); this time allows the viscosity of the medium to be assessed

Time	15 min	45 min at 4°C	90 min at 4°C
FT	6s	4s	5s

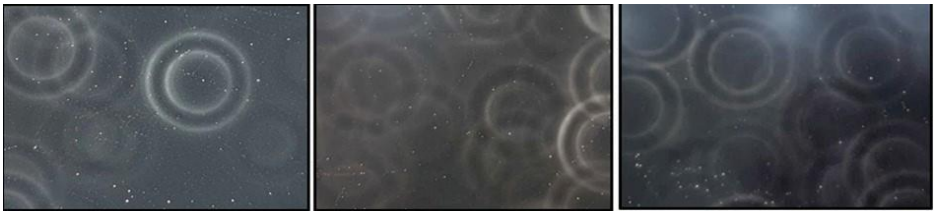


Time	2H at 4°C	4H at 4°C	6H at 4°C
FT	3s	4s	3s

3. "1st micrfiltration" microscopic images



Time	45 min at 4°C	90 min at 4°C
FT	3s	5s



Time	2H at 4°C	4H at 4°C	6H at 4°C
FT	4s	4s	3s

4. "2nd microfiltration" microscopic images

Fig. 6. Preliminary study results after double microfiltration

followed by two cycles of microfiltration at 0.22 µm, improved the microscopic appearance significantly (turbidity) with the complete disappearance of granules and a considerable reduction in solution viscosity (FT=3s vs. 8s).

Total and progressive motile spermatozoa rates. The post-thaw total motility (MOT) and progressive motility (PROG) of the canine spermatozoa are shown in Fig. 7.

According to our data, the average % of motile (MOT) and progressive motile (PROG) spz obtained with 0.05% SL medium were close to those obtained in the control extenders (EYP and CF), with 59% vs. 61.6% and 60.3% for motile spz, and 36.7% vs. 38% and 40.2% for progressive spz, respectively. In contrast, for the media with higher concentrations of SL (0.5% and 0.1%), the motility was lower compared to the control media

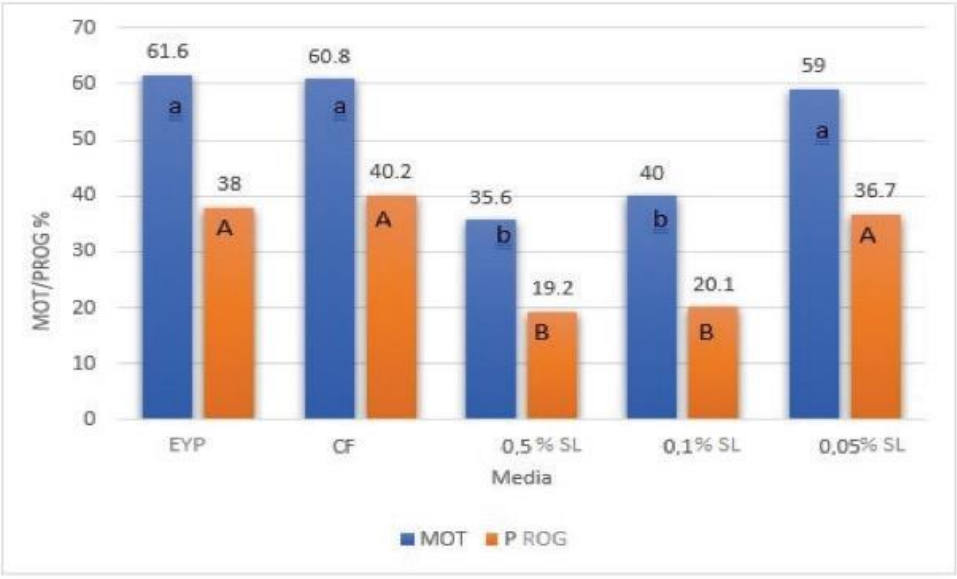


Fig. 7. Percentages of total and progressive motile spermatozoa

Different letters mean significant difference, P<0.05

(EYP and CF), with 35.6% and 40% vs. 61.6% and 60.3% for motile spz, and 19.2% and 20.1% vs. 38% and 40.2% for progressive spz, respectively.

Kinetic parameters. Sperm analysis using CASA revealed the average kinetic parameters in the five different media presented in Table 2. For VSL, VCL, VAP, LIN, STR, values were lower (P<0.05) for the 0.5%, 0.1%, and 0.05% SL extenders than for the control media (EYP and CF). The lowest rates for these parameters were obtained with the highest dose of SL, except for VAP and for another kinetic parameter (BCF), 0.05% SL presented decreased values. For ALH, the three concentrations of SL-based media and the control extenders showed close values (P>0.05).

Structural and functional integrity of spermatozoa. Fig. 8 shows the detailed results of plasma membrane functional integrity and acrosome integrity.

The mean percentages of spermatozoa with intact cytoplasmic membrane for each of the five studied media were: 69.1%, 65.3%, 50.9%, 54%, and 57.2%, respectively for EYP, CF, 0.5% SL, 0.1% SL, and 0.05% SL. The rates for acrosome integrity were: 67.1%, 64.2%, 52.6%, 49.7%, and 54.1%, respectively for EYP, CF, 0.5% SL, 0.1% SL, and 0.05% SL.

Table 2. Average spermatozoa velocities and linearities in the tested media

	VCL (µm/s)	VSL (µm/s)	VAP (µm/s)	STR (%)	LIN (%)	ALH (µm)	BCF (Hz)
EYP	156.3^{a}	92.26^{a}	103.2^{a}	89.3^{a}	59^{a}	6.5^{a}	26.6^{a}
CF	160.8^{a}	94.1^{a}	114.2^{a}	82.4^{a}	58.5^{a}	7.7^{a}	30.25^{a}
0.5% SL	115.2^{b}	36.2^{b}	80.2^{b}	45.1^{b}	31.4^{b}	5.1^{a}	24.6^{a}
0.1% SL	118.4^{b}	39.3^{b}	85.1^{b}	46.1^{b}	33.2^{b}	5.9^{a}	25.4^{a}
0.05% SL	125.6^{b}	40.1^{b}	58.2^{b}	68.9^{b}	31.9^{b}	6.9^{a}	18.9^{b}

Different letters in the same column mean significant difference, P<0.05

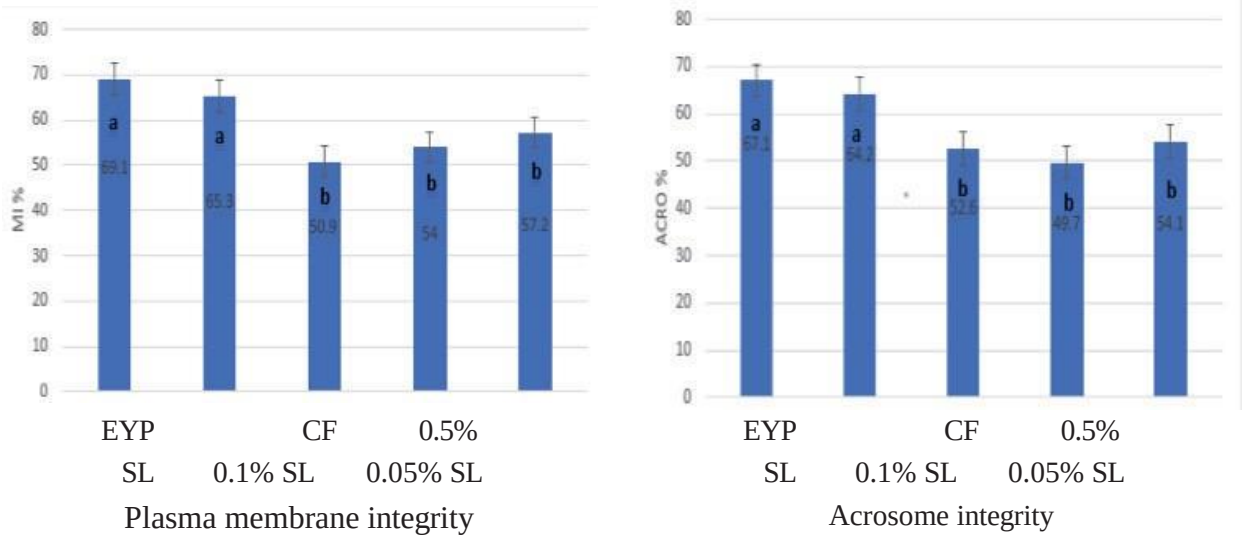


Fig. 8. Plasma membrane and acrosome integrity of canine spz in the different media

Different letters mean significant difference, P<0.05

Discussion

This study aimed to propose a plant-based extender for EY replacement, represented by SL, which contains a significant fraction of low-density lipoproteins (LDL) and could thus be an interesting alternative to animal-derived lecithin. In dogs, AXNÉR and LAGERSON (2016) tested different concentrations of lecithin for sperm cryopreservation; however, the doses used in the experiment were too high and therefore did not bring any significant improvement in sperm quality. In this context, our work focused on the use of diluents containing low rates of SL (0.5%, 0.1%, 0.05%), with the optimization of the medium preparation process through homogenization by magnetic stirring at +4°C for 6 hours, followed by two cycles of microfiltration (0.22 µm). These media were compared to two egg yolk-based extenders, one prepared and the other commercial (6% LDL, CaniFreeze®).

Several researchers have described extensively the standard protocol for preparing SL-based media. This protocol involves homogenization at room temperature for 15 minutes, followed by centrifugation at 2900xg for 15 minutes, and finally, two filtration steps at 11 µm and 0.45 µm (BECCAGLIA et al., 2009; DE PAZ et al., 2010; KMENTA et al., 2011; SALMANI et al., 2014; AXNÉR and LAGERSON, 2016). However, most of these studies revealed unsatisfactory results in terms of total and progressive motility, which they primarily attributed to viscosity and the difficulty in solubilizing the lecithin. These issues were directly related to the protocol used for preparing the media.

This was evident in our preliminary study in the turbid microscopic appearance with abundant granules and high solution viscosity. The optimal protocol that we retained for the subsequent experiments involved magnetic stirring at +4°C for 6 hours, followed by two cycles of microfiltration at 0.22 µm. This protocol significantly improved the microscopic appearance (turbidity), with the complete disappearance of granules, and a considerable reduction in solution viscosity compared to the usual protocol. Moreover, this new protocol simplified the preparation of the media and eliminated the need for centrifugation, which is equipment-intensive. The centrifugation was effectively replaced by extending the homogenization time at +4°C, followed by double filtration with a simple 0.22 µm syringe filter. This microfiltration offers the advantage of allowing cryoprotective particles, which do not exceed 20-70 nm in size, to pass through, while sterilizing the medium by preventing the passage

of bacteria, which are larger than the filter pores at 220 nm (DIVAR et al., 2023).

The mean percentages of MOT and PROG in the 0.05% SL medium were close to those obtained in the two EY-based control media (EYP and CF). In contrast, for the media with increased concentrations of SL (0.5% and 0.1%), the motility was lower compared to the control media. Our data are higher compared to those previously reported (DALMAZZO et al., 2019). Moreover, they differ significantly from the percentages observed in the 0.5% and 0.1% SL media. This could be explained by the better dispersibility of SL in the media due to the optimization of the preparation techniques (AXNÉR and LAGERSON, 2016; DALMAZZO et al., 2018; DALMAZZO et al., 2019; ZAKOŠEK PIPAN et al., 2020), primarily through homogenization at +4°C for 6 hours and double filtration of the solutions with 0.22 µm microfilters. This protocol differs from what was previously performed by using a single short homogenization step and two filtrations with 11 µm and 0.45 µm filters (HERMANSSON et al., 2021). Therefore, the previous low rates were explained by poor SL dispersibility and the presence of a significant number of granules in the diluent, which hinders spermatozoa movement and promotes collision phenomena (BELALA et al., 2019).

In the current study, the use of SL-based extenders significantly reduced the motion activities of canine sperm, as indicated by lower values of VCL, VSL, ALH, STR, LIN when high doses of SL were administered (except for VAP and BCF, for these parameters, the lowest rates were obtained with a reduced dose of SL). This could be due to the texture of the SL extender, which has higher viscosity than the traditional EY medium, as previously reported (PEARODWONG et al., 2019).

Sperm velocity-related parameters are an indirect indicator of mitochondrial function integrity (ŞENGÜL et al., 2024). Comparing the values of media based on 0.1%, 0.5%, and 0.05% SL with the two EY-based media (EYP and CF), revealed that the 0.5% and 0.1% SL media presented lower mean percentages of spz with intact cytoplasmic membrane and acrosome integrity compared to the control diluents. However, 0.05% SL medium showed the closest mean rates to these two-control extenders. It is well known that sperm membrane functional integrity and motility are correlated (ÖNDER et al., 2023). Our findings suggest that the integrity of the plasma membrane and acrosome was better preserved in the 0.05% SL, which is consistent with the motility results, in comparison with the two other media with increased SL levels.

The results obtained in the SL dilution media at 0.05% were similar to those obtained using native egg yolk plasma or the commercial solution (CaniFreeze®), in agreement with the data reported previously (BECCAGLIA et al., 2009; KMENTA et al., 2011; AXNÉR and LAGERSON, 2016).

Despite the significant difference compared to the two-control media (EYP and CF), the values of the spz with intact plasma membrane and acrosome integrity remain acceptable (above 50%) in the media containing 0.05% SL. We can add that the improved dispersibility of SL in the medium may have enhanced motility results, but this was insufficient to ensure the protection of the plasma membrane and acrosome integrity. This could be due to ineffective and inactive interaction between the membranes and the lecithin for phospholipid exchange (SALMANI et al., 2014; ZAKOŠEK PIPAN et al., 2020). The SL formulation used is based exclusively on a single type of phospholipid, phosphatidylcholines (TOKER and ALCAY, 2022). In contrast, egg yolk, considered a reference substance, contains at least three different fractions of phospholipids in addition to cholesterol molecules. Therefore, the total replacement of egg yolk with other ingredients might diminish the protective effects of cholesterol (PEARODWONG et al., 2019). This difference could limit the use of lecithin as a membrane stabilizer, highlighting the need for further research to optimize the formulation of this cryopreservation medium by enriching it with other phospholipids and membrane-stabilizing molecules.

Conclusions

The evaluation of SL-based extenders (0.5%, 0.1%, 0.05%) compared to EY-based media in preserving post-thaw canine spz enabled us to conclude

that the 0.05% SL effectively preserved the total and progressive motility of frozen-thawed spz. However, this medium did not protect the plasma membrane and acrosome integrity as effectively as the control media. SL thus can represent a good substitute to animal-derived membrane stabilizers if enriched with some other beneficial molecules acting synergically, and offers the advantage of meeting biosafety requirements in the handling, freezing, shipping, and use of frozen canine semen. Moreover, the optimal protocol for preparing SL-based extenders was found to be magnetic agitation at +4°C for 6 hours followed by two cycles of microfiltration. This protocol simplified the preparation and eliminated the need for centrifugation. The microfiltration also has the advantage of allowing cryoprotective particles to pass through, while sterilizing the medium by blocking bacteria. However, further studies are necessary to optimize the formulation and to standardize the preparation process of the cryopreservation medium and to evaluate its in vivo fertility.

Ethical statement

All the animal studies were conducted with the utmost regard for animal welfare, and all animal rights issues were appropriately observed. No animal suffered during the course of the work. All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA. 14).

Declaration of competing interest

No potential conflicting interest was reported by the authors

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SAŽETAK

Cilj je istraživanja bio procijeniti učinkovitost ekstendera na bazi sojina lecitina (SL) u krioprezervaciji psećeg sjemena. Uspoređeni su ekstender na bazi sojina lecitina u tri koncentracije (0,5%, 0,1%, 0,05%) s 40% nativne plazme žumanjka jajeta (EYP) i ekstender s komercijalnim medijem za krioprezervaciju na bazi žumanjka jajeta (EY) s 6% lipoproteina niske gustoće (LDL) (CaniFreeze®, CF, IMV Technologies, Aigle, France). Na temelju rezultata preliminarnog istraživanja, mediji su podvrgnuti produljenom miješanju uz pomoć magnetske mješalice na temperaturi +4°C tijekom 6 sati, nakon čega su uslijedila dva ciklusa mikrofiltracije (0,22 μm). Od 5 zdravih odraslih mužjaka, poznate plodnosti, tijekom 48 sati prikupljeno je 20 uzoraka ejakulata. Svaki je ejakulat podvrgnut inicijalnoj procjeni te je podijeljen u pet ekstendera (EYP 40%, CF, 0,5% SL, 0,1% SL, 0,05% SL). Razrijeđeno je sjeme zamrznuto, odmrznuto i analizirano primjenom sustava HT IVOS II CASA i protočnog citometra (Guava EasyCyte Plus) kako bi se procijenile kinetičke značajke plazmatske membrane i akrosoma spermatozoida. Podaci su pokazali da je medij za zamrzavanje koji je sadržavao 0,05 % sojina lecitina učinkovito očuvao ukupnu i progresivnu pokretljivost spermija nakon odmrzavanja u usporedbi s drugim ekstenderima na bazi sojina lecitina. No pokazatelji cjelovitosti membrane i akrosoma ostali su niži u usporedbi s kontrolnim medijima. Zaključeno je da ekstender na bazi sojina lecitina može biti dobra alternativa stabilizatorima membrane životinjskog podrijetla ako je nadopunjen drugim molekulama koje djeluju sinergijski kako bi sačuvale funkcionalni integritet spermatozoida. Prednost je sojina lecitina to što ispunjava zahtjeve biosigurnosti u proizvodnji i upotrebi zamrznutog psećeg sjemena. Ipak, potrebna su daljnja istraživanja kako bi se optimizirala formulacija i standardizirao postupak pripreme ovog ekstendera za krioprezervaciju.

Ključne riječi: pas; spermatozoidi; krioprezervacija; sojin lecitin; ekstender