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(Original Article)

Comparison Between Egg Yolk and Soybean Lecithin in a Tris-Based Extender on Ram Sperm Quality

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Abstract

This study aimed to compare between the addition of egg yolk (EG; 20%) and soybean lecithin (SL; 1%) to a Tris-based extender on total sperm motility, viability and reheotaxis parameters, including Positive Rheotaxis (PR%); Straight Line Velocity (VSL, µm/s); Curvilinear Velocity (VCL, µm/s); Linearity (LIN = VSL/VCL) Average Path Velocity (VAP, µm/s) and Beat Cross Frequency (BCF, Hz) in semen. Sperm kinetics and rheotaxis parameters were investigated using computer-assisted sperm analysis (CASA) with regulated flow velocity. Semen was collected using artificial vagina once weekly from 6 fertile rams at 9:00 a.m. during the late spring and autumn. Samples of pooled semen were diluted in one of the two fractions, with the primary extender composed of tris-based extender, with 20% egg yolk for the control group and the percentages of soybean lecithin (1%) for the treatment group.

Results revealed that there were no significant differences in total sperm motility, viability and PR % and VAP between the two extender groups. Also, there were significantly higher VCL and BCF in the soybean lecithin extender group compared to the egg yolk extender group. In the contrast there were significantly higher in VSL and LIN in the egg yolk extender group compared to the soybean lecithin group. In conclusion, the extenders were supplemented with 20% egg yolk, and 1.0% soybean lecithin had relatively similar effects on total sperm motility, viability and reheotaxis parameters. We concluded that a commercially available soybean lecithin (1%) was an alternative to an egg yolk-based extender in preserving motility, viability and reheotaxis parameters of semen.

Keywords: *Egg yolk extender, Ram semen, Soybean lecithin.*

Introduction

In light of the world's constantly expanding population, it is imperative to increase the sustainability and efficiency of animals used for food production. Thus, improving the fertility of livestock, especially cattle, sheep and goats around the world is essential to overcome this problem. Sheep and goats can provide meat, wool, skin, and milk for

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the modern livestock industry with the development of modern intensive agriculture and the use of few sires for breeding and increased sheep reproduction by selecting the best breeders (Lvchunrong *et al.,* 2019).

The modern animal industry is based on the use of artificial insemination (AI) and the cryopreservation of semen, which has preserved the species, allowed for improved animal production, an accelerated rate of genetic selection is key for genetic improvement programs. In addition, sperm cryopreservation is a useful method for maintaining the composition and functionality of semen and aids in the creation of a genetic resource bank for the livestock industry (Lone *et al.,* 2017). Also, it is decreasing the spread of sexually transmitted diseases (Manafi, 2011). Rams are less likely than other domestic animals to use artificial insemination due to the difficulty of using frozen-thawed sperm (Salamon and Maxwell, 2000).

However, the spermatozoa are exposed to temperature variations during cryopreservation, which lead to various types of cryoinjuries and significant biological and functional alterations that impair their capacity to function and reduce the quality of post-thawing sperm (Evans *et al*., 1987; Salamon and. Maxwell 2000; Grossfeld *et al.,* 2008; Sharafi *et al.,* 2009 and Chelucci *et al.,* 2015). During freezing and thawing, intracellular ice crystal formation, oxidative stress, osmotic pressure, pH, and cold shock all lower the functional maintenance of semen. (Januskauskas *et al*., 2003 and Gangwar *et al.,* 2018). So, semen is supplemented with extenders to maintain sperm viability and fertility during storage. Semen extenders are vital for sperm preservation, supporting fertilization by maintaining sperm metabolism, regulating pH, preventing bacterial contamination, and reducing cryogenic damage (Malik *et al.,* 2018 and Raheja *et al*., 2018). They regulate pH (Liu *et al.*, 2016), serve as an energy source (Mohamed and Shaarawy, 2019), provide antioxidant support (Mousavi *et al.,* 2019), contain antibiotics to prevent contamination (Schulze *et al.,* 2020) and help mitigate freezing shock (Tariq *et al.,* 2020). Extenders are employed in both long-term cryopreservation and short-term cooling (Johnston *et al.,* 2012). Cold shock and osmotic stress during the freeze-thaw process led to decreased post-thaw sperm quality (Salamon and Maxwell 2000). Appropriate extenders and cyoprotectant additives can stop the majority of this damage (Gil *et al.* 2003; Barbas and Mascarenhas 2009).

Glycerol is the most often used cryoprotectant for sperm cryopreservation (Polge *et al.,* 1949 and Holt, 2000). The discovery of the cryoprotective properties of glycerol in 1949 (Polge *et al.*, 1949) led to the ability to freeze spermatozoa. Glycerol can penetrate the cell membrane and act as a penetrating cryoprotectant (Aires *et al.,* 2003). In addition to the glycerol, an energy source substrate (glucose or fructose), a source of lipoprotein or high-molecular-weight material (like milk and egg yolk) to prevent cold shock, ionic or nonionic substances to maintain an appropriate pH and osmotic pressure, and additional additives like enzymes and antibiotics should all be included in the extender. (Vishwanath and Shannon, 2000 and Aires *et al*., 2003).

Animal products such as egg yolk are a reservoir of phospholipids and cholesterol that are used to protect the plasma membrane and acrosome and to prevent the spermatozoa from the damaging effects of freezing and cooling (Forouzanfar *et al.,* 2010; Hu *et al.,* 2010; Nishijima *et al.,* 2015; Swelum *et al.,* 2018). The effective

components of egg yolk are the lecithin and the low-density lipoprotein protein, which prevents cold shock (Salamon and Maxwell, 2000; Watson, 2000; Manjunath *et al.,* 2002; Medeiros *et al.* 2002; Moussa *et al*., 2002; Paulenz *et al.,* 2002; Bergeron *et al.,* 2004; Bencharif *et al*., 2010 and Forouzanfaretal., 2010 and Ustuner *et al.,* 2016).

However, in recent years, there were many who opposed the usage of milk and egg yolk because of the wide variability of their constituents, which makes evaluating their beneficial components complex (Moussa *et al*., 2002 and Gil *et al*., 2003). That makes it difficult preparations of standard extender (Aires *et al.,* 2003; Forouzanfar *et al.,* 2010 and Emamverdi *et al.,* 2013). Furthermore, milk and egg yolks increase the risk of microbial infection and exotic diseases through the transportation in the international exchange of stored semen (Aires *et al.,* 2003; Bousseau *et al.,* 1998; Sharafi *et al.,* 2015; Beccaglia *et al.,* 2009; Chelucci *et al.,* 2015; Alves *et al.,* 2019; Mafolo *et al*., 2020) and microbiological pollution that permits the subsequent synthesis of endotoxins (Forouzanfar *et al.,* 2010 and Vidal *et al.,* 2013). This has raised boundaries in international semen transport legislation in many countries because of biosecurity issues (Althouse, 2008). Furthermore, egg yolk has been identified as an antigenic substance that triggers the production of antibodies in the reproductive system and systemic circulation (Üstüner *et al.,* 2014). In addition, sperm agglutination and pre-capacitation are enhanced by egg yolk, which has impact on sperm quality (Emamverdi *et al*., 2013 and Hu *et al.,* 2011).

Therefore, replacing the egg yolk with other cryoprotectants, like soybean-based plant-derived lecithin, would be a clear, pathogen-free, and non-animal substitute for the cryopreservation of both human and animal sperm. Researchers have been studying to find a replacement for the main component of sperm extenders for a few years instead of avian egg yolk. Plant-originated soybean lecithin could be a better alternative to overcome of this matter (Chelucci *et al.,* 2015). The similar component found in egg yolk extenders such as (low-density lipoprotein and phospholipids, such as, Phosohatidylcholines) found in soy lecithin could be used in semen cryopreservation extenders in place of egg yolk (Bergeron and Manjunath, 2006; Forouzanfar *et al.,* 2010 and Salmani *et al.,* 2014). Therefore, a plant-based substitute for soy lecithin is utilized in semen extenders to protect sperm cell membranes from cold shock during cryopreservation (Motlagh *et al.,* 2014 and Layek *et al.,* 2016). According to our previous research, soy lecithin can enhance the quality and potential for fertility of post-thawed semen (Forouzanfar *et al.,* 2010). Additionally, soybean lecithin enhanced the ram sperm's acrosome integrity and mitochondrial capacity (Emamverdi *et al.,* 2013).

Recently, soybean lecithin (SL) has been successfully applied as a cryoprotectant that is extracellular for cryopreservation of semen in ram (Anel *et al*., 2006; Forouzanfar *et al*., 2010; Sharafi *et al*., 2009; Del Valle *et al.,* 2012 and Sharafi *et al.,* 2009), goat (Salmani *et al.,* 2013; Jiménez-Rabadán *et al.,* 2012; Roof *et al*., 2012; Vidal *et al.,* 2013), bull (Aires *et al.,* 2003), buffalo (Akhter *et al.,* 2012) and human (Reed *et al.,* 2009).

1% of soybean lecithin was successfully used in our previous study to cryopreserve semen in goat (Salmani *et al*., 2013) and in ram (Forouzanfar *et al*., 2010 and Emamverdi *et al.,* 2013). Soybean lecithin and egg yolk have optimal concentrations of

1% and 20%, respectively for extenders of ram semen and it suggests that soybean lecithin can be used as a substitute for egg yolk (Forouzanfar *et al*., 2010). Thus, this study aimed to compare between the addition of egg yolk (EG; 20%) and soybean lecithin (SL; 1%) to a Tris-based extender on total sperm motility, viability and reheotaxis parameters. Sperm kinetics and rheotaxis parameters were investigated using computer-assisted sperm analysis (CASA) with regulated flow velocity.

Materials and Methods

Chemicals

For this experiment, Elgomhoria Pharmaceuticals (Cairo, Egypt) provided all of the substances. SU-8-25 negative resist (MicroChem, Newton, CA), diacetone alcohol (Sigma Aldrich, Steinheim, Germany), glass wafers (Howard Glass, Worcester, MA), and poly-dimethylsiloxane (PDMS) (Syllgard-184, Dow Corning, Midland, MI) were among the materials used to fabricate the microchannel.

Experimental animals and management

The current study was conducted at Assiut University, Faculty of Agriculture, Experimental Farm of Animal Production Department.

The study started on the 28th of August 2024 and extended to the 15th of December, 2025. A total number of 6 apparently healthy, mature and fertile native rams (3-4 years old) were included in this study. Rams were housed in semi-open pens in normal environmental conditions. Rams were fed a concentrate feed mixture according to NRC (1985) that included 13.67% crude protein, 1.96% crude fat, 11.2% crude fiber, 2.32 Mcal/kg metabolizable energy, 1.22% limestone, 0.52% calcium, 0.31% phosphorus. Rams were fed *ad libitum* wheat straw in addition to the concentrate feed mixture. All day long, water was available.

Semen collection and initial evaluation

A sterile artificial vagina was used for semen collection from rams during the second mounting once weekly. A second and final semen sample was taken by artificial vagina following a 20-minute rest period if the first sample was low quality. After collection, the semen was transferred to the laboratory for semen evaluation within 5 min. The minimum standard for the quality of every semen ejaculate was: a volume of 0.5–1.5 mL, sperm concentration $\geq 2.5 \times 10^9$ sperm/ml, motility $\geq$ 75%, and abnormal morphology $\leq$ 15%., as described previously (Salmani *et al.,* 2014).

Preparation of extenders

Samples of pooled semen were diluted in one of the two fractions, with the primary extender composed of tris-based extender, with 20% egg yolk for the control group and the percentages of soybean lecithin (1%) for the treatment group. The primary extender composed of Tris (3.6342 g), glucose (0.5044 g), citric acid (1.8252 g), and glycerol (7 mL extenders per 100 mL).

Frozen semen and thawing

The ratio of 1:3 was used to dilute the semen samples into the cryodiluents and then gradually cooled for two hours to 4 ˚C. 0.5 mL Germany straws (transparent Ref.:

13408/0010) were used to pack the samples. After that, the straws were positioned horizontally on a rack, frozen for 15 minutes in liquid nitrogen vapor (3 cm above the liquid), dipped in liquid nitrogen, and kept until they thawed. For thawing, straws spent 30 seconds in a water bath at 37°C (Sun *et al.,* 2020).

Sperm motility and motion parameters

The samples of semen after thawing were analyzed for the total sperm motility at 37° C and viability. Reheotaxis parameters including**,** Positive Rheotaxis (PR%); Straight Line Velocity (VSL, µm/s); Curvilinear Velocity (VCL, µm/s)**;** Linearity (LIN = VSL/VCL) Average Path Velocity (VAP, µm/s) and Beat Cross Frequency (BCF, Hz) (Fig. 1). The total sperm motility**,** viability and parameter of rheotaxis were examined by a computer-assisted sperm analysis (CASA) system that we previously created to describe sperm movement in microfluidic settings (Elsayed *et al*., 2015) and described here in brief. An Optika XDS-3 inverted microscope with phase contrast and a 40× objective, a Tucsen ISH1000 camera (30 frames per second), and a CASA plugin for Image-J make up the CASA system. An open-source imaging processing program called Image-J was made available by the National Institutes of Health (NIH) at http://imagej.nih.gov/ij/.

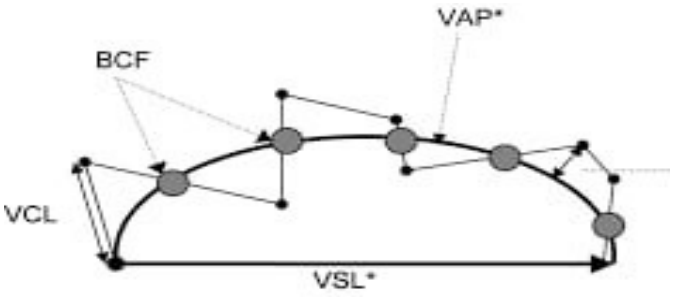

Figure 1. Diagram shows Straight Line Velocity (VSL, µm/s); Curvilinear Velocity (VCL, µm/s); **Linearity (LIN = VSL/VCL) Average Path Velocity (VAP, µm/s) and Beat Cross Frequency (BCF, Hz)**.

Microfluidic Device fabrication

PDMS microchannels

Microchannels were fabricated by using soft lithography, as extensively described by (Duffy *et al.,* 1998).

The monomer and curing agent were combined at a weight ratio of 10:1 to create polydimethylsiloxane (PDMS), which was subsequently degassed in a vacuum desiccator and applied to the SU-8 master. Channels were cut, peeled off the master, and punctured to enable tubing connections at microchannel inlets and outlets after PDMS was cured in an oven at 120°C for 30 minutes. Lastly, as previously mentioned, the PDMS microchannels were firmly bonded to a microscope glass slide using a portable corona treater (Electro-Technic Products, Chicago, IL) (Haubert *et al.,* 2006). 200 µm × 20 µm (WxH) PDMS microchannel dimensions were employed in this investigation. In this investigation, the PDMS (microchannel 1) is depicted in Figure 2a.

Lithography craved microchannel.

The chip is composed of two Polymethyl Methacrylate (PMMA) components; as illustrated in Fig. 2(b), the upper half has intake ports, while the lower part has the etched channel structure.

The direct write laser machining method created the generated channel. For channel manufacturing, VLS3.5 UNEVERSAL LASER SYSTEMS with a 30-Watt CO2 laser tube and a 100µm laser beam diameter were employed. In order to reduce roughness at the smallest possible dimensions, we achieved the best engraving by setting the laser beam power to 5 Watts (6%) and the engraving speed to 25 mm s-1 (10%) laser head translation speed. As seen in Fig. 2(c), the channel profile has a Gaussian shape.

The top portion and the bottom portion, which houses the channel, were bonded using the thermocompression method with acetic acid at 115°C and 1 N for seven minutes. Better bonding at lower temperatures and bonding times were attained by heating with acetic acid (Nasser *et al.*, 2019). The PMMA microchannel employed in this investigation had dimensions of 200 µm × 100 µm (W×H). The PMMA microchannel (microchannel 2) employed in this investigation.

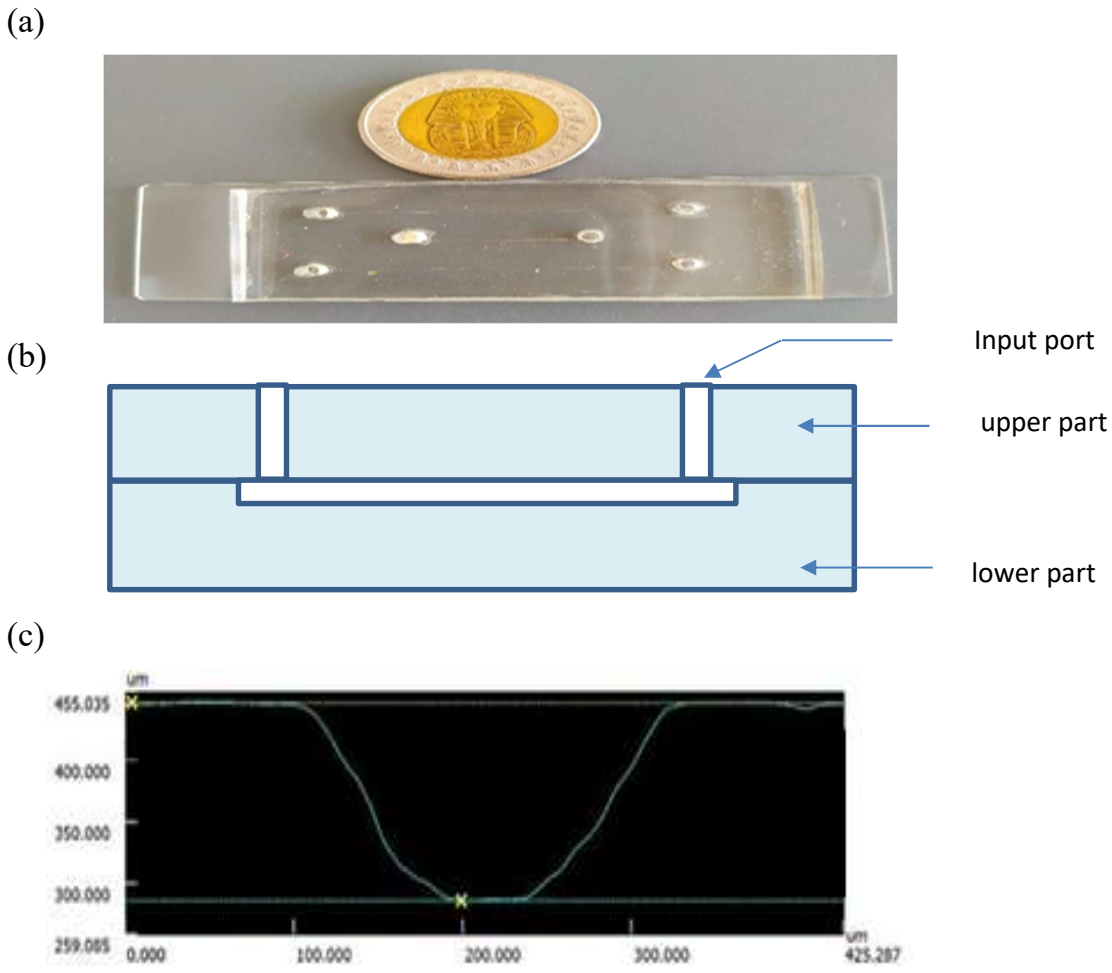

Figure 2. (a) Picture of microchannel 2 (PMMA channel with 100 µm height) used in the present study. (b) Chip schematic drawing for microchannel 2. (c) microchannel 2 profile.

Flow generation

By maintaining the liquid level at the inlet reservoir higher than that in the output reservoir by a height difference of Δh, hydrostatic pressure was utilized to create liquid flow inside the microchannel. A straightforward and inexpensive technique for creating flow inside microchannels, hydrostatic flow generation avoids the pulsing flow that characterizes syringe pumps (Moscovici *et al.*, 2010) Using the Darcy–Weisbach equation, one may determine the average velocity inside the microchannel (Munson *et al*., 2002).

$$\text{Vav} = \frac{(2\rho g Dh \Delta h)}{C\mu L} \quad (1)$$

where C is the friction factor (f) × Reynolds number (Re), ρ is the liquid density, g is the gravitational acceleration, Dh is the channel hydraulic diameter, Δh is the height difference between reservoirs, μ is the liquid viscosity, and L is the microchannel length (Munson *et al.,* 2002). For channels with an aspect ratio less than 0.5, equation (2) was used to determine the velocity profile inside the channel (Shah and AL, 1978).

$$\frac{V}{V_{av}} = \left(\frac{m+1}{m}\right)\left(\frac{n+1}{n}\right)\left[1 - \left(\frac{y}{b}\right)^n\right]\left[1 - \left(\frac{z}{a}\right)^m\right] \quad (2)$$

where a and b are the channel's width and height, respectively, and V is the liquid velocity at any point in the channel. However, z and y are the coordinates (measured from the centerline) of any point in the channel where V is required, and m and n are numerical parameters dependent on the channel aspect ratio α = b/a according to equations (3) and (4).

$$m = 1.7 + 0.5\alpha^{-1.4} \quad (3)$$

$n=2$

$$n = 2 + 0.3(\alpha - 1/3)\begin{cases} \alpha < \frac{1}{3} \\ \alpha \geq \frac{1}{3} \end{cases} \quad (4)$$

Average liquid velocity in all experiments reported kept here at 32 ± 0.06μm/s.

CASA analysis of sperm kinematics and rheotaxis parameters

According to Elsayed *et al*. (2015), all recorded videos were examined using a custom computer-assisted sperm analysis (CASA) system (Department of Mechanical Engineering, Faculty of Engineering, Assiut University, Egypt). The plugin is available for download at http://www.assiutmicrofluidics.com/research/casa.

An Optika XDS-3 inverted microscope with phase contrast (also at 40 × objectives) connected to a Tucsen ISH1000 camera at 30 frames per second was used to record videos of sperm cells. The following parameters were established after recorded movies were processed using a custom CASA: curvilinear velocity (VCL, μm/s),

straight-line velocity (VSL, µm/s), and average path velocity (VAP, µm/s) are examples of velocity parameters. Progression parameters include beat cross frequency (BCF, Hz) and linearity (LIN = VSL/VCL, %).

Statistical analysis

The mean ± (SEM) was used to represent all data. Dunn's multiple comparisons were used after Kruskal-Wallis ANOVA on ranks to compare mean values. Either Graphpad Prism v5 (Graphpad Software, Inc.) or JMP v5.0.1 (SAS campus drive) were used to calculate all statistics. Significant differences were defined as $p < 0.05$.

Results and Discussion

Table 1. The effect of different extenders on total sperm motility and viability (Lsmean ± SEM)

Parameters	Soya lecithin 1%	Egg yolk 20%	P value
Total sperm motility %	51.21 ± 1.21	50.57 ± 0.77	0.8017
Viability	58.20 ±1.65	60.40±1.78	0.7014

Table 2. The effect of different extenders on sperm rheotaxis and sperm kinematics (Lsmean ± SEM)

Parameters	Soya lecithin 1%	Egg yolk 20%	P value
PR %	48.60±0.3555	48.67±0.4856	0.9029
VCL (µm/s)	23.66±0.03211*	23.55±0.03830	0.0446
VAP (µm/s)	23.30 ±0.03324	23.24±0.03625	0.2636
VSL (µm/s)	17.60±0.05119	17.91±0.07995**	0.0015
LIN (VSL/VCL)	0.7537±0.001707	0.7689±0.003366***	0.0002
BCF (Hz)	1.441±0.009253**	1.389±0.01533	0.0050

PR%, positive rheotaxis; VCL, curvilinear velocity**;** VAP**,** average path velocity**;** VSL, straight line velocity; LIN, linearity; BCF, beat cross frequency. Significance: *$p < .05$, **$p < .01$, ***$p < .001$.

The results of this study showed that total sperm motility and viability after thawing of sperm showed no statistically significant difference between the two extenders, with values of soya lecithin (51.21 ± 1.21% and 58.2 ±1.68) and egg yolk (50.57 ± 0.77 and 60.4±1.68) respectively, (Table 1). These results are in agreement with those obtained by Emamverdi *et al.* (2013) in sheep and Salmani *et al*. (2014) in goats they observed that there was no significant difference between the extender contained 1% soybean lecithin and extender contained 20% egg yolk for ram total sperm motility and viability. Also, they found that the similar component found in egg yolk extender such as (low-density lipoprotein and Phosohatidylcholines) was found in soy lecithin and may be used in semen cryopreservation extenders in place of egg yolk (Bergeron and Manjunath, 2006; Forouzanfar *et al.,* 2010 and Salmani *et al.,* 2014). Therefore, it was concluded previously that a plant-based substitute for soy lecithin is utilized in semen extenders to protect sperm cell membranes from cold shock during cryopreservation (Motlagh *et al.,* 2014 and Layek *et al*., 2016).

As alternatives for extenders containing lecithins of animal origin, commercial soy lecithin and liposome-based extenders have been developed, for instance, for buffalo spermatozoa (Kumar *et al*., 2015) and bull spermatozoa (Leite *et al*., 2010 and Röpke *et al.,* 2011). Soy lecithin is believed to function similarly to lecithins found in milk or egg

yolks (Belala *et al.,* 2016) however Liposomes are molecules with a chemical definition that can transfer lipid and cholesterol (or other molecules of interest) to the sperm plasma membrane (Ansari *et al.*, 2016). Soybean lecithin (SL) has been successfully applied as an extracellular cryoprotectant for cryopreservation of semen in ram (Anel *et al.,* 2006; Forouzanfar *et al.,* 2010; Sharafi *et al.,* 2009; Del Valle *et al.,* 2012 and Sharafi *et al.,* 2009), goat (Salmani *et al.,* 2013; Jiménez-Rabadán *et al.,* 2012; Roof *et al.,* 2012; Vidal *et al.,* 2013), bull (Aires *et al.*, 2003), buffalo (Akhter *et al.,* 2012) and human (Reed *et al.,* 2009).

As shown in Table 2, the percentage of positive rheotaxis (PR%) showed no statistically significant difference between the two extenders, with values of 48.60±0.36% for soya lecithin and 48.67±0.49% for egg yolk (*P = 0.9029*). This indicates that both extenders maintain comparable levels of forward motility. Positive rheotaxis is an important parameter for assessing sperm motility, as it reflects the ability of spermatozoa to swim against fluid flow, which is crucial for successful fertilization (García-Álvarez *et al.,* 2011). The comparable PR% values between the two extenders suggest that both soy lecithin and egg yolk similarly support the maintenance of forward motility in ram spermatozoa during preservation.

This finding aligns with previous studies demonstrating that plant-based extenders, such as that containing soy lecithin, can effectively replace traditional egg yolk-based extenders without compromising sperm motility (Aboagla & Terada, 2003; Najafi *et al.,* 2013). The lack of significant differences may be attributed to the similar protective effects of soy lecithin and egg yolk lipoproteins on sperm membrane integrity, which is essential for maintaining motility (Bergeron *et al.,* 2004).

Further research could explore the long-term effects of these extenders on sperm functionality, including capacitation and acrosome reaction, to ensure that soy lecithin is a viable alternative to egg yolk in semen preservation protocols.

The results demonstrate that curvilinear velocity (VCL, μm/s) was significantly higher ($P < 0.05$) in the soy lecithin extender group (23.66 ± 0.03 μm/s) compared to the egg yolk group (23.55 ± 0.04 μm/s) (Table 2). These suggest slightly enhanced movement dynamics in the former. VCL is a key parameter of sperm kinematic activity, reflecting the actual path taken by spermatozoa, including all deviations and lateral movements (Mortimer, 1997). The higher VCL observed in the soy lecithin group suggests that this extender may better preserve sperm hyperactivation potential, which is crucial for sperm penetration through the female reproductive tract (Suarez and Ho, 2003).

The slight but statistically significant improvement in VCL with soy lecithin could be attributed to its phospholipid composition, which may provide better membrane fluidity and energy metabolism support compared to egg yolk (Aboagla & Terada, 2003). Previous studies have reported that plant-based extenders, such as that containing soy lecithin, can enhance sperm motility parameters by reducing oxidative damage and maintaining mitochondrial function (Najafi *et al*., 2013).

Further studies should investigate whether this slight increase in VCL translates into improved fertilization rates in vivo or in vitro.

The average path velocity (VAP, μm/s) showed no statistically significant difference ($P = 0.2636$) between the soy lecithin (23.3 ± 0.03 μm/s) and egg yolk (23.2 ± 0.04 μm/s) extenders (Table 2), indicating a stable velocity parameter across both treatments. VAP represents the smoothed trajectory of sperm movement and is an important indicator of progressive motility, which is essential for sperm migration through the female reproductive tract (El-Sherry *et al.,* 2014). The lack of significant variation suggests that both extenders similarly support the maintenance of sperm progressive motility during preservation.

This finding aligns with previous studies indicating that soy lecithin-based extenders can effectively replace egg yolk-based extenders without compromising key sperm kinematic parameters (El-Sherry *et al*., 2014; Najafi *et al*., 2013). The comparable VAP values between the two groups imply that both extenders provide adequate protection to sperm membranes, ensuring consistent energy metabolism and flagellar function (Aboagla and Terada, 2003).

The stability in VAP across both treatments reinforces the potential of soy lecithin as a viable alternative to egg yolk in semen extenders, particularly in terms of maintaining progressive motility. Future research could explore the long-term effects of these extenders on sperm functionality, including their impact on fertility rates in artificial insemination trials.

The results demonstrate a statistically significant difference ($P = 0.0015$) in straight line velocity (VSL, μm/s) between the two extenders, with higher values observed in the egg yolk group (17.91 ± 0.08) compared to the soy lecithin group (17.60 ± 0.05) (Table 2). This suggests that spermatozoa preserved with egg yolk move in a straighter trajectory. VSL is a critical parameter that measures the straight-line distance traveled by spermatozoa per unit time, reflecting their ability to maintain linear progressive motility (Amann and Waberski, 2014). The superior VSL values in the egg yolk group suggest that this extender better supports the maintenance of straight-line movement patterns in preserved ram spermatozoa.

This finding is consistent with previous research indicating that egg yolk-based extenders may provide better protection for sperm membrane integrity during cryopreservation (Muiño-Blanco *et al.,* 2008). The phospholipid composition of egg yolk, particularly its high content of phosphatidylcholine and cholesterol, may help in stabilizing sperm membranes and maintain the structural organization of the flagellum, thereby promoting straighter swimming trajectories (Purdy and Graham, 2004). In contrast, while soy lecithin provides similar membrane protection, its different lipid profile might result in slightly altered motility characteristics (Bousseau *et al.,* 1998).

The observed difference in VSL could have important biological implications. Spermatozoa with higher VSL values are generally more efficient at navigating the female reproductive tract and reaching the site of fertilization (Holt and Van Look, 2004). This may be particularly relevant for ovine reproduction, where sperm transport mechanisms favor progressively motile spermatozoa (Gadella, 2013). However, it's important to note that while statistically significant, the absolute difference in VSL

values between groups was relatively small (0.31 μm/s), and the clinical significance of this difference warrants further investigation.

These results are aligned with studies comparing different cryoprotectants in small ruminant semen preservation (Salvador *et al.,* 2006). The superior performance of egg yolk in maintaining VSL may be attributed to its ability to better protect sperm membranes from cold shock and ice crystal damage during the freezing process (Watson, 2000). However, the growing interest in plant-based extenders due to sanitary concerns and regulatory restrictions continues to drive research into optimizing soy lecithin formulations (Bergeron and Manjunath, 2006).

Future studies should examine whether these differences in VSL translate to improved fertility outcomes in artificial insemination programs. Additionally, research could focus on modifying soy lecithin formulations to better mimic the beneficial effects of egg yolk on sperm motility patterns (Aires *et al.,* 2003).

The results revealed a statistically significant difference (P = 0.0002) in linearity (LIN = VSL/VCL) between the two extenders, with higher values observed in the egg yolk group (0.7689 ± 0.0034) compared to the soy lecithin group (0.7537 ± 0.0017) (Table 2), reflecting improved directionality of sperm movement under egg yolk-based preservation. This parameter, which represents the straightness of sperm movement by calculating the ratio of straight-line velocity to curvilinear velocity, indicates that spermatozoa preserved in egg yolk exhibit significantly improved directional movement (El-Sherry *et al.,* 2014).

The superior LIN values in the egg yolk group suggest that this extender better maintains the structural integrity of sperm flagella during preservation, enabling more linear swimming patterns (Gillan *et al.,* 2005). This finding aligns with previous work demonstrating that egg yolk components, particularly low-density lipoproteins, provide superior protection to sperm membranes during cryopreservation (Bergeron *et al*., 2004). The enhanced directionality observed may be attributed to egg yolk's ability to better stabilize the axonemal structure and mitochondrial sheath, which are crucial for maintaining straight swimming trajectories (Verstegen *et al.,* 2002).

From a biological perspective, the improved linearity observed with egg yolk preservation could have important implications for sperm transport within the female reproductive tract (Suarez, 2008). Spermatozoa with higher linearity values are generally more efficient at navigating cervical mucus and progressing through the uterotubal junction, which may enhance their fertilizing potential (Katz *et al.,* 1990). This is particularly relevant for ovine reproduction, where efficient sperm transport is critical for successful fertilization (Holt, 2000).

The difference in LIN values between extenders may reflect variations in their protective mechanisms. While both extenders provide membrane stabilization, egg yolk appears to better preserve the structural components responsible for flagellar beating patterns (Rijsselaere *et al.,* 2003). This could be due to specific interactions between egg yolk lipoproteins and sperm membrane domains that influence motility characteristics (Muiño-Blanco *et al.,* 2008).

These findings support the continued use of egg yolk-based extenders in applications where optimal preservation of sperm motility patterns is crucial (Salamon and Maxwell, 2000). However, the relatively small absolute difference in LIN values (0.0152) suggests that soy lecithin may still be a viable alternative for many applications, particularly where regulatory or sanitary concerns favor plant-based extenders (Aires *et al.,* 2003).

Future research should investigate whether these differences in linearity translate to improved fertilization rates in vivo and explore modifications to soy lecithin formulations that might better preserve sperm movement characteristics (El-Sherry *et al.,* 2014).

The results demonstrate a statistically significant difference ($P = 0.0050$) in Beat cross frequency (BCF, Hz) between the two extenders, with higher values observed in the soy lecithin group (1.441 ± 0.009) compared to the egg yolk group (1.389 ± 0.015) (Table 2; Fig.3), indicating increased tail-beating activity and potentially higher energetic motion. BCF, which measures the frequency of sperm tail beats across their average path, suggests that spermatozoa preserved in soy lecithin exhibit more vigorous tail movement patterns (El-Sherry *et al*., 2017). This finding indicates that soy lecithin may better maintain the metabolic activity necessary for flagellar beating during cryopreservation.

The elevated BCF in the soy lecithin group could be attributed to differences in the lipid composition between the two extenders (Yeste *et al.,* 2017). Soy lecithin's specific phospholipid profile may enhance mitochondrial function and ATP production, leading to more frequent tail beats (Ortega Ferrusola *et al.,* 2009). This increased flagellar activity might reflect better preservation of axonemal structure and dynein ATPase activity in the soy lecithin-preserved spermatozoa (Mata-Campuzano *et al.,* 2012).

From a biophysical perspective, higher BCF values suggest greater energy expenditure and potentially improved sperm penetration capability (Mortimer and Mortimer 2021). However, it's important to note that excessive tail beating frequency doesn't always correlate with improved fertility, as optimal sperm movement requires coordination between beat frequency and progressive motility (Boryshpolets *et al*., 2013). The observed differences may reflect variations in how each extender modulates calcium flux and membrane fluidity, both of which influence flagellar beating patterns (Marquez and Suarez, 2007).

These findings align with emerging research on plant-based extenders showing they can effectively maintain sperm kinematic parameters. The increased BCF with soy lecithin might be particularly advantageous in cervical mucus penetration, where higher frequency beats could aid in overcoming viscous resistance (Suarez and Dai, 1992). However, the clinical significance of this 0.052 Hz difference warrants further investigation through fertility trials.

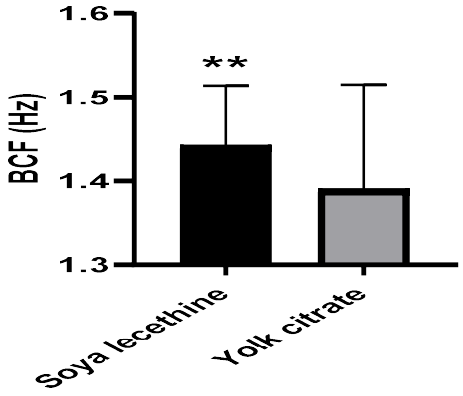

Figure 3. Beat Cross Frequency (BCF) of Ram Spermatozoa Preserved in Soybean Lecithin and Egg Yolk Extenders. Values are expressed as mean ± standard error of the mean (SEM). A statistically significant increase in BCF was recorded in the soybean lecithin group compared to the egg yolk group (*P < 0.05), indicating enhanced flagellar activity and potential energy output in the soybean-based medium.

Conclusion

In conclusion, the extenders were supplemented with 20% egg yolk and 1.0% soybean lecithin had relatively similar effects on total sperm motility, viability and reheotaxis parameters. We concluded that a commercially available soybean lecithin (1%) was an alternative to an egg yolk-based extender in preserving motility, viability and reheotaxis parameters of semen.

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مقارنه بين صـــفار البيض وليســـيثين فول الصـــويا فى مخفف الترس على جودة الحيوانات المنويه للكباش

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الملخص

الهدف من الدراسة هو دراسة مقارنه بين صفار البيض (20%) وليسيثين فول الصويا (1%) في مخفف Tris على الحركة الكلية للسائل المنوي، الحيوية وقياسات الانجذاب التيارى على حركة الحيامن (الريوتاكسيس) وتشمل: النسبة المئوية الموجبة للانجذاب التيارى (PR%)، السرعة المنحنية (VCL)، متوسط سرعة المسار الخطية (VAP)، سرعة الخط المستقيم للحيوانات المنوية (VSL) السرعة الخطية (LIN) وهي ســرعة الخط المســتقيم على الســرعة المنحنية للحيوانات المنوية ومعدل تردد النبضــات للحيوانات المنوية (BCF).

تم إستخدام تحليل وتقدير قياسات حركة الحيوانات المنويه والإنجذاب التياري بإستخدام الكمبيوتر (CASA) مع التحكم فى سرعة تدفق السائل المنوى. تم تجميع السائل المنوى مره كل إسبوع بإستخدام المهبل الإصـــطناعى. تم تخفيف عينات الســـائل المنوى فى مخفف Tris المحتوى على صـــفار البيض (20%) لمجموعة الكنترول والمحتوى على فول الصويا (1%) لمجموعة المعامله.

أظهرت النتائج عدم وجود فروق معنويه بين مخفف صـــفار البيض ومخفف فول الصـــويا في كل من الحركة الكلية والحيوية للحيامن وكذلك بعض قياسـات الإنجذاب التياري مثل النسـبه المئويه الموجبه للإنجذاب التيارى ومتوسـط سـرعة المسـار الخطيه للحيوانات المنويه. ووجد زيادة معنويه في السـرعة المنحنية ومعدل تردد النبضـات للحيوانات المنويه فى مخفف فول الصـويا مقارنة بمخفف صـفار البيض. وعلى العكس وجد زيادة معنويه في سرعة الخط المستقيم والسرعه الخطيه للحيوانات المنويه فى مخفف صفار البيض مقارنه بمخفف فول الصويا.

وخلصـــت النتائج على أن مخفف Tris المحتوى على صـــفار البيض (20%) والمخفف المحتوى على فول الصـويا (1%) تقريباً لهم تأثيرات متشـابهه على الحركه الكليه والحيويه للسـائل المنوى وكذلك قياسات الريوتاكسيس.

وتشـير هذه النتائج أن ليسـيثين فول الصـويا (1%) المتوفر تجاريا كان بديلا آمن لمخفف صـفار البيض في الحفـاظ على الحركـة الكليـة للســـائـل المنوي، الحيويـة وقيـاســـات الانجـذاب التيـارى (الريوتاكسيس).

الكلمات المفتاحية: *السائل منوي للكبش، مخفف صفار البيض، ليسيثين فول الصويا.*