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Is Tris-Egg Yolk Extender Better Than LDL Extender in Chilled Semen Preservation?

Falah Hasan Baiee[1] and Ghadeer Sabah Bustani[2*]

1 Department of Clinical Science, Faculty of Veterinary Medicine, University of Kufa, Kufa 54003, Najaf, Iraq.
2 College of Dentistry, The Islamic University, Najaf, Iraq.

Corresponding author: bustani@iunajaf.edu.iq
Co-authors: falahhali@uokufa.edu.iq

Abstract: -

Semen preservation is the most technology used in artificial insemination and animal breeding. There are two critical types of preservation, including preservation in freezing and chilled temperature, which cause reducing in sperm quality and finally lead to a decrease in the fertilization rate. Different extenders from international commercial sources are used to dilute and preserve the sperm during comparatively expensive chilling and cryoprotection operations. Several researchers will suggest applying the lab extenders prepared for semen storage, especially in chilled technic. In general, that are two famous laboratory prepared extenders, tris egg yolk and LDL extenders; thus, they are necessary to determine which of these are better used in semen preservation at chilling temperature.

Keywords: - Tris Egg Yolk Extender, LDL Extender, Artificial Insemination, chilling temperature and

Introduction: -

Artificial insemination is the most widely used method for fertilizing female cattle and other animals worldwide. In 1784, Lazzaro Spallanzani was reported and published the first artificial insemination in humans (Ombelet and Van Robays 2015; Bustani and Baiee 2021). Nowadays, many principles of insemination are used in humans and domestic (Baiee et al. 2020). During the past decades, researchers and scientists have been trying different experiments designed to prolong the sperm life and quality by resisting the circumstances airs from storage conditions (Tarig et al. 2017a; Baiee et al. 2020; Bustani and Baiee 2021). Chilled sperm is one of the most used methods for transporting and using sperm nowadays, which can store semen for days before being used in different reproductive techniques. The semen that is stored in chilled temperature preservation is collected and then held at 5˚C temperature for 4-5 depending on the sperm species and semen extender quality(Tarig et al. 2017b; Tarig et al. 2017a). Tris egg yolk-based extender is commonly used in chilled. Because of their low cost and acceptable outcomes, tris egg yolk semen extenders are widely employed in laboratory and field approaches (Bustani and Baiee 2021). In 1939 Philips was the first researcher to employ the tris egg yolk as a semen extender and proved its benefits in prolonging the life of sperm (Aboagla and Terada 2004). In addition, the main non-penetrative ingredient in extenders is egg yolk, which is used to dilute semen and protect sperm from freeze shock during the chilling process(Ahmad et al. 2018). It serves as a storehouse for cholesterol and phospholipids, which helps to preserve the sperm cell

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membrane and acrosome from cryogenic damage. It also protects membrane phospholipids from being lost during the freezing process(Memon et al. 2012). The low-density lipoprotein (LDL) of egg yolk preserves the phospholipids in sperm membranes during cryopreservation; previous research has shown that lipid-binding proteins from LDL in the tris egg yolk protect sperm during freezing. Thus our study aimed to cognition a better extender can used insemen preservation.

Material and method

Ethical standards: -

The present study is approved by the Kufa Institutional Animal Care and Use Committee (IACUC) in the Faculty of Veterinary Medicine, University of Kufa; Iraq (8580-2020). The percentage investigations of parameters were conducted at the Theriogenology and AI unit from September 15, 2019 to May 30, 2021.

Animal

The current study was used two fertile and mature bulls for semen collection at a farm in Iraq. Finally, 60 ejaculates were used in the present experiment. The two animals were fed the same way, with nutent and water.

Semen Processing

After being delivered to the laboratory, the semen extenders were manufactured before being preservation and kept at 36°C. Baiee's proprietary formula was used to dilute the sperm to a final concentration of dose 30 × 106, sperm per 0.25 ml straw (Gati and Bustani 2022).

To prepare manual extenders in the lab (tris egg yolk and LDL extenders), The sperm diluent was made according to the instructions of Baiee *et al.*, 2017 (Amirat et al. 2004; Kaka et al. 2015; Baiee et al. 2017; Bustani and Baiee 2021). Diluent was made according to the former researchers and was included in aliquots tris egg yolk extender; fructose: 1 g, Tris: 2.24 g, citric acid: 1.48 g, and 20% egg yolk v/v in 100 ml very pure of distilled water.

On the other hand, the LDL extender prepared and used in the 8% LDL (v/v) following the previous method (Moussa et al. 2002; Amirat-Briand et al. 2010), used egg yolk for extraction of LDL and diluted in citric acid; Tris; 2.42 g; 1.48 g, fructose 1.00 g, and v/v in 100 ml very pure of distilled water..

Study design: -

For the evaluation of the two semen extenders; used 60 semen samples and diluted each sample into two semen extender groups (Ege yolk extender group and LDL extender group) and preservation in chilling temperatures and assessment of the sperm parameters in four different periods, including 24, 48, 72, and 96 hours.

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Preservation assessment

The laboratory's preservation evaluation of sperm parameters using microscopic techniques under different tests included (motility, viability, morphology, and acrosomal integrity percentage) in the Hypo Osmotic Swelling Test (Bustani and Baiee 2021).

Statistical Analysis

The information of data were analysed and aragment by using the program PRISM Graphpad 8; The Shapiro–Wilk test was used to ensure that the acquired data had a normal distribution. A mixed-model analysis of variance (2-way ANOVA) was used to compare the differences of mean among groups; the significance was tested using A mixed model (repeated measures 2-way ANOVA) value less than ($P<0.05$) are measured statistically as meaningful and significant. Present founder and result were shown as mean ± and stander error means.

Result and Discussion:

The results in the figure-1 have illustrated the difference between the two groups of extenders on the first day of the experiment, which showed the tris egg yolk group superior significantly to the LDL group in the motility (general and progressive), HOST, and viability, which the previous studies proved the protective effect of the tris egg yolk extender as anti-shock (Tshabalala et al. 2019), a nutrient source for the sperm (Bustani and Baiee 2021) and productive agent during the thawing process (Gati and Bustani 2022). The previous studies demonstrated the cooling temperature has an adverse effect on the sperm, including a decrease in selective permeability of the cell and plasma membrane integrity. Moreover increased the sperm damage by increasing both the cellular reactive oxygen species (ROS) and mitochondria ROS (Aboagla and Terada 2004; Lubawy et al. 2022). On the other hand, the researcher reported that tris egg yolk is beneficial to sperm preservation; it has been routinely included in most preservation protocols. Low-density lipoprotein fraction is well-established as the active constituent(Pace and Graham 1974; Chaudhari et al. 2015).

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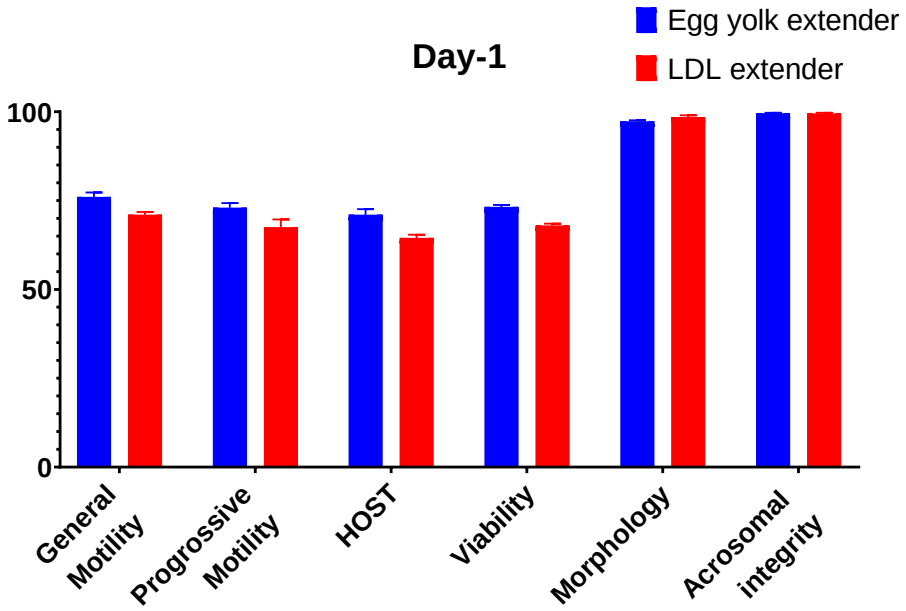
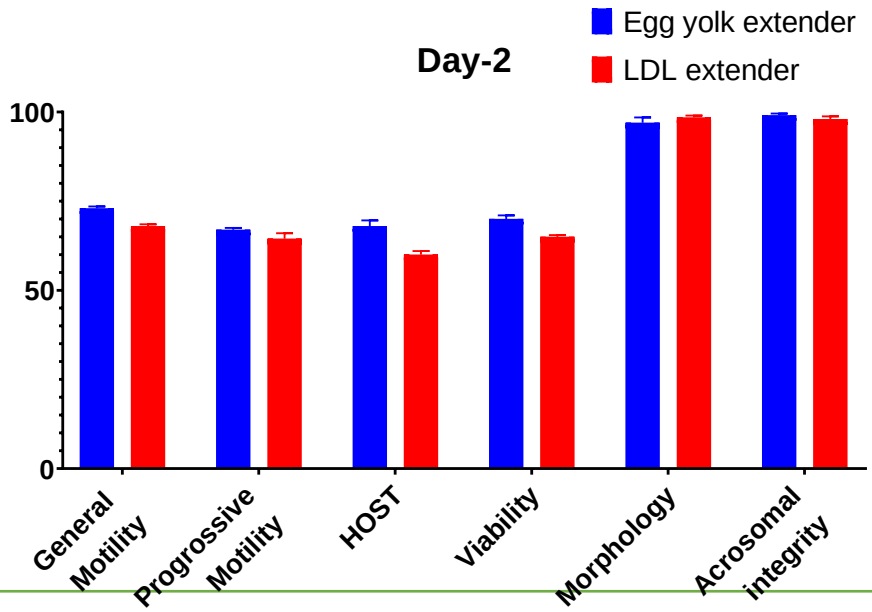


Figher-1 **:** At 24 hours of preparation and storage at cold temperature, values are given as means, and error bars reflect standard error (SE). .

egg yolk extenders depending on the morphology and acrosomal integrity as illustrated in the previous studies illustrated that combination of LDL in bovine extenders has given enhanced the sperm morphology and acrosomal integrity in comparison with other extenders that depending on egg yolk compounds (Amirat-Briand et al. 2010).



Figher-2 **:** At 48 hours of preparation and storage at cold temperature, values are given as means, and error bars reflect standard error (SE). .

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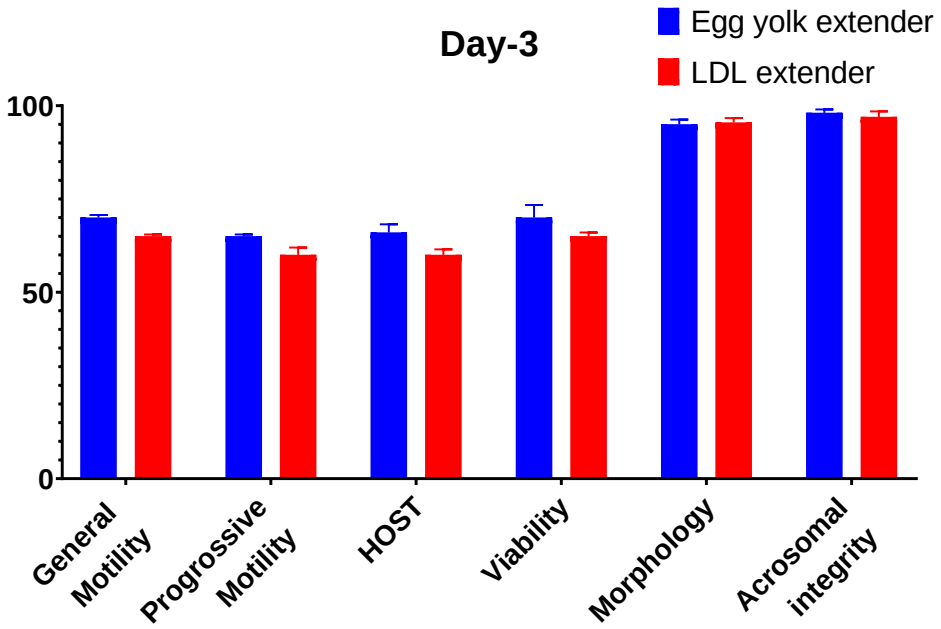
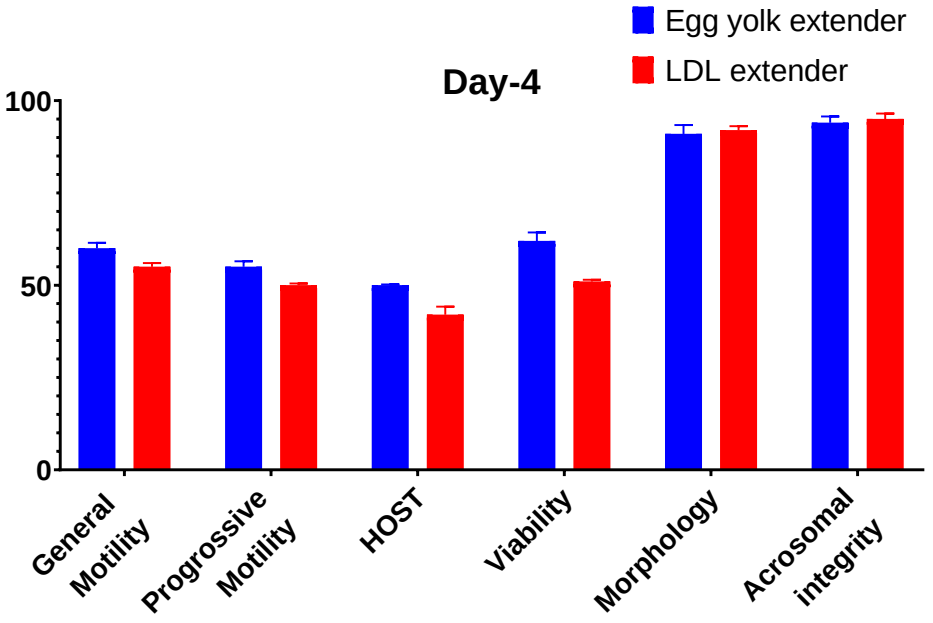


Figher-3 **:** At 72 hours of preparation and storage at cold temperature, values are given as means, and error bars reflect standard error (SE). .



Figher-4 **:** At 72 hours of preparation and storage at cold temperature, values are given as means, and error bars reflect standard error (SE). .

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The results in the figure-2,3 and 4 showed the excellence and superiority of the egg yolk group extender among the LDL group significantly in the motility, viability, and HOST. Moreover, the result showed a rise in the LDL group's morphology and acrosomal integrity among the tris egg yolk group but no significantly different between their values. In general, egg yolk was utilized at a concentration of 20 percent (w/v) (Baiee et al. 2020). Moreover, laboratory experiments found that this concentration makes it difficult to standardize data and interferes with biochemical assays and metabolic investigations. This problem might be solved by centrifuging specific components out of the egg yolk. (Amirat et al. 2004; Bustani and Baiee 2021). In addition, the presence of compounds in egg yolk that restrict spermatozoa respiration or reduce motility necessitates the use of cryoprotective fractions to replace whole egg yolk (Tshabalala et al. 2019). In the

Colocation: -

The current study's findings demonstrated the superiority of the egg yolk extender among the LDL extender in the sperm chilling preservation. Hence, further studies should be carried out to establish the egg yolk.

References

[1]. Aboagla, E.M.-E. and Terada, T. 2004. Effects of egg yolk during the freezing step of cryopreservation on the viability of goat spermatozoa. *Theriogenology* 62(6), pp. 1160–1172.

[2]. Ahmad, S.B., Ahmad, I., Ahmad, N., Qureshi, Z.I., Jamil, H., Ali, Q. and Ashfaq, K. 2018. Effect of Addition of Different Concentrations of Alpha Lipoic Acid to Tris Egg Yolk Citrate Glycerol Extender on Cryopreservation of Sahiwal Bull Spermatozoa. *Pakistan Veterinary Journal* 38(3)

[3]. Amirat-Briand, L. et al. 2010. In vivo fertility of bull semen following cryopreservation with an LDL (low density lipoprotein) extender: Preliminary results of artificial inseminations. *Animal reproduction science* 122(3–4), pp. 282–287.

[4]. Amirat, L., Tainturier, D., Jeanneau, L., Thorin, C., Gérard, O., Courtens, J.L. and Anton, M. 2004. Bull semen in vitro fertility after cryopreservation using egg yolk LDL: a comparison with Optidyl®, a commercial egg yolk extender. *Theriogenology* 61(5), pp. 895–907.

[5]. Baiee, F., Al-Wahab, B.A., Almusawi, A.A., Yu, L.L., Fitri, W.-N. and Bustani, G.S. 2020. Effect of vitrification on spermatozoa quality in bull semen. *EurAsian Journal of BioSciences* 14(2), pp. 3897–3904.

[6]. Baiee, F.H. et al. 2017. Hypo-Osmotic Swelling Test Modification to Enhance Cell Membrane Integrity Evaluation in Cryopreserved Bull Semen. *Pertanika Journal of Tropical Agricultural Science* 40(2)

[7]. Bustani, G.S. and Baiee, F.H. 2021. Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. *Veterinary World* 14(5), p. 1220.

[8]. Chaudhari, D. V, Dhami, A.J., Hadiya, K.K. and Patel, J.A. 2015. Relative efficacy of egg yolk and soya milk-based extenders for cryopreservation (− 196 C) of buffalo semen. *Veterinary world* 8(2), p. 239.

[9]. Gati, M.A. and Bustani, G.S. 2022. In Vivo and Vitro Evaluation of Bovine Sperm Cryopreservation; by Used Manual Laboratory Prepared Extenders and Different Commercial

Extenders. *Revista Electronica de Veterinaria* , pp. 1–13.

[10]. Kaka, A. et al. 2015. α-Linolenic acid supplementation in BioXcell® extender can improve the quality of post-cooling and frozen-thawed bovine sperm. *Animal reproduction science* 153, pp. 1–7.

[11]. Lubawy, J., Chowański, S., Adamski, Z. and Słocińska, M. 2022. Mitochondria as a target and central hub of energy division during cold stress in insects. *Frontiers in zoology* 19(1), pp. 1–19.

[12]. Memon, A.A., Wahid, H., Rosnina, Y., Goh, Y.M., Ebrahimi, M. and Nadia, F.M. 2012. Effect of antioxidants on post thaw microscopic, oxidative stress parameter and fertility of Boer goat spermatozoa in Tris egg yolk glycerol extender. *Animal reproduction science* 136(1–2), pp. 55–60.

[13]. Moussa, M., Martinet, 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(6), pp. 1695–1706.

[14]. Ombelet, W. and Van Robays, J. 2015. Artificial insemination history: hurdles and milestones. *Facts, views & vision in ObGyn* 7(2), p. 137.

[15]. Pace, M.M. and Graham, E.F. 1974. Components in egg yolk which protect bovine spermatozoa during freezing. *Journal of animal science* 39(6), pp. 1144–1149.

[16]. Tarig, A.A. et al. 2017a. Effect of different concentrations of egg yolk and virgin coconut oil in Tris-based extenders on chilled and frozen-thawed bull semen. *Animal reproduction science* 182, pp. 21–27.

[17]. Tarig, A.A. et al. 2017b. Effect of different concentrations of soybean lecithin and virgin coconut oil in Tris-based extender on the quality of chilled and frozen-thawed bull semen. *Veterinary world* 10(6), p. 672.

[18]. Tshabalala, M.M., Nephawe, K.A., Mphaphathi, M.L., Pilane, C.M. and Nedambale, T.L. 2019. 21 Effect of egg yolk extracted low-density lipoprotein on cryopreserved Nguni bull semen. *Reproduction, Fertility and Development* 31(1), p. 136.