The optimum concentration of soya-lecithin for replacing egg yolk in INRA-82-modified semen extenders improved the cryopreserved semen quality and DNA integrity of Arabian stallions


Abstract The cryopreservation of stallion spermatozoa varies greatly between animals. Different equine semen extender supplements were invented to maximize the post-thaw semen quality. Soy lecithin is one of those additives that contain plant origin phospholipids used to replace egg yolk to minimize disease transmission. This study aimed to determine which concentrations of soy lecithin can replace egg-yolk in modified INRA-82 semen extenders for the cryopreservation of horse semen. Semen was collected from six Arabian stallions and extended with modified INRA-82 semen extender supplemented with 0.0, 1.25, 2.50, and 5.0% soya lecithin. Frozen-thawed sperm motility was measured at 0, 1, 2, and 3 h. Viability index, sperm plasma membrane integrity (SPMI), and acrosome integrity in addition to the comet assay non-fragmented DNA, head DNA, tail DNA, tail length, and tail moment were statistically analyzed. The addition of 2.5% soya lecithin had the highest post-thaw sperm motility at 0, 1, 2 h (P < 0.001) and 3 h (P < 0.0001), the optimum viability index (P < 0.0001), SPMI (P < 0.05), acrosome integrity (P < 0.0001), non-fragmented DNA (P < 0.01), DNA in the comet head (P < 0.05), minimum DNA in the comet tail (P < 0.05), shortest comet tail length (P < 0.0001), and lowest comet tail moment (P < 0.01). Soy lecithin at concentrations other than 2.5% deteriorated all post-thaw semen parameters. Soy lecithin (2.5%) can replace egg yolk in the modified INRA-82 semen extender for the cryopreservation of good-quality stallion semen.

Keywords: modified INRA-82, semen cryopreservation, post-thaw DNA, soy lecithin, stallion

1. Introduction

Soy bean-based semen extenders have become commercial semen extenders and have been used for the chilling and cryopreservation of buffalo semen (Akhter et al 2011; Singh et al 2012) and for the cryopreservation of dairy bull semen (Layek et al 2016; Miguel-Jimenez et al 2020). In rams, soybean lecithin-based extenders preserved frozen semen when glycerol was replaced with ethylene glycol or dimethyl sulfoxide (Najafi et al 2017) and when it was supplemented with green tea extract to increase its antioxidant capacity to improve the post-thawing semen quality (Mehdipour et al 2016). In bulls, soya-lecithin semen extenders were used to replace egg yolk in Tris-glycerol semen extenders as a source of plant lipoproteins (Ondřej et al 2019) and to prevent microbiological contamination by bacteria or mycoplasma contamination by using egg yolk for its lipoprotein content (Bousseau et al 1998; Hinsch et al 1997). Compared to Tris-based extenders supplemented with equal quantities of egg yolk and different concentrations of glycerol, commercial soya-lecithin-based extenders did not significantly reduce the non-return rate or conception rate of Holsteins (van Wagendonk-de Leeuw et al 2000) but improved post-thaw motility and field fertility with no influence on live acrosome-reacted sperm in buffaloes (Akhter et al 2012). Compared to the tris-egg yolk extender, the chilling of bull semen for 18 h before freezing in two commercial soy-based extenders resulted in poor semen quality (Muiño et al 2008). Soya milk-tris extender improved conception regardless of the declined post-thaw sperm motility (Arifiantini and Yusuf 2010).

The optimum concentration of soy lecithin as a source of plant lipoproteins in semen extenders to replace egg yolk was investigated in different animals. In rabbits, 1.5% soybean lecithin improved post-thaw semen motility compared to 0.5%, 2.5%, and 3.5% (Nishijima et al 2015). When goat semen egg yolk-based extenders were replaced by different concentrations of soy lecithin, either 1.5% (Salmani et al 2014) or 3% (Fathi et al 2019) achieved optimum post-thaw semen quality. In rams, the replacement of egg yolk tris-based semen extender with 2% soy lecithin resulted in better or similar
post-thaw semen characteristics (El-Azazi and Yaseen 2016). Zhao et al (2021) substituted Tris-fructose egg yolk extender with different concentrations of Tris-fructose soy extenders and reported that the addition of 0.5% soy lecithin resulted in better post-thaw ram semen quality preserved at 0°C. In stallions, supplementation with different concentrations of soya-lecithin 20 g/L (2.0%) in a commercial semen extender revealed non-significant improvement in post-thaw semen quality but better fertility outcome (Papa et al 2011). Duan et al (2013, 2014) compared the addition of 10, 20, and 30% soy lecithin to 5% egg yolk in stallion semen extenders and recommended a supplement semen extender with 20% soy lecithin to replace egg yolk.

In the same context, our study aimed to investigate the optimum concentration of soy lecithin (0, 1.25, 2.5, 5.0%) to replace egg yolk in a modified INRA-82 semen extender for the cryopreservation of stallion semen.

2. Materials and Methods

2.1. Preparation of Extender

Modified INRA-82 (El-Badry et al 2014) consisted of 25 g/L glucose monohydrate, 1.5 g/L lactose monohydrate, 1.5 g/L raffinose pentahydrate, 0.4 g/L potassium citrate monohydrate, 0.3 g/L sodium citrate dihydrate, 4.76 g HEPES, pH 7.0, 500 mg/L gentamycin, 0.035% sodium dodecyl sulfate (SDS), and 0.15% skim milk. Aliquots of INRA-82 extenders were supplemented with different concentrations of soya lecithin: 0 (control group), 1.25%, 2.5% and 5.0% (Duan et al 2014). For semen cryopreservation, the extender was supplemented with 5% glycerol (El-Badry et al 2017). All chemicals and reagents were purchased from Sigma–Aldrich Co. (St. Louis, MO).

2.2. Animals

The present study was conducted on six Arabian stallions of known field fertility and aged 8-11 years. Animals were individually housed at Horse Police stud (El-Basateen, Police Training department, Ministry of Interior, Cairo, Egypt) and a private horse stud at Menofya Governorate. The authors followed the ARRIVE guidelines and carried out the study in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The study does not include any laboratory animals.

2.3. Semen Collection and Processing

On a biweekly schedule collection, 10 ejaculates per stallion were obtained from six stallions. Semen collection was performed early in the morning. A mare in estrus was used as a mount animal. Semen was collected using a lubricated and pre-warmed (45°C-50°C) Colorado model artificial vagina with an inline filter to separate the gel fraction. Immediately following collection, the gel-free portion of the ejaculate was evaluated for volume and progressive motility, and the concentration was determined with a hemocytometer. Only ejaculates with at least 60% progressively motile sperm and 250 ×10⁶ sperm cell/mL were used for freezing. Semen was extended 1:1 (semen: extender) using INRA-82 extender that had been warmed to 38°C. The diluted samples were divided into two portions, placed into 15-mL Falcon conical centrifuge tubes and then centrifuged for 10 minutes at 400×g (Cochran et al 1984). At least 95% of the supernatant was removed (Loomis 2006), and pellets were resuspended to a final sperm concentration of 100 × 10⁶ motile sperm/mL with modified INRA-82 (supplemented with different concentrations of soya lecithin containing glycerol). Each aliquot was cooled slowly to 5°C over 1 hour under aerobic conditions and then incubated at 5°C for 30 minutes (Crockett et al 2001). Afterward, semen was drawn into 0.5-mL straws (Minitube, Germany), sealed by powder and placed 4 cm above liquid nitrogen in the vapor phase in a foam box for 10 minutes before being plunged into the liquid phase (Cristanelli et al 1985). The straws were then stored in gobbets on canes and kept immersed in liquid nitrogen. For thawing, two straws per treatment were warmed in a water bath at 38°C for 30 seconds.

2.4. Evaluation of Frozen-Thawed Semen

Spermatozoa motility was examined and recorded using a warmed stage of a phase-contrast microscope (200×) just after thawing 0, 1, 2, and 3 hours post-thawing. The post-thawing viability indices were calculated to be equal to half of the post-thaw motility in addition to the summation of recorded motility at the first-, second-, and third-hours post-thawing (Milovanov 1962). The procedure described by Nie and Wenzel (2001) was used to determine the sperm membrane integrity (SMI) by counting the percentage of hypo-osmotic stress test (HOST)-positive cells in each sample. A 100-mL aliquot of each semen sample was mixed in 1.0 mL of a prewarmed 100 mOsm sucrose solution (1.712 g sucrose dissolved in 50 mL of sterile deionized water). The mixture was incubated at 37°C for 60 minutes in a 1.5-mL microcentrifuge tube. Following incubation, a small drop of sample was placed on a microscope slide and cover slipped for examination by using phase-contrast microscopy (400×) to evaluate 100 spermatozoa for evidence of swelling and curling changes. The percentage of spermatozoa with acrosomal integrity was determined by mixing 0.5 mL of each sample with 50 mL of a 1% formal citrate (1 mL of 37% formaldehyde and 2.92 g trisodium citrate dihydrate dissolved in 100 mL distilled water). One drop of the above
mixture was placed on a glass slide and covered with a cover slip, and 100 spermatozoa were evaluated under a light microscope (oil immersion, 1,000×) (Wells and Awa 1970).

2.5. Comet (Single-Cell Gel Electrophoresis Assay)

The alkaline comet assay for frozen thawed spermatozoa was carried out (Hughes et al 1996). Fully frosted glass slides were covered with 100 µl of 0.5% normal melting point agarose (Sigma), a coverslip was added, and the agarose was allowed to solidify. The coverslips were removed, and one straw of frozen-thawed sperm cells (just after thawing) in 50 µl of phosphate-buffered saline (pH 7.2) was mixed with 50 µl of 1.2% low-melting-point agarose and used to form the second layer. The slides with removed coverslips were then placed in lysis buffer for 1 hour (2.5 M NaCl, 100 mM Na ethylenediaminetetraacetic acid, 10 mM Tris, 1% Triton X at a pH of 10, and 10% DMSO). The slides were then incubated at 37°C in 100 µl/ml proteinase K in lysis buffer overnight. After draining the proteinase K solution from the slides, they were placed in a horizontal electrophoresis unit filled with freshly prepared alkaline electrophoresis solution containing 300 mM NaOH and 1 mM ethylenediaminetetraacetic acid for 20 minutes to allow the DNA to denature. Electrophoresis was performed at room temperature at 25 V (0.714 V/cm) and 300 mA, obtained by adjusting the buffer level for 10 minutes. The slides were then washed with a neutralizing solution of 0.4 M Tris at pH 7 to remove alkali and detergents. After neutralization, the slides were stained with 50 µl of 20 mg/mL ethidium bromide and mounted with a coverslip. A total of 200 sperm cells were examined under a fluorescence microscope (400×). The intensity of the stain in the comet tail region is presumed to be related to the DNA content, and DNA damage is estimated from measurements of the percent DNA in the tail, tail length, and Olive tail moment. The olive tail moment was calculated by multiplying the tail DNA% by the tail moment length. The tail moment length was measured from the center of the head to the center of the tail of the comet using image analysis software (Comet-Score program). Spermatozoa with fragmented DNA displayed increased migration of the DNA from the nucleus toward the anode, whereas spermatozoa with non-fragmented DNA did not form a “comet” (Fraser, 2004).

2.6. Statistical Analysis

Data were analyzed by simple one-way analysis of variance (ANOVA), followed by Duncan’s multiple range test when the treatments exhibited significant differences at P <0.05 using IBM-SPSS version 20.0 computer software.

3. Results

The post-thaw sperm motility of the semen extender supplemented with 2.5% soya-lecithin showed the highest motility at 0 h (60.5±2.0; P < 0.001), 1 h (54.0±1.69; P < 0.001), 2 h (48.0±2.0; P < 0.001) and 3 h (40.0±1.58; P < 0.0001) compared to those supplemented with 0.0%, 1.25%, and 5.0% soya-lecithin at 0 h (45.0±3.54; 47.5±2.0; 40.0±2.85), 1 h (40.0±3.54; 41.5±2.18; 35.0±2.37), 2 h (35.0±3.54; 36.0±1.87; 29.5±2.00), and 3 h (27.0±2.55; 27.5±2.24; 20.5±2.15) (Figure 1).

Figure 1 Mean semen post-thaw progressive motility percentage 0, 1, 2, and 3 hours after adding soy lecithin at concentrations of 0.0, 1.25%, 2.5%, and 5.0% to Stallion semen extender with error bars. Different superscripts (a, b, c) indicate significance at P<0.05.

The post-thaw sperm viability index achieved the highest value (P < 0.0001) when the semen extender was supplemented with 2.5% soya-lecithin (Table 1) and reached the lowest value when the semen extender was supplemented with 5.0% soya-lecithin. The hypo-osmotic swelling test indicating sperm membrane integrity (P < 0.05) and sperm acrosome
integrity (P < 0.0001) maintained high values when the semen extender was supplemented with 2.5% soya-lecithin compared to the other concentrations (Table 1).

The non-fragmented DNA (P < 0.01; Figure 2), DNA in the head of the comet (P < 0.05), and DNA in the tail of the comet (P < 0.05) attained the highest values when the semen extender was supplemented with 2.5% soya-lecithin compared to the other concentrations (Table 1). The shortest comet tail length (P < 0.0001) and the lowest Ollve tail moment (P < 0.01) were observed when semen extender was supplemented with 2.5% soya-lecithin compared to the other concentrations (Table 1).

The stallion semen extenders supplemented with increasing concentrations of soya lecithin (0.0, 1.25, 2.5, and 5.0%) showed fair nonsignificant negative correlations with sperm acrosome integrity and comet head DNA (Table 2). Soya lecithin treatments showed significant correlations (Table 2) with comet tail length (r = 0.50; P < 0.05) and comet tail moment (r = 0.47; P < 0.05) and tended to have a negative correlation with non-fragmented DNA (r = -0.30; P > 0.05). The acrosome integrity had negative correlations with comet tail DNA (r = -0.87; P < 0.0001), comet tail length (r = -0.63; P < 0.01) and comet tail moment (r = -0.42; P < 0.05).

Table 1 Mean ± S.E.M post-thaw stallion semen viability, HOST, acrosome integrity, and DNA integrity extended in modified INRA-82 semen extender supplemented with 0.0, 1.25%, 2.5, and 5.0% soya lecithin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.0%</th>
<th>Soya Lecithin concentration %</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.25%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Viability index</td>
<td>0.0%</td>
<td>124.5±11.33a</td>
<td>128.75±16.35a</td>
</tr>
<tr>
<td>SPMI (HOST)</td>
<td></td>
<td>44.20±1.49a</td>
<td>48.00±0.71ab</td>
</tr>
<tr>
<td>Acrosome integrity</td>
<td></td>
<td>48.00±0.71b</td>
<td>47.00±0.71b</td>
</tr>
<tr>
<td>Non-fragmented DNA</td>
<td></td>
<td>91.5±1.5b</td>
<td>90.72±1.41b</td>
</tr>
<tr>
<td>DNA in head of comet (%)</td>
<td></td>
<td>84.52±2.04ab</td>
<td>83.98±1.28ab</td>
</tr>
<tr>
<td>DNA in tail of comet (%)</td>
<td></td>
<td>15.48±2.03ab</td>
<td>16.02±1.28ab</td>
</tr>
<tr>
<td>Comet tail length (pixel)</td>
<td></td>
<td>3.27±0.36ab</td>
<td>3.62±0.13b</td>
</tr>
<tr>
<td>Comet tail moment</td>
<td></td>
<td>0.74±0.07a</td>
<td>0.77±0.06a</td>
</tr>
</tbody>
</table>

Means with different superscripts within rows (a, b, c) are significant at P<0.05, hypo-osmotic swelling test (HOST).

Table 2 Correlations between sperm motility (at 0, 1, 2, 3 h), viability index, HOST, with non-fragmented DNA, comet head and tail DNA, comet tail length, and tail moment.

<table>
<thead>
<tr>
<th>Post-Thaw semen parameters</th>
<th>Non-fragmented DNA</th>
<th>Comet</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head DNA</td>
<td>Tail DNA</td>
<td>Tail length</td>
</tr>
<tr>
<td>Motility 0 h</td>
<td>0.84***</td>
<td>-0.95***</td>
<td>-0.40*</td>
</tr>
<tr>
<td>Motility 1 h</td>
<td>0.83***</td>
<td>-0.94***</td>
<td>-0.41*</td>
</tr>
<tr>
<td>Motility 2 h</td>
<td>0.84***</td>
<td>-0.94***</td>
<td>-0.42*</td>
</tr>
<tr>
<td>Motility 3 h</td>
<td>0.86***</td>
<td>-0.89***</td>
<td>-0.53**</td>
</tr>
<tr>
<td>Viability index</td>
<td>0.85***</td>
<td>-0.94***</td>
<td>-0.45*</td>
</tr>
<tr>
<td>Hypo-osmotic swelling test</td>
<td>0.39*</td>
<td>-0.67**</td>
<td>-0.08NS</td>
</tr>
<tr>
<td>Acrosome integrity</td>
<td>0.82***</td>
<td>-0.87***</td>
<td>-0.63**</td>
</tr>
<tr>
<td>Treatments</td>
<td>-0.30a</td>
<td>-0.14NS</td>
<td>0.14NS</td>
</tr>
</tbody>
</table>

NS (non-significant), #P > 0.05), *P < 0.05, **P < 0.001, ***P < 0.0001.

Figure 2 Comet image of stallion spermatozoa extended in modified INRA-82 supplemented with 5% soya lecithin with (arrow) or without (arrowhead) DNA fragmentation.
4. Discussion

Soy lecithin components of phospholipids are more abundant than any other vegetable source (Taladrid et al 2017). Plant phospholipids protect the sperm plasma membrane during cryopreservation processes and during storage better than animal source phospholipids found in bird egg yolk (3). Soy bean lecithin-based semen extenders or semen extenders supplemented with soy lecithin aimed to replace egg yolk during semen cryopreservation of bulls (Miguel-Jimenez et al 2020, Ondřej et al 2019), buffalo (Singh et al 2012; Singh et al 2018), rams (18), goats (Fathi et al 2019), rabbits (Nishijima et al 2015), and stallions (Papa et al 2011- Duan et al 2014). The significant improvement in the post-thaw semen motility in the current study after adding 1.25% and 2.5% soy lecithin and their decrease after supplementing semen extender with 5% soy lecithin indicate that there is one optimum concentration of soy lecithin suitable to replace egg yolk for each animal species, and concentrations below this optimum one show slight improvements, but those above the optimum concentrations deteriorated post-thaw semen characteristics. From the supplemented concentrations of soy lecithin to the modified INRA-82 semen extender to replace egg yolk during the cryopreservation of Arabian stallion semen, the addition of 2.5% soy lecithin significantly increased the sperm progressive motility at 0, 1, 2, and 3 h post thawing. In comparison to the modified INRA-82 stallion semen extender used in the current study, a commercial stallion freezing extender containing pasteurized egg yolk and supplementing commercial skim milk-based extender used for stallion semen with 4.5% soy lecithin instead of egg yolk did not improve post-thaw sperm progressive or sperm total motility but showed slight improvement in the sperm with intact plasma membranes, and adding concentrations of soy lecithin from 1.0, 1.25, 1.5, to 1.75% showed nonsignificant improvement in post-thaw sperm progressive or total motility but showed slight improvement in the sperm with intact plasma membranes (Papa et al 2011). This disagreement may refer to the different types of extenders and the use of commercial extenders and different concentrations of soy lecithin that either exceeded or were lower than our optimum concentration tested in the current study. Using the same semen extender of this study (INRA-82) and in concentrations lower than our optimum one (2% soy lecithin) resulted in similar post-thawing motility compared with egg yolk (Duan et al 2014). Similar to the decrease in sperm post-thaw progressive motility at 0, 1, 2, and 3 h when soy lecithin concentration increased from 2.5% to 5%, the supplementation of semen extender with 3% soybean lecithin indicated the lowest progress motility (Duan et al 2013). In agreement with horses, the supplementation of rabbit semen extender with 20% egg yolk or 0.5% 1.5% 2.5% or 3.5% soy lecithin indicated improvement in post-thaw sperm motility with the addition of lower concentrations, but concentrations above 1.5% soy lecithin decreased sperm post-thaw motility (Nishijima et al 2015). Contrary to horses, the addition of 1% or 2% soya bean lecithin was not suitable to replace egg yolk in Tris-based extenders for preserving dog semen (Axnér and Lagerson 2016). Both concentrations decreased the post-thaw sperm motility compared to Tris-based extenders containing 20% egg yolk (Axnér and Lagerson 2016). The lower concentration of soy lecithin indicated better motility than the higher concentration (Axnér and Lagerson 2016). The use of 3.5% soy lecithin to replace egg yolk in ram semen extenders revealed better post-thaw motility than egg yolk (Khalifa and Abdel-Hafez, 2014). In goats, compared to two commercial semen diluents and supplementation of the tris-based semen extender with 1.5%, 3.0%, 6.0%, and 10.0%, 3% soy lecithin was recommended to replace egg yolk, as indicated by the improvement in sperm post-thaw motility, the lowest defective sperm percentage, normal sperm head ultrastructure, and the lowest production of lipid peroxidation (Fathi et al 2019). Mahabadi goats’ semen extender supplemented with 1.0% and 1.5% soy lecithin indicated similar post-thaw sperm motility compared to egg yolk and indicated better sperm movement characteristics compared to 0.5%, 2.0% and 2.5% concentrations of soy lecithin (Salmani et al 2014). The use of commercial soya lecithin-based extender for the cryopreservation of buffalo semen maintained post-thaw sperm motility, plasma membrane integrity, acrosomal integrity, and produced acceptable fertility rates (36) and was recommended to replace egg yolk (Akhter et al 2012). In buffalo, commercial soy-based semen extenders indicated slight nonsignificant improvement in post-thaw sperm motility compared to the egg yolk-based extender (Singh et al 2018).

The current study reported significant improvement in sperm membrane integrity, acrosome integrity, and DNA integrity when 2.5% soy lecithin replaced egg yolk in the INRA-82 semen extender. Similarly, 1.0% and 1.5% (Salmani et al 2014) or 3% (Fathi et al 2019) soy lecithin replaced egg yolk in goat semen extenders indicated no differences in plasma membrane integrity (Salmani et al 2014), and the higher concentration decreased the percentage of sperm with defective acrosomes and improved their plasma membrane integrity (Fathi et al 2019). Similarly, the supplementation of tris-fructose ram semen extender with 0.5% soy lecithin preserved post-thaw motility at 216 h and 288 h when cryopreserved at 0°C compared to the same extender supplemented with egg yolk, 0.25%, 0.75%, 1.0%, and 1.25% soy lecithin, with no differences in acrosome integrity (Zhao et al 2021). In rams, Khalifa and Abdel-Hafez (2014) supplemented Tris-citric acid extender with either egg yolk or 3.5% soy lecithin and reported better post-thaw sperm viability and acrosome integrity when soy lecithin replaced egg yolk. They attributed this improvement to the lower lipid peroxidation when soy lecithin was used for supplementing semen extenders. Compared to egg yolk, commercial soy-based semen extenders significantly decreased post-thaw buffalo sperm viability, plasma membrane integrity, and acrosome integrity (Singh et al 2018). This decrease in post-thaw semen quality may refer to the lower percentage of soy lecithin (1.0%), which may not be optimum for buffalo bull semen. In horses (Papa et al 2011; Duan et al 2014), goats (Fathi et al 2019), and rabbits (Nishijima et al 2015), a similar
decrease in post-thaw sperm plasma membrane integrity, acrosome integrity, and DNA integrity with higher concentrations of soy lecithin could be attributed to an increase in the percentages of protease inhibitors, amylases, lipoxygenases, urease and seed lectins (Speroni et al 2010). In agreement with our results, the cryopreservation of stallion semen in INRA-82 semen extender containing 2% soy lecithin improved plasma membrane integrity detected by the hypoosmotic swelling test and mitochondrial membrane integrity compared to 5% egg yolk and 3.0% soy lecithin, indicating similar plasma and mitochondrial membrane integrity to egg yolk (Duan et al 2014). Supplementing the stallion’s semen extender with both plants (2.0% soy lecithin) and animals (5.0% egg yolk) achieved the highest mitochondrial membrane potential and the lowest lipid peroxidation (Duan et al 2014). In contrast, supplementing commercial skim milk-based extender with soy-lecithin in concentrations lower than the optimum one reported in the current study (1.0, 1.25, 1.5, and 1.75%) resulted in no change in the plasma membrane integrity of equine semen (Papa et al 2011). The lack of improvement in post-thaw semen quality when a lower concentration of soy lecithin was supplemented may be due to the lower amount of phospholipids in soy lecithin that were not enough to protect spermatozoa from oxidative damage (Medica et al 2021). The improvement in comet parameters in post-thaw semen of stallions recorded in the current study was also noticed when 2% soy lecithin and 2% soy lecithin with 5% egg yolk were added to INRA-82 semen extender and referred to minimum lipid peroxidation and oxidative stress leading to the preservation of both sperm plasma membrane and mitochondrial membrane integrity (Duan et al 2013, 2014).

5. Conclusions

It could be concluded that traditional semen extenders used for the cryopreservation of stallion semen could be supplemented by soy lecithin rather than egg yolk based on the post-thaw semen quality. There is one optimum concentration of soy lecithin for every species, including horses; above this concentration, semen quality declines. This study recommended the use of 2.5% soy lecithin to supplement the modified INRA-82 semen extender for the cryopreservation of stallion semen.

Acknowledgments

We send our merci to the soul of our colleague Prof. Dr. Gamal Attia ElSisi, who was the former principal investigator of this project and passed away during the coronavirus pandemic.

Ethical Considerations

The authors declare that they followed ethical standards when using animals in research.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

The authors wish to thank The Academy of Scientific Research and Technology for funding the project entitled “Investigation and production of extenders for storage of equine semen” in collaboration with the Bulgarian Academy of Science.

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