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USAGE OF SOYBEAN (*GLYCINE MAX*) DILUENT IN STORAGE OF SEMEN OF BULLS AT 5^{O}C

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Abstract

Egg yolk tris extender is the most appropriate media for cryopreservation in most domestic animals (sperms). In this study we try to discuss that soybean tris extender is more economical and less contaminated to replace the egg yolk tris extender .three semen parameters were studied, motility, dead and live and acrosome integrity at zero h, 24h, 48h, 72h after cooling at 5°C. There is significant difference ($p<0.05$) between (EYT) and 30% concentration of SBT in sperm motility respectively (62.2± 1.05, 54.15 ± 1.05), live sperm three concentrations of SBT respectively (71.55 ± 2.85, 60.4 ± 4.0, 70.2 ± 2.5, 63.55 ± 2.7) and acrosome integrity with 30% concentration SBT respectively (84.75 ± 1.25, 73.9 ± 1) after 24 hours of cooling. There is significant difference ($p<0.05$) between (EYT) and SBT 10%, 30% in sperm motility respectively (45.95 ± 2.15 , 38.65±2.5, 41.1 ±2.1). There is significant difference ($p<0.05$) between (EYT) and SBT 10%, 20%, 30% in sperm livability respectively (71.55± 2.85, 60.4 ±4.0, 70.2 ± 2.5, 63.55) and there is significant difference ($p<0.05$) between (EYT) and SBT 20% in acrosome integrity respectively (77.8 ± 1.25, 80.5 ± 1.3) after 48 hours of cooling. There is significant difference ($p<0.05$) between (EYT) and SBT 30% in sperm motility respectively (37.3 ± 2.3, 32.15 ± 1.5). There is significant difference ($p<0.05$) between (EYT) and SBT extender concentrations 10% and 30% in sperm livability respectively (48.9 ± 2.35, 40.3 ± 1.15, 43.4 ± 1.9) and there is significant difference ($p<0.05$) between (EYT) and SBT extender concentrations 20% respectively (72 ± 1.4, 76.25 ± 1.75) after 72 hours of cooling. Conclusion the SBT concentration 20% is more reliable to replace YET. More research needed to confirm our result.

Key words: bulls semen; soybean; plants

Introduction

According to the World Organization for Animal Health (2003) the semen processing compound should be free of any biological risk (Aires *et al*., 2003). Egg Yolk Tris Extender (EYT) is an important constituent of diluents used for cryopreservation for the semen's bull and other domestic animals. It contains low-density lipoprotein (lecithin) which protects the phospholipids of the sperm membrane during preservation and thawing (Marco *et al*., 2004). Microbial contaminations of the egg are the challenge for extension lead to lower the fertilizing rate of the sperm (Marco *et al*., 2004). In addition to the egg yolk, globules make the sperm evaluation difficult (Marco *et al*., 2004), because microbial contamination of egg yolk leads to reduce the sperm fertility (Bousseau *et al*, 1998). Soybeans Tris Extender (SBT) is more suitable for semen extension because of it rich in protein with balanced amino acid and low cost.

Different stressors will generate Reactive Oxygen Species (ROS) and lipid peroxidation of the cell membrane, which will ultimately affect the spermatozoa (Wang *et al*, 1997). Soya extender with milk containing conventional diluents and found that mitochondrial membrane potential was increased in the soy-lecithin based extender. Moreover, DNA fragment index and sperm motility were improved too in soy lecithin diluent as compare to milk based extenders (Rehmana *et al*, 2013). Penicillin and streptomycin are still used at the rate of one gram per liter (Rehmana *et al*, 2013). The bad smell and test for soya are due to Lipoxygenase enzyme (WiIkens *et al*, 1967).

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Materials and Methods

1- Semen collected via artificial vagina from three Holstein bulls aged (4-5) year weekly for eight weeks from local farmers in Baghdad / Iraq.

2- Hydroxymethyle amino-methane (Tris)-yolk-fructose extender prepared by dissolving 3.03 gm of Tris + 1.67 gm of citric acid + 1.25 gm of fructose in a complete volume of 92 ml of distilled water, 24 ml of egg yolk and 500 µg/ml Gentamycin + 100 µg/ml Tylosin were added to the mixture.

3- Soybean was bought from the local market and Prepared according to (EL-keraby *et al.*, 2010) summarized by 10 grams of Soybean were washed and soaked in 100 ml distilled water and then boiled for 30 min. then water was discarded. The whole Soybean grains were washed again, finally cooled down with 50 ml of distilled water containing 0.25% $NaHCO_3$. Soybean grains were grounded in a blender for 5 min and the slurry cooled. Soybean milk was extracted by filtration through a clean cotton cloth, centrifuged and boiled again for 10 min. allowed to cool down. 500 µg/ml Gentamycin and 100 µg/ml Tylosin were added to the mixture. Soya extraction prepared in three different concentrations in Tris extender 10%, 20% and 30%. To assess the movement and livability of the sperm, tow Eppendorf tube (0.25) ml for each extender put them in the water bath 37°C to warm it. For Sperm motility examination we put 10 µl for each extender on the warmed slide (37°C) at 0, 24, 48 and 72 hours of cooling, The motile sperm percentages (%) were assessed by visual examination under power magnification (×40). Assessment of sperm livability performed using an Eosin–Nigrosine staining mixture, 200 spermatozoa counted from five fields at 0, 24, 48 and 72 hours of cooling to each extender (Blom, 1950; Singh *et al.*, 2012).

Acrosome integrity, *Well and Awa* stain used for acrosome integrity, the stain prepared by Fast green fast 1gm, Eosin 1gm ,Ethanol 1.9 ml (96%) and complete the volume to 100 ml by sodium citrate (2.9%). Semen smear doing on the warmed slide (37°C) and fixed by ethanol 1% for three minutes after drying it dipped in the stain for 50 minutes, 200 spermatozoa from five fields tested by using (microscope 100x) at 0, 24, 48 and 72 hours of cooling to each extender (Wells *et al.*, 1970).

Statistical analysis

Crude data were collected and analyzed using SPSS (Statistical program for social studies, Version 12, Illinois, USA) for descriptive statistics involving means and standard error of the mean (SEM) and for comparing means using paired *t*–test to detect the significant differences between extenders groups in this study. *P*-value less than 0.05 were considered statistically significant.

Results

There is no significant difference between egg yolk extender and three different concentrations of soybean extender 10%, 20%, 30% in sperm motility respectively (76.2± 2.9, 75.75 ± 2.8, 76.45 ± 1.0, 74.9 ± 3.3) at zero time of cooling .there is no significant difference ($p>0.05$) within soybean extraction extender 10%, 20%, 30% at zero time of cooling. Table 1.

There is no significant difference between egg yolk extender and three different concentrations of soybean extender 10%, 20%, 30% in sperm livability respectively (80.45 ± 1.85, 78.35 ± 2.45, 80.4 ± 2, 77 ± 2.65) at zero time of cooling.

There is no significant difference within soybean extraction extender 10%, 20%, 30% in sperm livability respectively (78.35 ± 2.45, 80.4 ± 2, 77 ± 2.65) at zero time of cooling. Table 1.

There is no significant difference between egg yolk extender and three different concentrations of soybean extender 10%, 20%, 30% in acrosome integrity respectively (90.15 ± 1.4, 90.6 ± 1, 92 ± 1, 90.5 ± 1) at zero time of cooling.

There is no significant difference within soybean extraction extender 10%, 20%, 30% in acrosome integrity respectively (90.6 ± 1, 92 ± 1, 90.5 ± 1) at zero time of cooling Table 1.

There is significant difference ($p<0.05$) between egg yolk extender and soybean extender concentrations 30% in sperm motility respectively (62.2 ± 1.05, 54.15 ± 1.05) after 24 hours of cooling.

There is significant difference ($p<0.05$) in sperm motility within soybean extraction extender 20% and 30% respectively (59 ± 3.3, 54.15 ± 1.05) Table 2 after 24 hours of cooling.

There is significant difference ($p<0.05$) between egg yolk extender and three different concentrations of soybean extender 10%, 20%, 30% in sperm livability respectively (71.55 ± 2.85, 60.4 ± 4.0, 70.2 ± 2.5, 63.55 ± 2.7) after 24 hours of cooling.

There is significant difference ($p<0.05$) within soybean extraction extender 10%, 20%, 30 in sperm livability respectively (60.4 ± 4.0, 70.2 ± 2.5, 63.55 ± 2.7) after 24 hour of cooling. Table 2.

There is significant difference ($p<0.05$) between egg yolk extender and 30% concentrations of soybean

extender in acrosome integrity respectively (84.75 ± 1.25, 73.9 ± 1) after 24 hours of cooling.

There is significant difference ($p<0.05$) between soybean extraction extender 10% and soybean extraction extender 20% and 30% respectively (82.3 ± 1.75, 87.1 ± 1.4, 73.9 ± 1). There is significant difference ($p<0.05$) between soybean extraction extender 20% and soybean extraction extender 30% in acrosome integrity respectively (87.1 ± 1.4, 73.9 ± 1) after 24 hours of cooling table 2.

There is significant difference ($p<0.05$) between egg yolk extender and two different concentrations of soybean extender 10%, 30% in sperm motility respectively (45.95 ± 2.15 , 38.65 ± 2.5, 41.1 ± 2.1) after 48 hours of cooling table 3.

There is no significant difference ($p>0.05$) between three different concentrations of soybean extender 10%, 20%, 30% in sperm motility after 48 hours of cooling table 3.

There is significant difference ($p<0.05$) between egg yolk extender in 10% concentrations of soybean extender in sperm livability respectively (61.7 ± 2.15, 36.2 ± 3.6) after 48 hours of cooling.

There is significant difference ($p<0.05$)between soybean extraction extender 10%, 20%, 30% respectively in sperm livability (36.2 ± 3.6, 60.25 ± 3.7, 58.8 ± 3.3) after 48 hours of cooling. Table 3.

There is significant difference ($p<0.05$) between egg yolk extender and 20% concentrations of soybean extender in acrosome integrity respectively (77.8 ± 1.25, 80.5 ± 1.3) after 48 hours of cooling Table 3.

There is significant difference ($p<0.05$) between soybean extraction extender 10% and soybean extraction extender 20% and 30% respectively (76.45 ± 1.6, 80.5 ± 1.3, 77.65 ± 1). There is significant difference ($p<0.05$) between soybean extraction extender 20% and soybean extraction extender 30% in acrosome integrity respectively (80.5 ± 1.3, 77.65 ± 1) after 48 hours of cooling Table 3.

There is significant difference ($p<0.05$) between egg yolk extender and soybean extender concentrations 30% in sperm motility respectively (37.3 ± 2.3, 32.15 ± 1.5) after 72 hours of cooling table 4.

There is significant difference ($p<0.05$) between three different concentrations of soybean extender 10%, 20% with 30% in sperm motility respectively (36.15 ± 1.15, 36.8 ± 1.4, 32.15 ± 1.5) after 72 hours of cooling table 4.

There is significant difference ($p<0.05$) between egg yolk extender concentrations 10% and 3% of soybean extender in sperm livability respectively (48.9 ± 2.35, 40.3 ± 1.15, 43.4 ± 1.9) after 72 hours of cooling Table 4.

There is significant difference ($p<0.05$) between soybean extraction extenders 10%, 20%, 30% respectively in sperm livability (40.3 ± 1.15. 47.1 ± 2, 43.4 ± 1.9) after 72 hours of cooling Table 4.

There is significant difference ($p<0.05$) between egg yolk extender and 20% concentrations of soybean extender in acrosome integrity respectively (72 ± 1.4, 76.25 ± 1.75) after 72 hours of cooling Table 4.

There is significant difference ($p<0.05$) between soybean extraction extender 20% and soybean extraction extender 10% and 30% in acrosome integrity respectively (76.25 ± 1.75, 72.35 ± 1.9, 72.7 ± 0.9) after 72 hours of cooling table 4.

Discussion

The power of spermatozoa fertility could be maintained using freezing or cooling system in which semen was diluted using dilution material that could provide the physical and chemical needs (Aires *et al*., 2003). Extender or diluent is a medium of chemical components used for preservation, extension and protection of sperm cells against various shocks during processing, storage and transportation used for artificial insemination. Beneficial and useful extender should provide energy for metabolic activities with in sperm cell; maintain osmotic pressure and pH of the medium, also keeps a check on the contamination of the medium to protect semen from microorganisms growth. Different semen extenders provide sufficient nutrition in the form of fructose sugar to sperm cells during storage. It also prevents sperm cells against Cryo-Shocks during cold storage at extreme temperature (Marco *et al*., 2004).

There are many types of extenders, in present study Soybean Tris and Egg Yolk Tris extender had been using. The study showed a significant differences ($P<0.05$) in sperm motility in all groups in different period time of cooling. The best individual sperm motility up to 48 hours indicated that citric acid and fructose in Tris amino-methane egg yolk had a function to maintain sperm motility percentage in (EYT) extender group. Low Density Lipoprotein (LDL) fraction especially phospholipids which already existed in egg yolk were effective component in preventing motile sperms from cold shock (Aires *et al*., 2003).

As well, there is an equal effect with a significant differences ($P < 0.05$) after 24 to 48 hours in sperm motility observed in Soybean Tris extender in preserving the motility and viability of liquid ram sperm at 15 and

Table 1: Semen parameters in different Soy Bean Tris extender and Egg Yolk Tris extender in 5^{0}C at zero hour of cooling. (Mean ±SE).

Sperm parameters	Egg yolk-Tris extender	Soybean - Tris extender		
		10 %	20 %	30 %
Motility (%)	76.2± 2.9 A	75.75 ± 2.8 A	76.45 ± 1.0 A	74.9 ± 3.3 A
Live Sperm (%)	80.45 ± 1.85 A	78.35 ± 2.45 A	80.4 ± 2 A	77± 2.65 A
Acrosome Integrity (%)	90.15 ± 1.4 A	90.6 ± 1 A	92± 1 A	90.5 ±1 A

A = Non significant difference between groups.

Table 2: Semen parameters in different Soybean Tris extender and Egg Yolk Tris extender in 5°C after 24 hours of cooling. (Mean ±SE).

Sperm parameters	Egg yolk-Tris extender	Soybean - Tris extender		
		10 %	20 %	30 %
Motility (%)	62.2± 1.05 A	57.7 ± 3.05 A	59± 3.3 A	54.15 ± 1.05 B
Live Sperm (%)	71.55± 2.85A	60.4 ±4.0 B	70.2 ± 2.5 B	63.55 ± 2.7 B
Acrosome Integrity (%)	84.75 ±1.25 A	82.3 ±1.75 A	87.1 ± 1.4 A	73.9 ± 1 B

A = significant difference (p < 0, 05) between groups.

Table 3: Semen parameters in different Soybean Tris extender and Egg Yolk Tris extender in 5°C after 48 hours of cooling. (Mean ±SE).

Sperm parameters	Egg yolk-Tris extender	Soybean - Tris extender		
		10 %	20 %	30 %
Motility (%)	45.95 ± 2.15 A	38.65±2.5 B	43.2 ±2.3 A	41.1 ±2.1 C
Live Sperm (%)	61.7±2.15 A	36.2 ±3.6 B	60.25 ±3.7 A	58.8 ±3.3 A
Acrosome Integrity (%)	77.8 ±1.25 A	76.45 ±1.6 A	80.5 ±1.3 B	77.65 ± 1 A

A= significant difference (p < 0, 05) between groups

Table 4: Semen parameters in different Soybean Tris extender and Egg Yolk Tris extender in 5°C after 72 hours of cooling. (Mean ±SE).

Sperm parameters	Egg yolk-Tris extender	Soybean - Tris extender		
		10 %	20 %	30 %
Motility (%)	37.3±2.3 A	36.15±1.15 A	36.8±1.4 A	32.15±1.5 B
Live Sperm (%)	48.9±2.35 A	40.3±1.15 B	47.1± 2 A	43.4±1.9 B
Acrosome Integrity (%)	72±1.4 A	72.35±1.9 A	76.25±1.75 B	72.7±0.9 A

A= significant difference (p<0, 05) between groups.

5ºC as an egg-yolk extender. The added of Soybean extender enhanced improvement of sperm parameter due to its low viscosity and less debris so phospholipids from Soybean may integrate with sperm membrane phospholipids (that may be replaced which maintain its structure and function) to form a protective film against the lethal factors.

Soybean has large amount of lipoprotein called soya lecithin. It is similar to egg yolk lecithin, which help in protecting sperm membrane against cold shock (Marco *et al.*, 2004).

Viable sperm are the most important indicator to determine spermatozoa quality during the cooling process (Aires *et al.*, 2003). The study found that after 24 hours and 48 hours of cooling storage resulted the highest viable sperms in Tris amino-methane egg yolk followed by Soybean Tris extender 20% that showed a significant differences (P<0.05) than other groups.

The decreasing number of viable sperms was due to the inconsistent temperature changes during storage time and the sperm metabolism that produced lactic acid that became one of inhibitor factors, which could decrease sperm viability (Aires *et al.*, 2003).

Lecithin protects the plasma membrane by restoring phospholipid that is lost because of the heat and protects the cell viability. Soy-lecithin is a very good alternative to phospholipids present in egg yolk for semen cryopreservation. A total of 25% soy lecithin based extenders increased the sperm membrane and acrosome integrity, live percentage, and motility in bovines. Soy-lecithin was considering as a good replacement source for semen extension (Bousseau *et al.*, 1998).

Sperm quality was negatively affected when Soybean concentration was increased beyond the 30%. Low sperm motility to the higher concentration of lecithin in the extender. The higher soya concentration in semen extender decreased the sperm visibility under microscope (Marco *et al*., 2004). Our study results are supported these findings).

In the current study, all groups were produced significantly ($P<0.05$) higher HOST values in all periods time of cooling process. We infer that the sperm membrane integrity could not remain intact possibly due to decreased velocity and higher debris in the 30% Soybean Tris extender. Soybean Tris extender 20% is the optimum concentration in which the sperm exhibited better membrane integrity.

Lecithin also consisted of fatty acid glycerol, phosphate acid and choline that had a function to develop membrane metabolism structure. The increasing sperm membrane integrity function will then increase substrate absorption on spermatozoa that could promote the motility (Aires *et al*., 2003; Marco *et al*., 2004; Blom, 1950; Singh *et al*., 2012).

The current study results findings are supported of other author's results were published.

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