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REVIEW

DERLEME

Role of Low Density Lipoproteins in Semen Preservation

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SUMMARY

The discovery that egg yolk has a beneficial effect on fertility in semen extenders led to its widespread use in semen extenders. Egg yolk is commonly found in semen extenders to preserve mammalian spermatozoa against cold shock during freeze-thaw process. Protective action of yolk is largely presumed to be due to low density lipoproteins (LDL). In recent years, there have been increasing demands to replace whole yolk in semen extenders because of presence of substances in yolk that inhibit respiration of spermatozoa or diminish their motility. Consequently, it could be of great benefit to remove these detrimental substances in yolk rather than complete egg yolk. Recent studies have confirmed that LDL extracted from egg yolk is successful for cryopreservation of semen from various species. Density gradient ultracentrifugation has been generally used for purification of egg yolk LDL. LDL are composed of 84-89% lipids and 11-17% proteins. Lipids of LDL are composed of approximately 69% triglycerides, 26% phospholipids and 5% cholesterol. Phospholipids play an essential role in the stability of LDL structure because association forces are essentially hydrophobic. LDL presents a density of 0.982 g/ml. They are spherical molecules of 17-60 nm diameters. The interactions of lipid and protein are disrupted and under freezing, but interactions between proteins are increased and adhere to cell membranes, thus preserving spermatozoa membranes. In conclusion, researches are still carried out needed to evaluate and understand the respective roles of lipids and apoproteins from LDL and to isolate the antagonistic agent contained in egg yolk.

Key Words
Cryopreservation
Egg Yolk
LDL
Semen

Anahtar Kelimeler
Kriyoprezervasyon
Yumurta sarısı
LDL
Sperma

ÖZET

Sperma Saklanmasında Düşük Yoğunluklu Lipoproteinlerin Rolü

Fertilite üzerine olumlu etkisinin keşfinden sonra yumurta sarısı, sperma sulandırıcılarında yaygın olarak kullanılmaktadır. Yumurta sarısının kullanılmasındaki temel amaç; dondurma çözdürme sonrasındaki soğuk şokuna karşı spermatozoanın korunmasıdır. Bu koruyucu etkisini, içerdiği düşük yoğunluklu lipoproteinler (LDL) ile gerçekleştirdiği düşünülmektedir. Son yıllarda yumurta sarısının tamamının kullanılması yerine, spermatozoon motilitesini olumsuz etkileyen içeriğinin uzaklaştırılarak, faydalı kısımlarının sulandırıcıya ilave edilmesi tercih edilmeye başlanmıştır. Çeşitli çalışmalarda yumurta sarısından elde edilen düşük yoğunluklu lipoproteinlerin farklı türlere ait spermaların dondurularak saklanmasında başarıyla kullanıldığı bildirilmektedir. Yumurta sarısından LDL elde etmek için genellikle ultrafaz santrifüj ile yoğunluk farkına göre ayırma yöntemi kullanılır. LDL, %84-89 lipid, %11-17 oranında proteinlerden oluşmaktadır. Lipid içeriğin yaklaşık olarak %69'unu trigliseridler, %26'sını fosfolipidler ve %5'ini de kolesterol oluşturmaktadır. Fosfolipidler özellikle hidrofobik karakterleri ile LDL yapısının korunmasında etkilidirler. LDL 0,982 g/ml yoğunluğa sahip bir moleküldür. 17-60 nm çapında olan ve küresel biçime sahip bu moleküllerin, dondurma sırasında spermatozoon membranına bağlanarak ve seminal plazma proteinleri ile birleşerek koruyucu etkilerini gösterdikleri düşünülmesine rağmen koruma mekanizması henüz tam olarak aydınlatılamamıştır. Çeşitli çalışmalarda bildirildiği üzere; sulandırıcılarda standart olarak kullanılan %20 yumurta sarısı yerine %8 LDL kullanımı, sperma parametrelerine olumlu katkı sağlamakta ve antioksidan enzim aktivitesini önemli ölçüde arttırmaktadır. Buna karşın sulandırıcıda %10'un üzerindeki oranlarda kullanılan LDL ise spermatozoa kalitesini azaltmaktadır. Sonuç olarak hem LDL yapısındaki lipid ve apoproteinlerin etki mekanizmalarının aydınlatılmasına yönelik hem de yumurta sarısındaki zararlı bileşenlerin izole edilmesine yönelik araştırmalar halen devam etmektedir.

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INTRODUCTION

In the field of reproductive physiology, the earliest studies involved the cryopreservation of spermatozoa. Since the development of artificial insemination with frozen semen, studies have been conducted to develop an extender that can be used to improve the cryopreservation of semen. (Luster SM 2004). The freezing process exposes the spermatozoa to thermal shock, which results in damage to the plasma membrane and acrosome. Various extenders have been tested in an attempt to limit cellular injury (Woelders et al 1997, Celeghini et al 2007). The discovery that egg yolk has a beneficial effect on fertility in semen extenders led to its widespread use in semen extenders. Egg yolk is commonly found in semen extenders to preserve mammalian spermatozoa against cold shock during the freeze-thaw process (Luster SM 2004). The precise cryoprotective mechanism of egg yolk is unknown. The protective action of yolk was largely attributed to the low-density lipoproteins (LDL) (Watson and Martin 1975, Quinn and Chow 1980, Babiak et al 1999, Moussa et al 2002, Bergeron and Manjunath 2006). In 1974, Pace and Graham purified egg yolk and observed that LDL fraction displayed a cryoprotective property. Afterwards, numerous authors have proposed that the low density fraction of yolk, which is mainly composed of LDL, could be largely responsible for the resistance against cold shock and the improved motility after storage. (Pace and Graham 1974, Foulkes 1977, Moussa et al 2002). In this review, we focused on the latest progressions about using of LDL in semen cryopreservation.

LDL

LDL make up about two-thirds of the total solids of egg yolk and are localized in the soluble fraction of egg yolk called plasma. LDL present a density of 0.982 g/ml. They are spherical molecules of 17-60 nm diameters, with a lipid core of triglycerides and cholesterol esters surrounded by a phospholipid and protein film. Phospholipids play an essential role in the stability of LDL structure due to hydrophobic characteristics. LDL contain 84-89% lipids and 11-17% proteins. Lipids of LDL are composed of approximately 69% triglycerides, 26% phospholipids and 5% cholesterol (Moussa et 2002).

Why LDL are demanded to replace whole egg yolk in semen extenders?

The first aim of sperm-freezing protocols, including the use of extenders, is to prevent lethal intracellular ice crystal formation and to reduce membrane damage during and after cryopreservation. Egg yolk helps the sperm cells in resisting against cold shock, in association with other components (Phillips 1939, Bogart and Mayer 1950). But egg yolk contains substances (granules) that prevent the metabolic exchanges of the spermatozoa or reduce their motility. It would therefore be advantageous to replace this complex solution with its component molecules that are responsible for its cryoprotective effect. Many studies have attempted to determine which components of egg yolk are responsible for cellular protection with the objective of preparing a chemically defined freezing extender (Ali Al Ahmad et al 2008). Moreover, egg yolk was usually used at the concentration of 20% (w/v) and laboratory studies revealed that this concentration makes results difficult to standardize and interferes with biochemical assays and metabolic investigations, which can be overcome by removing some components in the egg yolk by centrifugation (Evenson and Melamed 1983, Wall and Foote 1999). Furthermore, the presence of substances in yolk that inhibit respiration of spermatozoa or reduce their motility, increases demands to replace whole egg yolk by the cryoprotective fraction (Pace and Graham 1974, Haidl and Schilling 1994). Effectively, in addition to elements (LDL) that are highly favourable to freezing, yolk contains other noxious substances (Kampshmidt et al 1953, Pace and Graham 1974, Watson and Martin 1975).

Extraction of LDL from egg yolk

For purification of egg yolk LDL has been generally used via density gradient ultracentrifugation. (Kampschmidt et al 1953, Pace and Graham 1974, Watson and Martin 1975). The method used to extract LDL was developed by Moussa et al (2002), LDL purity of 97% was achieved. Fresh eggs are manually broken. The yolks are separated from an albumen. The yolks are diluted with an isotonic saline solution (0.17 M NaCl) (w/w) and stirred for 1 h before being centrifuged at 10 000 x g for 45 min at +4 °C. The supernatant (plasma) is separated from the sediment (granules). The plasma is centrifuged again to remove all traces of contamination with granules; then it is mixed with 40% ammonium sulfate to precipitate out the livetins. After 1 h of stirring at 4–8 °C, the mixture is centrifuged at 10 000 x g for 45 min. The sediment is discarded and the supernatant is dialyzed against distilled water to eliminate the ammonium sulfate. Then the solution is centrifuged (10 000 x g, 45 min, 4–8 °C) and LDL-rich supernatant is collected (Moussa et al 2002). This extraction technique is currently protected under the patent no. 0100292

held by the ENVN and INRA of Nantes (Moussa et al 2002, Bencharif et al 2012).

The Mechanism of LDL in semen protection

LDL are composed lipids and proteins. The interactions of lipid and protein are disrupted under freezing, but interactions between proteins are increased. Consequently, LDL are disrupted under the freezing and thawing. Triglycerides and phospholipids are liberated in the medium and apoproteins form a gel. Furthermore, phospholipids could form a protective film at the surface of spermatozoa membranes after disruption of LDL (Hu et al 2006). Moussa et al (2002) and Hu et al (2006) demonstrated that LDL were responsible for the gelation process in freezing–thawing. The first gelation was occur the disruption of LDL structure and this disruption favored the spermatozoa dehydration caused by the freezing–thawing process.

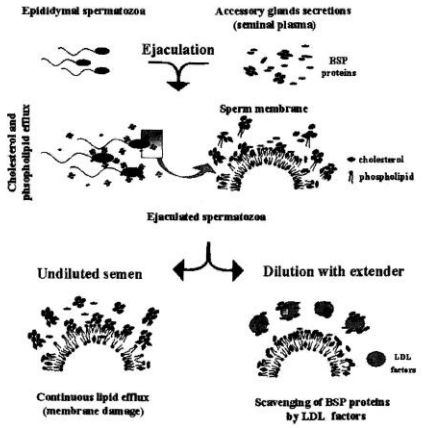

Figure 1. Mechanism of sperm protection by egg yolk. Modified from Manjunath et al (2002)
Şekil 1. Yumurta sarısı ile spermazotoon korunma mekanizması. Manjunath ve ark (2002)'dan uyarlanmıştır

LDL would promote the entry of phospholipids and cholesterol into the cell membrane (Bergeron et al 2004), building a complex with seminal plasma proteins, making them unavailable to function in the membrane (Manjunath et al 2002, Bergeron et al 2004) (Figure 1). However, the respective roles of the protein and lipid components of LDL during interactions with the spermatozoa membrane have yet to be clearly established (Bencharif et al 2008). The major proteins of bull seminal plasma (BSP) bind to sperm surface at ejaculation and stimulate cholesterol and phospholipid efflux from the sperm membrane. Since LDL interacts specifically with BSP proteins, it is proposed that the discriminate of BSP proteins in seminal plasma by LDL represents the major mechanism of sperm protection by egg yolk. LDL is one of the constituent of egg yolk that prevents binding of the BSP proteins to sperm and lipid efflux from the sperm membrane and is beneficial to sperm functions during sperm cryopreservation (Bergeron et al 2004).

Observations

There have been many attempts to find out which component in egg yolk provides cell protection with the aim to prepare chemically defined extender. Pace and Graham have purified egg yolk using ultracentrifugation and observed that LDL had a cryoprotective action. Many investigations confirmed that LDL have a cryoprotective action in the egg yolk (Foulkes 1977, Moussa et al 2002). In recent years, numerous studies have confirmed that LDL extracted from egg yolk is successfully used for cryopreservation of semen from boars, bulls and dogs (Moussa et al 2002, Hu et al 2006, Jiang et al 2007, Bencharif et al 2008, Varela et al 2009, Bencharif et al 2010, Hu et al 2010, Hu et al 2011) (Table 1).

CONCLUSION

Egg yolk has become a common component of extenders for cryopreservation of human and different animals semen for 60 years. It had been shown that egg yolk could help in resisting against cold shock and improving sperm fertility ability. Moreover, the protective action of yolk was largely attributed to LDL (Bogart and Mayer 1950, Watson and Martin 1975, Quinn and Chow 1980, Babiak et al 1999, Wall and Foote 1999, Manjunath et al 2002, Moussa et al 2002, Bergeron et al 2004, Bergeron and Manjunath 2006). In recent years, there have been increasing demands to replace whole egg yolk in semen extenders because of the presence of substances in yolk that inhibit respiration of spermatozoa or diminish their motility. LDL is one of the constituent of egg yolk that prevents binding of the BSP proteins to sperm sperm membrane. Therefore, LDL is beneficial to sperm functions during sperm cryopreservation (Bergeron et al 2004). The replacement of egg yolk by LDL in the composition of extenders was beneficial for semen cryopreservation (Demianowicz and Strezek 1996, Moussa et al 2002, Hu et al 2011). The extenders based on 6-10% LDL gave higher proportions of motile sperm, acrosome-intact sperm, plasma membrane-intact sperm and anti-oxidant enzymes activities following the freezing–thawing and thus improved frozen semen quality in comparison with the standard medium based (Tris-citric acid-glucose/fructose) on egg yolk (Hu et al 2011). However, the increase of LDL concentration in the

extender above 10% leads to a decrease in spermatozoa performance after freeze-thaw and the similar results were also reported by Moussa et al (2002). More researches are needed to evaluate and understand the respective roles of lipids and apoproteins from LDL and to isolate the antagonistic agent contained in egg yolk.

Table 1. Current observations about using LDL to replace whole egg yolk
Tablo 1. Yumurta sarısının tümünün yerine LDL kullanımı hakkında yapılan güncel araştırmalar

Name/Year	Species	Replacment	Used LDL average % (w/v)	Results
Moussa et al. 2002	Bull	20 % Egg Yolk	2.5 – 20% Optimum: 8%	Higher percentage and better movement characteristic were achieved.
Hu et al. 2010	Bull	20 % Egg Yolk	7–10% Optimum: 8%	LDL showed beneficial cryoprotective effects on frozen–thawed bull spermatozoa.
Hu et al. 2011	Bull	20 % Egg Yolk	7–9% Optimum: 8%	8% LDL gave the highest results than the egg yolk media in terms of semen quality parameters and anti-oxidant enzymes activities.
Hu et al. 2006	Boar	20 % Egg Yolk	6-10% Optimum: 9%	Significantly enhanced motility, acrosome and plasma membrane integrity of boar sperm after freezing and thawing.
Jiang et al. 2007	Boar	20 % Egg Yolk	6-10% Optimum: 9%	LDL showed better protection in the straw of 0.25 ml than 0.5 ml.
Bencharif et al. 2008	Dog	20 % Egg Yolk	4-10% Optimum: 6%	6% LDL medium provides improved protection of the spermatozoa during the freeze–thaw process and a marked improvement in the motility parameters of canine spermatozoa.
Varela et al. 2009	Dog	20 % Egg Yolk	6, 8, 10% Optimum: 8%	The replacement of egg yolk by LDL in the composition of extenders was beneficial for sperm motility and membrane integrity of cooled and frozen dog sperm.
Bencharif et al. 2010	Dog	20 % Egg Yolk	6%	The 6% LDL extender gave superior results to the Tris egg yolk extender.

REFERENCES

Ali Al Ahmad MZ, Chatagnon G, Amirat L, Moussa M, Tainturier D, Anton M, Fieni F. Use of Glutamine and Low Density Lipoproteins Isolated from Egg Yolk to Improve Buck Semen Freezing. Reprod Dom Anim. 2008; 43: 429–436.

Amirat L, Bencharif D, Vera-Munoz O, Pineau S, Thorin C, Destrumelle S, Desherces S, Anton M, Jouan M, Shmitt E, Tainturier D. In vivo fertility of bull semen following cryopreservation with an LDL (low density lipoprotein) extender: Preliminary results of artificial inseminations. Anim Reprod Sci. 2010; 122: 282–287.

Babiak I, Glogowski J, Luczynski MJ, Luczynski M, Demianowicz W. The effect of egg yolk, low density lipoproteins, methylxanthines and fertilization diluent on cryopreservation efficiency of northern pike (*Esox lucius*) spermatozoa. Theriogenology. 1999 ; 52: 473–479.

Bencharif D, Tainturier D, Amirat L, Barriere JP. Comparison of diluents supplemented with egg yolk or LDL for the cryopreservation of canine semen. Reprod Domest Anim. 2006 ; 41: 316.

Bencharif D, Amirat L, Anton M, Schmitt E, Desherces S, Delhomme G, Langlois ML, Barrie`re P, Larrat M, Tainturier D. The advantages of LDL (Low Density Lipoproteins) in the cryopreservation of canine semen. Theriogenology. 2008; 70: 1478–1488.

Bencharif D, Amirat L, Garand A, Anton M, Schmitt E, Desherces S, Delhomme G, Langlois ML, Barrière P, Destrumelle S, Vera-Munoz O, D Tainturier D. Freezing canine sperm: Comparison of semen extenders containing Equex® and LDL (Low Density Lipoproteins). Anim Reprod Sci. 2010; 119: 305-313.

Bencharif D, Amirat L, Guillou JL, Garand A, Anton M, Schmitt E, Desherces S, Delhomme G, Langlois ML, Destrumelle S, Vera-Munoz O, Barrie`re P, Tainturier D. Canine-chilled Sperm: Study of a Semen Extender Made with Low-density Lipoproteins from Hen Egg Yolk Supplemented with Glutamine. Reprod Dom Anim. 2012; 48(2): 258-66.

Bergeron A, Crete MH, Brindle Y, Manjunath P. Low-Density Lipoprotein Fraction from Hen's Egg Yolk Decreases the Binding of the Major Proteins of Bovine Seminal Plasma to Sperm and Prevents Lipid Efflux from the Sperm Membrane. Biol Reprod . 2004; 70: 708–717.

Bergeron A, Manjunath P. New Insights Towards Understanding the Mechanisms of Sperm Protection by Egg Yolk and Milk. Mol Reprod Dev. 2006; 73(10): 1338-44.

Bogart R, Mayer D. The effects of egg yolk on the various physical and chemical factors detrimental to spermatozoa viability. J Anim Sci. 1950; 9: 143.

Celeghini ECC, Arruda RP, Andrade AFC, Nascimento J, Raphael CF, Rodrigues PHM. Effects that bovine sperm cryopreservation using two different extenders has on sperm membranes and chromatin. Anim. Reprod. Sci. 2007; 100: 1–13.

Demianowicz W, Strezek J. The effect of lipoprotein fraction of egg yolk on some of the biological properties of boar spermatozoa during storage of the semen in liquid state. Reprod Domest Anim. 1996; 31: 279–280.

Evenson DP, Melamed MR. Rapid analysis of normal and abnormal cell types in human semen and testis biopsies by flow cytometry. J Histochem Cytochem. 1983; Jan;31(1A Suppl): 248–53.

Foulkes JA. The separation of lipoproteins from egg yolk and their effect on the motility and integrity of bovine spermatozoa. J Reprod Fertil. 1977; 49(2):277-284.

Haidl G, Schilling WB. Assessment of sperm chromatin condensation: an important test for prediction of IVF outcome. Arch Androl. 1994; May-Jun;32(3): 263–6.

Hu J, Li OW, Li G, Chen XY, Yang H, Zhang SS, Wang LQ. The Cryoprotective Effect on Frozen-thawed Boar Semen of Egg Yolk Low Density Lipoproteins. Asian Australas J Anim Sci. 2006; 19(4): 486-494.

Hu JH, Li QW, Zan LS, Jiang ZL, An JH, Wang LQ, Jia YH. The cryoprotective effect of low-density lipoproteins in extenders on bull spermatozoa following freezing–thawing. Anim Reprod Sci. 2010; 117: 11–17.

Hu JH, Jiang ZL, Lv RK, Li QW, Zhang SS, Zan LS, Li YK, Li X. The advantages of low-density lipoproteins in the cryopreservation of bull semen. Cryobiology. 2011; 62: 83–87.

Jiang ZL, Li QW, Li WY, Hu JH, Zhao HW, Zhang SS. Effect of low density lipoprotein on DNA integrity of freezing–thawing boar sperm by neutral comet assay. Anim Reprod Sci. 2007; 99: 401–407.

Kampshmidt RF, Mayer DT, Herman HA. Lipid and lipoprotein constituents of egg yolk in the resistance and storage of bull spermatozoa. J Dairy Sci. 1953; 36: 733–742.

Luster SM. Cryopreservation of bovine and caprine oocytes by vitrification. A Thesis of Master of Science, Louisiana State University and Agricultural and Mechanical College, Urbana-Champaign, USA. 2004.

Manjunath P, Nauc V, Bergeron A, Me´nard M. Major proteins of bovine seminal plasma bind to the low-density lipoprotein fraction of hen's egg yolk. Biol Reprod. 2002; 67: 1250–1258.

Moussa M, Martinet V, Trime`che A, Tainturier D, Anton M. Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen–thawed bull semen. Theriogenology. 2002; 57: 1695–1706.

Pace MM, Graham EF. Components in egg yolk which protect bovine spermatozoa during freezing. J Anim Sci. 1974; 39: 1144–1149.

Phillips PH. The preservation of bull semen. J Bio Chem. 1939; 130: 415.

Quinn PJ, Chow PYW. Evidence that phospholipids protects spermatozoa from cold shock at a plasma membrane site. J Reprod Fertil. 1980; 60: 403–407.

Varela AS, Corcini CD, Ulguim RR, Alvarenga MVF, Bianchi I, Corrêa MN, Lucia T,

Deschamps JC. Effect of low density lipoprotein on the quality of cryopreserved dog semen. Anim Reprod Sci. 2009; 115: 323–327.

Wall RJ, Foote RH. Fertility of bull semen frozen and store in clarified egg yolk-Tris-glycerol extender. J Dairy Sci. 1999; 82: 817–821.

Watson PF, Martin CA. The influence of some fractions of egg yolk on the survival of ram spermatozoa at 5^0C. Aust J Biol Sci. 1975; 28(2): 145–152.

Woelders H, Matthijs A, Engel B. 1997. Effects of trehalose and sucrose, osmolality of the freezing medium, and cooling rate on viability and intactness of bull sperm after freezing and thawing. Cryobiology. 1977; 35(2): 93-105.