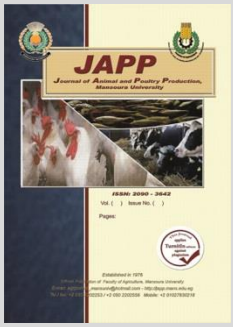
Journal of Animal and Poultry Production

Journal homepage: www.japp.mans.edu.eg
Available online at: www.jappmu.journals.ekb.eg

Soybean Lecithin as an Alternative to Egg Yolk in Tris-Based Extender of Cryopreserved Apri Rabbit Semen

Younan, G. E.[1*]; A. M. Shehab El-Din[1] ; A. E. Abdel-Khalek[2] ; M. A. El-Sherbieny[1] and Azza A. Helmy[1]

[1] Animal Production Research Institute, Agricultural Research Center, Egypt.
[2] Animal Production Department, Faculty of Agriculture, Mansoura University, Egypt.



ABSTRACT

The present study was conducted to demonstrate whether soy lecithin could be used as an alternative to egg yolk (EY) in tris-extender for cryopreservation of APRI rabbit semen. In tris-extender (E1), 18% EY was replaced by 1% (E2), 1.5% (E3), or 2% (E4) SBL (1:4). Semen was evaluated visually after cryopreservation stages and by CASA only post-thawing. Results showed that visual sperm motility, livability, abnormality, acrosome integrity, and membrane integrity were improved (P<0.05) by E3 after all cryopreservation stages. Slow, total progressive, and total motility were the highest (P<0.05), while non-motility and non-progressive motility were the lowest (P<0.05) with E3 compared with other extenders. In comparing with E1, E3 showed the best dynamic sperm parameters, in terms of the highest VCL, STR (P<0.05), VSL (P≥0.05), and the lowest (P<0.05) WOB. Prolificacy of rabbits showed the highest (6.68 kits/doe, P<0.05) by E3 compared with 4.96, 4.72, and 2.8 kits/doe for E1, E2, and E4, respectively. The correlation coefficient was the lowest (r=0.691, P<0.05) between acrosome integrity and litter size, and the highest (r= 0.921, P<0.001) between membrane integrity and prolificacy. Progressive motility and slow motility correlated with pregnancy (r=0.811and r=0.778, P<0.01) and prolificacy (r=0.898, P<0.001 and 0.801, P<0.01). In conclusion, addition of soybean lecithin at a level of 1.5% as an alternative to 18% egg yolk in tris extender may has a beneficial impact on motility parameters of cryopreserved APRI-rabbit spermatozoa, and prolificacy of doe rabbits.

Keywords: Rabbit, semen, egg yolk, lecithin, sperm function, sperm dynamic.

INTRODUCTION

In rabbits, cryopreserved semen has been used for experiments or as a purpose of genetic resource bank (Mocé and Vicente, 2009). In commercial rabbit farms, artificial insemination (AI) programs evaluated cryopreserved semen with good quality and high fertility, because there are many factors affecting the quality of the semen, following freezing (Iaffaldano *et al.*, 2012; Lavara *et al.*, 2013; Rosato and Iaffaldano, 2013). There are several differences in fresh and cryopreserved semen (Bolet *et al.*, 2000; Vicente *et al.*, 2000), belonging to different breeds or lines of rabbits (Mocé *et al.*, 2005).

Semen cryopreservation is an excellent tool for storage of semen with valuable genetic materials (Bolet *et al.*, 2000; Blash *et al.*, 2005). During this process, spermatozoa are suffering from several stressors when they are exposing to decreased temperature (cooling and freezing), cryoprotectants, formation of ice and increased osmolality level of the medium (Watson, 2000). In rabbit semen cryopreservation, sperm damage in terms of decreasing the acrosome integrity, motility and livability of spermatozoa. Also, there were impaired sperm transportation, premature capacitation, and curtailed longevity occurred *in vitro* are often observed. These changes lead to a pronounced decrease in the fertilization capacity of spermatozoa after cryopreservation (Graham and Mocé, 2005; Rodriguez-Martinez and Barth, 2007). The successful cryopreservation is affected by several factors, such as the initial semen quality, processes of cryopreservation and freezing diluents. Therefore, extender composition, concerning type and level of cryoprotectants, and protocol of cryopreservation is essential to obtain excellent extender during cryopreservation of rabbit semen.

In rabbits, tris-based extender is the commune extender frequently used for cryopreservation, being with egg yolk with different levels from 10-20% (Mocé and Vicente, 2009). Egg yolk is normally used as a cryoprotective agent in semen freezing extenders, but its use has sanitary and practical disadvantages. Plasma contains mainly Low-Density Lipoproteins (LDL), which are widely presumed to be the cryoprotective agent in egg yolk. Some authors used egg yolk plasma in a ready-to-use extender compared to whole egg yolk (Pillet *et al.*, 2011). Also, the protection afforded by egg yolk has not yet been completely elucidated. Thus, a comparison effect of egg yolk and egg yolk plasma of different avian species in the extender on the efficiency of chilling preservation of rabbit sperm is desirable.

Experiments on different species demonstrated that a sterile soybean extract is a new generation of semen diluents often used instead of egg yolk or milk (Anel *et al.*, 2006, 2008). Soybean lecithin (SBL) has recently been suggested as a replacement for egg yolk in extenders for semen conservation (Aires *et al.*, 2003; Aurich *et al.*, 2007). Therefore, spermatozoa can be preserved in

* **Corresponding author.**
E-mail address: ggezzate@yahoo.com
DOI:10.21608/jappmu.2021.149460

commercial diluents containing SBL, such as Biociphos and Andromed for bovine (Bousseau *et al.,* 1998; Aires *et al.,* 2003) and equine (Aurich *et al.,* 2007). The SBL-based diluents were introduced by the commercial AI companies at the beginning of year 2000 (Gil *et al.,* 2000; Thun *et al.,* 2002). In comparing with an extender with a conventional egg yolk extender, many researchers found that the latter generally gave acceptable preserve effect and better fertility, but that results varied between breeds (van Wagtendonk-de Leeuw *et al.,* 2000; Kmenta *et al.,* 2011). In this context, Aires *et al.* (2003) reported clear benefits from using SBL-extender as compared to an egg yolk containing extender. These findings indicate that SBL may thus be a good alternative, if there is a concern regarding microbial contamination that may negatively affect semen quality during low temperature storage, and impair fertilizing capacity of spermatozoa (Aurich and Spergser, 2007).

There is a lack in the information on the effect of using SBL in Tris-based extender, as a replacement of egg yolk, on semen quality of cryopreserved semen of APRI rabbit in Egypt. Therefore, the present study was conducted to demonstrate whether soy lecithin could be used as an alternative to egg yolk in tris-extender for cryopreservation of APRI rabbit semen.

MATERIALS AND METHODS

The current study was conducted in the Rabbit breeding Farm, Sakha Animal Production Research Station, Animal Production Research Institute (APRI) during the period from January to November 2020.

Animals:

Five sexually mature APRI rabbit bucks (3.25±0.50 kg body weight and about 6-9 months of age) were used as semen donors. Bucks were housed in flat-deck cages with semi-opened system. Animals were fed a commercial standard diet that contains 18% CP and 2600 Kcal/kg digestible energy. Drinking water was automatically offered freely.

Semen collection:

Semen was collected twice a week by using an artificial vagina from all bucks. Only ejaculates with more than 70% mass motility was were used in this study. On each day of semen collection, the ejaculates were pooled and divided into four portions to dilute with different types of extenders.

Semen extenders:

For preparing 100 ml of the control extender, 3.028 g tris, 1.675 g citric acid, 1.250 g fructose, antibiotics (500 IU Penicillin and 500 µg Streptomycin/ml), 18 ml egg yolk, and 5.5 ml dimethyl sulfoxide (DMSO) were dissolved in distilled water up to 100 ml. These ingredients were well mixed to prepare Tris-egg yolk extender (E1, control). In other three treatment extenders, egg yolk in E1 was replaced by soybean lecithin at a level of 1 g SBL (E2), 1.5 g SBL (E3), or 2 g SBL (E4). Semen was diluted with all types of extenders at ratio of 1 semen: 4 extender.

Freezing process:

The diluted semen was equilibrated at 4^{o} C for 4 hours, packaged in 0.25 ml plastic straws (IMV Technologies, L'Aigle, France) for cryopreservation. The cooled straws were suspended horizontally in liquid nitrogen vapor 5 cm above the liquid nitrogen surface for 10 min before being plunged in liquid nitrogen.

Semen evaluation:

Semen was evaluated visually after dilution, equilibration, and thawing; however, semen was evaluated by computer assisted semen analysis (CASA) in post-thawed semen. Thawing was carried out by immersing the straws in a water bath at 37°C for 30 s before semen evaluation.

Visual semen evaluation:

Motility percentage was estimated for fresh semen by examining a drop of diluted semen under microscope at 400x. The percentages of sperm livability and abnormality was determined by mixing a drop of semen with a drop of eosin-nigrosin stain on pre-warmed slide in microscopic field of 100 sperm cells for semen samples of each extender. Abnormal features of spermatozoa were recorded according to the defects of head, mid-piece and tail (Pérez-Sánchez *et al.,* 1997).

Plasma membrane integrity:

Hypo-Osmotic Swelling Test (HOS-t) was used to estimate the integrity of sperm membrane. In this test, spermatozoa responded to be with curled tails were counted in 200 sperm cells, then the percentage of curled spermatozoa was computed. Briefly, sperm cells were incubated at 37◦C for 1 h with hypo-osmotic solution (150 mOsm/L, Jeyendran *et al.,* 1984, 1992). The hypoosmotic solution contained sodium citrate (3.75 g) and fructose (13.51 g) /L distilled water. Post-incubation, spermatozoa in an aliquot (50 µl) were attained with an equal volume of 2% glutaraldehyde/0.165 M sodium cacodylate buffer (pH 7.3 at 25◦C).

Acrosome integrity:

Acrosome integrity was estimated in term of percentage of sperm cells with intact acrosome. Briefly, 5 µl of semen was extended with 0.16 MNaCl (50 µl), then spermatozoa were fixed at room temperature for 30 min at minimum with 1% (v/v) glutaraldehyde by mixing the extended semen with an equal volume of 2% (v/v) glutaraldehyde/0.165 M and sodium cacodylate buffer (pH 7.3 at 25◦C). Sperm samples were examined after incubation by microscope (1000×) for sperm acrosome integrity. Sperm cells with a dense, thick apical ridge on the head in a field of 400 sperm cells were considered as intact acrosome (Shams-Borhan and Harrison, 1981). Spermatozoa were fixed using 1% glutaraldehyde in 0.165 M sodium cacodylate buffer (Almadaly *et al.,* 2012).

Semen evaluation by CASA:

Computer assisted semen analysis (CASA, SPERMOLAB®, Cairo, Egypt) was applied to evaluated the diluted semen after thawing. A drop of semen (5 µL) extended with different levels of SBL was loaded into a pre-warmed slide (dis-posable Leja). Before the analysis, sample was allowed to settle on the mini-thermal heating stage (38 °C). For each specimen, about 200 spermatozoa from 2-3 drops of each sample were evaluated. The final analysis was done for each sample, including the following parameters:

- Percentages of total sperm motility (TSM), progressive sperm motility (PSM), rapid progressive sperm motility (RPSM), slow progressive sperm motility (SPSM),

non-progressive sperm motility (NPSM), and immotile spermatozoa (IMS). Where:

TSM = PSM + NPSM; PSM = RPSM + SPSM; IMS= 100 – TSM.

- **Sperm kinetic parameters included:**

- **Curve linear velocity (VCL):** Average velocity of the sperm through its real path, (reference value > 45 µm/s).
- **Straight linear velocity (VSL):** Average velocity of the sperm through the straight line connecting the first position of the last track (reference value > 25 µm/s).
- **Average path velocity (VAP):** Average velocity of the sperm through its average trajectory (reference value > 35 µm/s).
- **Linearity (LIN):** The straightness of the sperm path (reference value 59%).

LIN=VSL/VCL

- **Straightness (STR):** The righteousness of motion (reference value> 80%).

STR=VSL/VAP

- **Wobble (WOB):** Is the degree of oscillation of the actual path of the sperm head in his relationship with the VAP.

WOB=VAP/VCL

Fertility test:

Fertility test was done to determine the fertilizing ability of spermatozoa of all types of extenders. Total of 100 nulliparous APRI female rabbits (about 6 months old and 3-3.50 kg live body weight. At the time of insemination, rabbit does were induced for ovulation by administration of 20 µg GnRH analogue (1 ml contains 0.0042 mg buserelin acetate equivalent to 0.004 mg buserelin; Receptal® VET.; MSD Animal Health) according to Morrell (1995). Each female was artificially inseminated (AI) with a dose of 50x106 motile sperm in a straw of 0.5 ml semen (Quintela et al., 2004). Immediately before insemination, semen straws were thawed at 37oC for 30 s. For AI procedure, each female was placed carefully on a stable bench and inseminating pipettes (5 mm diameter) was inserted 10–15 cm into vagina to ensure appropriate delivery of sperm. Doe rabbits were diagnosed for pregnancy on day 10-12 d post-insemination by abdominal palpation, then pregnancy rate was calculated as a number of pregnant does divided on number of inseminated does. After parturition, kindling rate (number of delivered does/number of inseminated does x100) was calculated, and total litter size/doe was recorded. Doe rabbit prolificacy was calculated according to the following equation:

Prolificacy = (pregnancy rate x litter size/doe).

Statistical analysis:

The obtained data were statistically analyzed by one-way ANOVA using the General Linear Model procedure of SAS (2004). Differences among means were tested using Range Multiple test of Duncan (1955). All percentage values were subjected to arcsine transformation before the statistical analysis. All differences were considered statistically significant at P<0.05. Correlation coefficients were conducted between each of sperm parameter and pregnancy, litter size, or prolificacy using SAS (2004).

RESULTS AND DISCUSSION

Results

Visual sperm characteristics:

Results in Table 1 showed that the percentages of progressive motility, livability, membrane integrity, and acrosome integrity of spermatozoa were significantly (P<0.05) improved in E3 as compared to E1, E2 and E4 in semen after dilution, equilibration and thawing stages. However, sperm abnormality didn't differ significantly with different SBL levels as compared to EY-extender all stages. Generally, sperm motility, livability, and membrane and acrosome integrities were improved when semen was diluted with E3 containing 1.5% SBL as compared to free extender (E1) and other extender types.

Table 1. Sperm characteristics in rabbit semen diluted with different extenders at cryopreservation stages.

Item	Experimental extender			
	E1 (18% EY)	E2 (1% SBL)	E3 (1.5% SBL)	E4 (2% SBL)
Post-diluted semen:				
Progressive sperm motility (%)	64.55±1.71^{b}	62.73±2.55^{b}	71.82±1.22^{a}	58.64±2.87^{b}
Sperm livability (%)	65.45±2.20^{b}	64.73±2.61^{b}	73.45±1.53^{a}	57.45±2.94^{c}
Sperm abnormality (%)	19.27±1.34	17.73±1.38	17.27±1.35	19.55±1.26
Sperm membrane integrity (%)	69.30±1.46^{ab}	67.57±1.93^{ab}	73.07±3.89^{a}	61.47±4.45^{b}
Acrosome integrity (%)	79.47±3.60^{ab}	78.20±2.90^{ab}	81.98±5.47^{a}	68.47±2.27^{b}
Post-equilibrated semen:				
Progressive sperm motility (%)	56.82±3.38^{ab}	54.55±4.01^{bc}	65.45±2.17^{a}	45.91±3.61^{c}
Sperm livability (%)	59.18±3.61^{ab}	57.36±3.87^{b}	68.91±2.57^{a}	49.18±3.58^{b}
Sperm abnormality (%)	15.36±1.01	13.73±1.00	14.64±1.38	16.09±1.16
Sperm membrane integrity (%)	59.17±5.97	55.73±3.57	64.20±4.65	50.13±6.06
Acrosome integrity (%)	65.93±4.36^{ab}	63.97±2.46^{ab}	70.67±4.33^{a}	57.17±2.63^{b}
Post-thawed semen:				
Progressive sperm motility (%)	37.73±3.65^{b}	38.18±4.06^{b}	48.64±2.03^{a}	25.45±4.23^{c}
Sperm livability (%)	39.27±3.92^{a}	39.64±4.18^{a}	49.91±2.71^{a}	27.73±4.14^{b}
Sperm abnormality (%)	12.00±0.85^{ab}	10.27±0.46^{b}	9.54±0.95^{b}	13.63±1.14^{a}
Sperm membrane integrity (%)	38.23±1.93^{b}	40.67±1.90^{b}	49.20±1.93^{a}	27.27±2.54^{c}
Acrosome integrity (%)	44.13±2.55^{ab}	42.67±2.06^{ab}	52.37±3.70^{a}	32.87±4.83^{b}

a, b and c: Means within the same row reflected significant differences at P<0.05.

Sperm characteristics examined by CASA:

Sperm motility:

Data in Table 2. Revealed that the percentages of sperm motility as rapid, slow, total progressive, and total motility were significantly (P<0.05) the highest, while immotile spermatozoa and non-progressive motility percentages were the lowest (P<0.05) in semen extended with E3 (1.5% SBL) as compared to control and other extenders SBL levels.

Table 2. Sperm motility in post-thawed semen extended with different extenders using CASA.

Item	Experimental extender			
	E1 (18% EY)	E2 (1% SBL)	E3 (1.5% SBL)	E4 (2% SBL)
Sperm motility (%)				
Rapid progressive	9.62±1.80^{b}	12.61±3.31^{ab}	20.06±2.35^{a}	9.21±2.29^{b}
Slow progressive	26.41±1.26^{ab}	23.19±3.41^{b}	34.12±2.11^{a}	18.39±3.79^{b}
Total progressive	36.03±2.08^{b}	35.80±3.60^{b}	54.18±2.32^{a}	27.60±1.51^{b}
Non-progressive	34.95±2.93^{a}	30.68±3.94^{ab}	21.42±2.48^{b}	24.65±4.11^{ab}
Total motility	70.98±2.55^{b}	66.48±2.23^{b}	75.58±3.15^{a}	52.25±3.55^{c}
Immotile	29.02±3.08^{b}	33.52±1.26^{b}	24.42±3.89^{b}	47.75±5.63^{a}

a, b and c: Means within the same row reflected significant differences at P<0.05.

Sperm dynamic parameters:

Results of dynamic parameters of all spermatozoa (Table 3) revealed different effects of extender type on sperm dynamic parameters. In comparing with E1, semen extended with E3 showed the best dynamic sperm parameters in term of the highest VCL, STR (P<0.05), and VSL (P≥0.05), and the lowest (P<0.05) WOB as compared to other extenders. However, VAP was lower, while LIN did not differ significantly in E2 as compared to control. It is worthy noting that E4 showed insignificant differences with E1 regard to all sperm dynamic parameters.

Table 3. Average of dynamic sperm characteristics in post-thawed semen extended with different extenders.

Item	Experimental extender			
	E1 (18% EY)	E2 (1% SBL)	E3 (1.5% SBL)	E4 (2% SBL)
VCL (µm/s)	49.10±5.01^{b}	25.67±3.84^{c}	93.47±3.43^{a}	68.00±11.93^{b}
VSL (µm/s)	15.43±0.81	17.90±1.24	18.50±2.29	14.53±1.07
VAP (µm/s)	79.83±2.52^{a}	60.60±2.96^{b}	58.00±1.53^{b}	72.07±5.30^{a}
LIN (%)	32.48±5.214^{b}	73.63±13.17^{a}	19.84±2.629^{b}	22.73±4.225^{b}
STR (%)	19.37±1.158^{b}	29.47±0.677^{a}	32.11±4.636^{a}	20.27±1.447^{b}
WOB (%)	166.07±18.254^{ab}	248.03±40.124^{a}	62.14±1.795^{c}	116.28±30.763^{bc}

a, b and c: Means within the same row reflected significant differences at P<0.05.

Fertility rate:

The pregnancy rate was affected significantly (P<0.05) by extender type. Semen extended with E3 increased pregnancy rate to 84% as compared to 76% in E1, but the difference was not significant. On the other hand, pregnancy rates of semen extended with 1% (E2) and 2% (E4) significantly (P<0.05) decreased as compared to 1.5% SBL (E3), but bid not differ significantly from that of E1. All conceived does delivered with 100% kindling rate, but litter size of semen extended with E2 and E3 was significantly (P<0.05) higher than E1. Prolificacy of doe rabbits inseminated with semen extended with different extender types, E3 showed significantly (P<0.05) the highest value (6.68 kits/doe) as compared to 4.96, 4.72, and 2.8 kits/doe for semen extended with E1, E2, and E4, respectively (Table 4).

Table 4. Reproductive traits of doe rabbits inseminated with cryopreserved semen extended with different types of extenders.

Item	Experimental extender			
	E1 (18% EY)	E2 (1% SBL)	E3 (1.5% SBL)	E4 (2% SBL)
Pregnancy rate	76.0±7.48^{ab}	60.0±6.32^{b}	84.0±7.48^{a}	56.0±7.48^{b}
Kindling rate	100	100	100	100
Litter size/doe	6.59±0.31^{b}	7.96±0.34^{a}	7.97±0.37^{a}	4.96±0.53^{c}
Doe rabbit prolificacy	4.96±0.41^{b}	4.72±0.40^{b}	6.68±0.66^{a}	2.80±0.50^{c}

a, b and c: Means within the same row reflected significant differences at P<0.05.

Correlation confidents:

Results in Table 5. showed significant and positive correlation between reproductive parameters and all visual observation, except for sperm abnormality which showed significantly negative correlation with all reproductive parameters. It is of interest to not that the correlation coefficients between all visual observations and prolificacy was the strongest, followed by litter size, and the lowest with pregnancy.

Results also showed the highest correlations between CASA parameters and prolificacy, followed by litter size, and pregnancy rate, respectively. It is worthy noting that sperm progressive motility (visually or by CASA correlated with all reproductive parameters. All sperm dynamic parameters had insignificant correlation with pregnancy rate, but only the correlation of VSL, VAP and STR with liter size was significant. Only STR correlated significantly (P<0.05) with prolificacy.

Table 5. Correlation coefficients between sperm parameters and reproductive traits of doe rabbits.

Item	Pregnancy rate	Litter size	Doe prolificacy
Visual observations:			
Progressive motility	0.600*	0.650*	0.717**
Livability	0.610*	0.647*	0.724**
Sperm abnormality	-0.465	-0.774**	-0.705*
Membrane integrity	0.744**	0.861**	0.921***
Acrosome integrity	0.730**	0.691*	0.816**
CASA parameters:			
Progressive motility	0.811**	0.670*	0.898***
Rapid progressive motility	0.431	0.459	0.541*
Slow progressive motility	0.778**	0.587*	0.801**
Immotile spermatozoa	-0.752**	-0.708*	-0.820**
VCL	0.515	-0.097	0.325
VSL	0.292	0.593*	0.508
VAP	-0.116	-0.608*	-0.444
LIN	-0.394	0.417	-0.056
WOB	-0.460	0.181	-0.240
STR	0.291	0.712**	0.592*

Discussion

The usage of egg yolk (EY) as a diluent during semen cryopreservation has been questioned for certain potential negative aspects (Aires *et al.,* 2003; Gil *et al.,*

2003). During semen freezing, using EY-extenders has been developed for semen of different species (Curry, 2007, Dinnyes *et al.,* 2007, Barbas and Mascarenhas, 2009) including rabbits (Mocé and Vicente, 2009). The EY is very important to prevent cold shock and maintaining sperm membranes in post-thawed semen (Bergeron and Manjunath, 2006). It reduces the toxicity of the seminal plasma, and provide substrates to neutralize the production of H_2O_2 following sperm metabolism (Vishwanath and Shannon, 2000). Rabbit spermatozoa are relatively sensitive to cryoprotectants containing hydroxyl groups such as glycerol, compared with those containing amide or methyl groups (Chen *et al.,* 1989; Castellini *et al.,* 1992). Egg yolk is often included as an additional protective additive in rabbit sperm-freezing extenders (Mocé and Vicente, 2009). The dilution of rabbit buck semen before freezing with INRA-82 extender supplemented with a combination of 4% dimethyl sulphoxide (DMSO) and 4% dimethyl formamide (DMF) improved quality of frozen-thawed New Zealand White rabbit spermatozoa. Supplementation of INRA-82 with DMSO or with DMF alone at higher concentrations deteriorates the sperm quality (Fadl *et al.,* 2019). Therefore, DMSO was used as cryoprotectant in tris-extender used in our study.

The present study aimed to evaluate the effects of SBL on semen characteristics when in replacing EY in tris-extender for cryopreservation of APRI rabbit semen. In our study we used SBL at three levels (1, 1.5, and 2%) instead of EY (18%). The present results showed the best results of tris-extender with SBL at a level of 1.5%, in terms of increasing motility and live spermatozoa, acrosome integrity and membrane integrity after all freezing stages. More attention has been given to determined integrity of plasma membrane and acrosome due to the require to a proper sperm function, which is important for metabolism, capacitation, ova binding, and acrosome reaction of spermatozoa. In our study, improving the plasma membrane integrity along with acrosome integrity proven in our study be used to predict the sperm fertilizability (Brito *et al.,* 2003; Bacinoglu *et al.,* 2008; Santolaria *et al.,* 2015).

Our results on rabbit semen proved beneficial effects as SBL as EY did during cryopreservation of bull semen (Aires *et al.,* 2003), rams (Forouzanfar *et al.,* 2010), stallions (Papa *et al.,* 2011), goats (Salmani *et al.,* 2014), boars (Simpson *et al.,* 1987), dogs (Beccaglia *et al.,* 2009), cats (Vick *et al.,* 2012), fish (Yildiz *et al.,* 2013), and human (Reed *et al.,* 2009). In the present study, sperm characteristics (progressive motility and livability) in 1.5% SBL was improved as compared to EY or other levels of SBL. In agreement with our results, rate and classes of sperm motility after freezing and thawing significantly increased in the 1.5% SBL compared with in the EY (Nishijima *et al.,* 2015). Also, it maintained motility, viability values of chilled-stored spermatozoa and preserved their fertilizing capacity (EL-Azzazi and Hanafy, 2015). Thus, SBL could be included in rabbit semen extenders as a non-animal origin instead of EY. In semen extender, EY could be safely replaced by SBL as a cryoprotectant, and EY had no harmful effects on sperm characteristics in post-thawed rabbit semen (EL-Azzazi and Hanafy, 2015; Nishijima *et al.,* 2015).

The superiority of SBL in semen extenders may be related to lacking the cytotoxicity of lecithin (Fiume, 2001). However, increasing the level SBL to 2% in the current study impaired post-thawed semen quality. Similarly, several authors mentioned to decline in osmotic pressure by using SBL (Salmani *et al.,* 2014) or low-density lipoprotein (Moussa *et al.,* 2002), as a result of fructose and salts inclusion in the extender, leading to sperm cell damage.

In the recent decades, CASA is considered as one of the newest techniques for semen evaluation (Hoogewijs *et al.,* 2012; Gloria *et al.,* 2013; Amann and Waberski 2014). In accordance with the obtained results of parameters of sperm dynamics, some authors reported similar results on rrabbit semen (Błaszczyk *et al.,* 2013; Safaa *et al.,* 2008). Despite the positive impacts observed on motility, livability, and acrosome and membrane integrities, the present results revealed some improvement in most dynamic parameters (VCL, VSL, VAP, LIN, STR, and WOB) in extender with SBL at a level of 1.5% as compared to EY-extender. It was confirmed that visually examined sperm motility significantly affected the fertility, and there was significant effect of the percentages of motility and progressive rapid motility on the prolificacy. Evaluation of semen with CASA provides pronounced markers of fertility (Theau-Clément et al. 2015). Although Lavara et al. (2005) recorded significantly negative correlation between LIN and fertility ($r = -0.32$), our results revealed similar negative correlation of LIN with pregnancy rate and litter size ($r=-0.3936$, $P \geq 0.05$).

It is worth to note that, the obtained results regard to visual sperm characteristics were in association with sperm dynamic parameters and both were correlated with pregnancy rate and doe prolificacy. These finding were proved by the correlation coefficients between each of membrane integrity and acrosome integrity, and each reproductive trait, being the lowest ($r=0.691$, $P<0.05$) between acrosome integrity and litter size, and the highest ($r= 0.921$, $P<0.001$) between membrane integrity and prolificacy. Regarding CASA parameters, progressive motility, in particular slow motility, can be used an indicator for prediction of sperm fertility, whereas both parameters correlated significantly with pregnancy ($r=0.811$and $r=0.778$, $P<0.01$) and prolificacy ($r=0.898$, $P<0.001$ and 0.801, $P<0.01$).

Artificial insemination (AI) in rabbits may be accompanied with some detrimental factors affecting fertilizing ability of spermatozoa during preservation. Alvariño (2000) evidenced that rabbit semen preservation is one of the main problems for a wide use of AI in industrial Rabbitries (Castellini, 1996). The improvement in sperm parameters as affected by supplementing tris-extender with 1.5% SBL, resulted in the highest pregnancy and prolificacy of doe rabbits inseminated with E2 semen. This in accordance with Nishijima *et al.* (2015), who showed that motility of spermatozoa is in closed relationship with reproductive efficiency. Also, Nishijima *et al.* (2015) reported that increased litter size from 3.3±3.8 for semen extended with EY to 5.1±1.9 for that extended with SBL at a level of 1.5%. Accordingly, the present results may indicate that EY contains additional components that decrease sperm characteristics and

fertilizing ability (Aires *et al.*, 2003), which may lead to reduction in sperm fertility (van Wagtendonk-de Leeuw *et al.*, 2000).

CONCLUSION

In conclusion, inclusion of soybean lecithin at a level of 1.5% as an alternative to 18% egg yolk in tris extender may has a beneficial impact on physical sperm characteristic of cryopreserved APRI-rabbit spermatozoa and prolificacy of doe rabbits inseminated with this semen. Further studies are required for studding the effect of soy bean lecithin in tris-extender on dynamic parameters and sperm metabolism in rabbit semen.

REFERENCES

Aires, V.A.; Hinsch, K.D.; Mueller-Schloesser, F.; Bogner, K.; Mueller- Schloesser, S. and Hinsch, E. (2003). *In vitro* and *in vivo* comparison of egg yolk based and soybean lecithin-based extenders for cryopreservation of bovine semen. Theriogenology, 60:269–279.

Almadaly, E.; El-Kon, I.; Heleil, B.: Fattouh, E.; Mukoujima, K.; Ueda, T.; Hoshino, Y.; Takasu, M. and Murase T. (2012). Methodological factors affecting the results of staining frozen–thawed fertile and sub-fertile Japanese Black bull spermatozoa for acrosomal status. Anim. Reprod. Sci., 136: 23–32.

Alvariño, J.M.R. (2000). Reproductive performance of male rabbits. 7th World Rabbit Cong., Valencia, Spain, 4-7 July, 13-30.

Amann, R.P. and Waberski, D. (2014). Computer-assisted sperm analysis (CASA): Capabilities and potential developments. Theriogenology, 81: 5-17.

Anel, L.; Alvarez, M.: Martínez-Pastor, F.; García-Macías, V.; Anel, E. and de Paz, P. (2006). Improvement strategies in ovine artificial insemination. Reprod. Dom. Anim., 41 Suppl 2:30–42.

Anel, L.; Alvarez, M.; Martínez-Pastor, F.; Gomes, S.; Nicolas, M.; Mata, M.; Martínez, A.F.; Borragan, S.; Anel, E. and de Paz, P. (2008). Sperm Cryopreservation in Brown Bear (*Ursus arctos*): Preliminary Aspects. Reprod. Dom. Anim., 43 Suppl 4: 9–17.

Aurich, C. and J. Spergser, (2007). Influence of bacteria and gentamicin on cooled-stored stallion spermatozoa. Theriogenology, 67: 912-918.

Aurich, C., Seeber P. and Müller-Schlösser F. (2007). Comparison of different extenders with defined protein composition for storage of stallion spermatozoa at 5^0C. Reprod. Dom. Anim., 42: 445–448.

Bacinoglu, S.; Tas, M.; Cirit, U.; Ozdas, O.B. and Ak, K. (2008). The potential fertility estimation capacity of the hypoosmotic swelling test, the thermal stress test and a modified cervical mucus penetration test in the bovine. Anim. Reprod. Sci., 104: 38–46.

Barbas, J. P. and Mascarenhas, R. D. (2009). Cryopreservation of domestic animal sperm cells. Cell Tissue Bank, 10:49–62.

Beccaglia, M.; Anastasi, P.; Chigioni, S. and Luvoni, G.C. (2009). Tris-lecithin extender supplemented with antioxidant catalase for chilling of canine semen. Reprod. Domest. Anim., 44:345–349.

Bergeron, A. and Manjunath, P. (2006). New insights towards understanding the mechanisms of sperm protection by egg yolk and milk. Mol. Reprod. Dev., 73:1338–1344.

Blash, S.; Chen, L.; Harvey, M. and Gavin, W.G. (2005). Reestablishment of a transgenic rabbit line by artificial insemination using cryopreserved semen. Lab. Anim., 34: 61-63.

Błaszczyk, M.; Slanina, T.; Massanyi, P. and Stawarz, R. (2013). Semen Quality Assessment of New Zealand White Rabbit Bucks. Journal of Microbiology, 2 (Special issue 1): 1365-1376.

Bolet, G.; Brun, J.M.; Monnerot, M.; Abeni, F.; Arnal, C.; Arnold, J.; Bell, D.; Bergoglio, G.; Besenfelder, U.; Bosze S.; Boucher, S.; Chanteloup, N.; Ducourouble, M.C.; Durand-Tardif, M.; Esteves, P.J.; Ferrand, N.; Gautier, A.; Haas, C.; Hewitt, G.; Jehl, N.; Joly, T.; Koehl, P.F.; Laube, T.; Lechevestrier, S.; López, M.; Masoero, G.; Menigoz, J.J.; Piccinin, R.; Queney, G.; Saleil, G.; Surridge, A.; Van der Loo, W.; Vicente, J.S.; Viudes de Castro, M.P.; Virag, J.S. and Zimmermann, J.M. (2000). Evaluation and conservation of European rabbit (Oryctolagus cuniculus) genetic resources. First results and inferences. World Rabbit Science, 8:281–315.

Bousseau, S.; Brillard, J.P.; Marquant-Le Guienne, B.; Guérin, B.; Camus, A. and Lechat, M. (1998): Comparison of bacteriological qualities of various egg yolk sources and the *in vitro* and *in vivo* fertilizing potential of bovine semen frozen in egg yolk or lecithin-based extender. Theriogenology, 50: 699–706.

Brito, F.C.; Barth, A.D.; Bilodeau-Goessels, S.; Panich, P.L. and Kastelic, P. (2003). Comparison of methods to evaluate the plasmalemma of bovine sperm and their relationship with *in vitro* fertilization rate. Theriogenology, 60:1539–1551.

Castellini, C. (1996). Recent advances in rabbit artificial insemination. 6th World Rabbit Science, Cong., Toulouse, France, 2: 13-28.

Castellini, C.; Battaglini, M. and Lattaioli, P. (1992). Effects of cryoprotectants and freezing on rabbit semen quality. Journal of Applied Rabbit Research, 15: 431–438.

Chen, Y.; Yang, X. and Foote, R.H. (1989). Timed breeding of rabbits with fresh and frozen-thawed semen and evidence of acrosome alteration of following freezing and thawing. Anim. Reprod. Sci., 18:35–41.

Curry, M.R. (2007). Cryopreservation of mammalian semen. Methods Mol. Biol., 368:303–311.

Dinnyes, A.; Liu, J. and Nedambale, T.L. (2007). Novel gamete storage. Reprod. Fertil.,19:719–731.

Duncan, D.B. (1955). Multiple range and multiple F test. Biometrics, 11: 1-42.

El-Azzazi, F.E. and Hanafy, A.M. (2015). Impact of soy-lecithin based extender on quality and fertility of preserved rabbit semen. Egyptian Journal of Rabbit Science, 25(2): 197 – 209.

Fadl, A.M.; Ghallab, A.M. and Abou-Ahmed, M.M. (2019) Quality Assessment of Cryopreserved New Zealand White Rabbit Spermatozoa in Inra-82 Extender Containing Different Cryoprotectants. World Rabbit Sci., 27: 77-83.

Fiume, Z. (2001). Final report on the safety assessment of lecithin and hydrogenated lecithin. Int. J. Toxicol., 20:21–45.

Forouzanfar, M.; Sharafi, M.; Hosseini, S.M.; Ostadhosseini, S.; Hajian, M. and Hosseini L. (2010). *In vitro* comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of ram semen. Theriogenology, 73:480–487.

Gil, J.; Januskauskas, A.; Haard, M.; Haard, M.G.M.; Johanis, A.; Söderquist, L. and Rodriguez-Martinez, H. (2000). Functional sperm parameters and fertility of bovine semen extended in biociphos plus and triladyl. Reprod. Dom. Anim., 35:69-77.

Gil, J.; Rodriguez-Irazoqui, M.; Lundeheim, N.; Soderquist, L. and Rodriguez- Martinez, H. (2003). Fertility of ram semen frozen in Bioexcell and used for cervical artificial insemination. Theriogenology, 59:1157–70.

Gloria, A.; Carluccio, A.; Contri, A.; Wegher, L.; Valorz, C. and Robbe, D. (2013). The effect of the chamber on kinetic results in cryopreserved bull spermatozoa. Andrology, 1: 879-885.

Graham J.K. and Mocé E. (2005). Fertility evaluation of frozen/ thawed semen. Theriogenology, 64: 492-504.

Hoogewijs, M.K.; de Vliegher, S.P.; Govaere, J.L.; de Schauwer, C.; de Kruif, A.; van Soom, A. (2012). Influence of counting chamber type on CASA outcomes of equine semen analysis. Equine Vet J., 44, 542-549.

Iaffaldano, N.; DiIorio, M. and Rosato, M.P. (2012). The cryoprotectant used, its concentration, and the equilibration time are critical for the successful cryopreservation of rabbit sperm: Dimethyl acetamide versus dimethyl sulfoxide. Theriogenology, 78: 1381–1389.

Jeyendran, R.S.; Van der Ven, H.H. and Zaneveld, L.J.D. (1992). The hypoosmotic swellingtest: an update. Arch. Androl., 29: 105–116.

Jeyendran, R.S.; Van der Ven, H.H.; Perez-Pelaez, M.; Crabo, B.G. and Zaneveld, L.J.D. (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod.Fertil., 70: 219–228.

Kmenta, I.; Strohmayer, C.; Müller-Schlösser, F. and Schäfer-Somi, S. (2011). Effects of a lecithin and catalase containing semen extender and a second dilution with different enhancing buffers on the quality of cold-stored canine spermatozoa. Theriogenology, 75:1095–1103.

Lavara, R.; David, I.; Mocé, E.; Baselga, M. and Vicente, J.S. (2013). Environmental and male variation factors of freezability in rabbit semen. Theriogenology, 79: 582–589.

Mocé, E. and Vicente, J.S. (2009). Rabbit sperm cryopreservation: a review. Anim. Reprod. Sci., 110:1–24.

Mocé, E.; Lavara, R. and Vicente, J.S. (2005). Influence of donor male on the fertility of frozen-thawed rabbit semen after artificial insemination of females from different genotypes. Reproduction in Domestic Animals, 40: 516–521.

Morrell, J.M. (1995). Artificial insemination in rabbits. Br. Vet. J., 151: 477-488.

Moussa, M.; Matinet, V.; Trimeche, A.; Tainturier, D. and Anton, M. (2002). Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen-thawed bull semen. Theriogenology, 57:1695–706.

Nishijima, K.; Kitajima, S.; Koshimoto, C.; Morimoto, M.; Watanabe, T.; Fan, J. and Matsuda, Y. (2015). Motility and fertility of rabbit sperm cryopreserved using soybean lecithin as an alternative to egg yolk. Theriogenology, 84:1172–1175.

Papa, F.O.; Felício, G.B.; Melo-Oña, C.M.; Alvarenga, M.A.; De Vita B. and Trinque C. (2011). Replacing egg yolk with soybean lecithin in the cryopreservation of stallion semen. Anim. Reprod. Sci., 129:73–77.

Pérez-Sánchez, F.; Tablado, L. and Soler, C. (1997). Sperm Morphological Abnormalities Appearing in the Male Rabbit Reproductive Tract. Theriogenology, 47:893-901.

Pillet, E.; Duchamp, G.; Batellierc, F.; Beaumald, V.; Antond, M.; Deshercese, S.; Schmitte, E. and Magistrini, M. (2011). Egg yolk plasma can replace egg yolk in stallion freezing extenders. Theriogenology, 75:105–114.

Quintela, L.A.; Pena, A.I.; Vega, M.D.; Gullon, J.; Prieto, C.; Barrio, M.; Becerra, J.J.; Maseda F. and Herradon, P.G. (2004). Ovulation induction in rabbit does submitted to artificial insemination by adding buserelin to the seminal dose. Reprod. Nutr. Dev., 44: 79–88.

Reed, M.L.; Ezeh, P.C.; Hamic, A.; Thompson, D.J. and Caperton, C.L. (2009). Soy lecithin replaces egg yolk for cryopreservation of human sperm without adversely affecting post-thaw motility, morphology, sperm DNA integrity, or sperm binding to hyaluronate. Fertil. Steril., 92: 1787–1790.

Rodriguez-Martinez, H. and Barth, A.D. (2007). *In vitro* evaluation of sperm quality related to *in vivo* function and fertility. Soc. Reprod. Fertil. Suppl., 64: 39–54.

Rosato M.P. and Iaffaldano N. (2013). Cryopreservation of rabbit semen: comparing the effects of different cryoprotectants, cryoprotectant-free vitrification, and the use of albumin plus osmo-protectants on sperm survival and fertility after standard vapor freezing and vitrification. Theriogenology, 79:508–516.

Safaa, H.M.; Vicente, J.S.; Lavara, R. and Viudes de Castro, M.P. (2008). Semen evaluation of two selected lines of rabbit bucks. World Rabbit Sci., 16: 141 – 148.

Salmani, H.; Towhidi, A.; Zhandi, M.; Bahreini, M. and Sharafi, M. (2014). *In vitro* assessment of soybean lecithin and egg yolk based diluents for cryopreservation of goat semen. Cryobiology, 68:276–280.

Santolaria, P.; Vicente-Fiel, S.; Palacín, I.; Fantova, E.; Blasco, M.E.; Silvestre, M.A. and Yániz, J.L. (2015). Predictive capacity of sperm quality parameters and sperm sub populations on field fertility after artificial insemination in sheep. Anim. Reprod. Sci., 163: 82–88.

SAS (2004). SAS/STAT User's Guide: Version 9.1.3. SAS Institute Inc., Cary, NC., USA. Ani. Sci., 6, 291-299.

Shams-Borhan, G. and Harrison, R.A.P. (1981). Production, characterization, and use of ionophore-induced: calcium-dependent acrosome reaction in ram spermatozoa. Gamete Res., 4: 407–432.

Simpson, A.M.; Swan, M.A. and White, I.G. (1987). Susceptibility of epididymal boar sperm to cold shock and protective action of phosphatidylcholine. Gamete. Res., 17:355–373.

Thun, A., Hurtado, M. and Janett, F. (2002). Comparison of biociphos-plus R and tris-egg yolk extender for cryopreservation of bull semen. Theriogenology, 57:1087–1094.

van Wagtendonk-de Leeuw, A.M.; Haring, R.M.; Kaal-Lansbergen, L.M. and den Daas, J.H. (2000). Fertility results using bovine semen cryopreserved with extenders based on egg yolk and soy bean extract. Theriogenology, 54:57–67.

Vicente, J.S.; Viudes de Castro, M.P.; Lavara, R. and Lavara, F. (2000). Effect of male line on prolificacy from does inseminated with low sperm doses. Paper presented at the 7th World Rabbit Congress, 4–7 July, Valencia, Spain, pp., 1273–1277.

Vick, M.M.; Bateman, H.L.; Lambo, C.A. and Swanson, W.F. (2012). Improved cryopreservation of domestic cat sperm in a chemically defined medium. Theriogenology, 78:2120–2128.

Vishwanath, R. and Shannon, P. (2000). Storage of bovine semen in liquid and frozen state. Anim. Reprod. Sci., 62:23–53.

Watson, P.F. (2000). The causes of reduced fertility with cryopreserved semen. Anim. Reprod. Sci., 60: 481-492.

Yildiz, C.; Bozkurt, Y. and Yavas, I. (2013). An evaluation of soybean lecithin as an alternative to avian egg yolk in the cryopreservation of fish sperm. Cryobiology, 67:91–94.

ليسيثين فول الصويا كبديل لصفار البيض بمخفف الترس لتجميد السائل المنوي لأرانب الأبري

جورج عزت يونان[١]، أحمد محمد شهاب الدين[١]، عبد الخالق السيد عبد الخالق[٢]، محمد عبد الجواد الشربينى[١] وعزة احمد حلمي[١]

[١] معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، مصر .

[٢] قسم الإنتاج الحيواني، كلية الزراعة، جامعة المنصورة، مصر.

تهدف الدراسة المقدمه لتوضيح إمكانية إستخدام ليسيثين الصويا كبديل لصفار البيض لحفظ السائل المنوى لأرنب الأبرى بالتجميد. استخدم في هذه الدراسة مخفف الترس مع صفار البيض كمخفف أساسي، وتم استبدال صفار البيض (١٨ ٪) بليسيثين فول الصويا (١ جم) كمخفف ثاني و١,٥ جم في المخفف الثالث أو ٢ جم في المخفف الرابع. تم تخفيف السائل المنوي بمعدل ١: ٤ وتقييم خصائص الحيوانات المنوية مجهريا بعد التخفيف والموازنة والإسالة وبواسطة الحاسب الالي (CASA) فقط في السائل المنوي بعد الإسالة. أظهرت النتائج تحسن معنوي ($P < 0.05$) في النسبة المئوية ومعدلات الحركة التقدميه وسلامة الغشاء البلازمى والأكروسوم للحيوانات المنوية بعد جميع مراحل الحفظ (تخفيف – موازنة وتجميد). ارتفعت الحركة الكلية، التقدمية الكلية والبطيئة، بينما انخفضت الحركة الغير تقدمية والحيوانات المنوية الغير متحركة في السائل المنوي للمخفف الثالث الخصائص الديناميكية للحيوانات المنوية (سرعة المنحنى الخطية والاستقامة) ارتفعت بشكل معنوي ($P < 0.05$) بينما انخفض التمايل في السائل المنوي المخفف مع ليسيثين الصويا (١,٥جم) مقارنة بصفار البيض. أظهرالسائل المنوى للمخفف الثالث أعلى خصوبة (6.68 خلفة/أم) مقارنة بـ ٤,٩٦ و٤,٧٢ و٢,٨ خلفة/أم للسائل المنوى للمخففات الأول، الثاني والرابع، على التوالي. كان معامل الارتباط منخفض معنويا بين اختبار سلامة الأكروسوم للحيوانات المنوية وعدد الخلفات في البطن ($r = 0.691$)، وكان الأعلى معنويا مع الخصوبة ($r = 0.921$). وارتبطت الحركة التقدمية والحركة التقدمية البطيئة مع معدل الحمل معنويا ($r=0.811$) و ($r=0.778$) وكذلك مع الخصوبة معنويا ($r=0.898$) و($r=0.801$). وتوصى الدراسة بأدراج ليسيثين فول الصويا عند مستوى ١,٥ ٪ كبديل لصفار البيض (١٨ ٪) في مخفف الترس لما له من تأثير إيجابي على خصائص الحيوانات المنوية الفيزيائية ومعدل الخصوبة في الحيوانات المنوية للأرانب المجمده. هناك حاجة إلى مزيد من الدراسات لدراسة تأثير ليسيثين فول الصويا في مخفف الترس على الخصائص الديناميكية والتمثيل الغذائى للحيوانات المنوية في السائل المنوي للأرانب.