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Sperm Characteristics of Holstein Bull Semen Cryopreserve by using Three Various Types of Extenders

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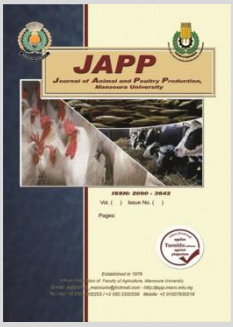

ABSTRACT

The goal of this study was to assess effect of three Tris-extender types, of egg yolk (E1), soy lecithin (E2), and coconut milk (E3), on sperm characteristics of Holstein bull semen at freezing stages. Semen was collected and pooled on each collection day. Semen should have good mass motility ≥70%. The pooled semen was divided into three divisions; E1: extended by E1: Tris-citric with 15% egg yolk; E2 and E3: diluted with 0.5% soy-lecithin and 15% coconut milk at a rate of 1:15, respectively. Semen with each extender was diluted and cooled at 5ºC for 4 hours, as equilibration period. Then, semen was packaged in 0.25 ml French straws and frozen in Liquid nitrogen (-196 °C) for one month. Results showed that E2 showed higher positive impact on sperm characteristics than E1 and E3 after dilution and equilibration, and thawing in terms of livability, plasma membrane integrity, motility, intact acrosome of spermatozoa, but the differences were not significant. In post-thawed semen -E2 was showed higher (P<0.05) progressive motility, and livability than other semen extenders. Also, E2 showed (P<0.05) higher sperm plasma membrane integrity, and intact acrosome, but the differences were not significant. The percentage of sperm abnormalities was similar in all extenders. In conclusion, this study recommended that 0.5% soy bean lecithin can be used instead of 15% egg yolk or 15% coconut milk for dilution and cryopreservation of bull semen.

Keywords: Bovine semen, dilution, cryopreservation, sperm characteristics.

INTRODUCTION

Artificial insemination was the most widely used method of buffalo and cattle requires convenient culture to dilute and keep semen samples of best bulls from high superiority male germplasm to the extreme probable range. Several compounds which enter in preparing semen diluent play a vital role in preserving sperm characteristics and protecting them from impaired effects of temperature during cryopreservation process. Spermatozoa face some problems through storage procedure including cold shock, osmotic changes, pH fluctuations, cryo-damages, energy depletion through metabolism throughout freezing-thawing stages. Through cryopreservation steps, phospholipid to cholesterol percentage of sperm bio-membranes becomes unbalanced principally because of cholesterol efflux and generation of several reactive oxygen species (ROS). All of these imbalances have a straight effect on sperm fertility. Consequently, a composition of perfect medium of semen diluent and semen additives should be used throughout semen preservation (Nitin Raheja *et al.*, 2018). Semen cryopreservation process considers an effective technique for enhancing animal breeding systems. Membrane integrity and post-thaw sperm motility are decreased because of osmotic stress and cold shock throughout cryopreservation procedures (Maxwell and Salamon, 2000). At the present time, egg yolk is the greatest public ingredient from utmost semen diluents to keep sperm from damaging through freezing–thawing technique (Forouzanfar *et al.* 2010). Although, egg yolk as a semen extender have many difficulties such as it leads to rising the danger of microbial infection this increase have to lead to endotoxin creation which can decrease the sperm ability of fertilizing and rise the chance of danger of spreading disease transmission in the international interchange of frozen semen (Beccaglia *et al.*, 2009).

Soy bean lecithin (SL) is a normal composition of palmitic, oleic, and many fatty acids like as stearic phosphatidylcholine. All these fatty acids are principal phospholipids recognized to deliberate structural stability to cells and are in maximum of mammalian biological membranes (Oke *et al*., 2010). Lecithin had been proven as the main origin source of lipoprotein in semen diluents (Papa *et al*., 2010). Most reports indicated that adding SL to semen diluent improves acrosome integrity, viability, membrane structure, and post thawed motility in human spermatozoa (Reed *et al.*, 2009).

Coconut milk extract is an hydrolipidic extraction of the not-dehydrated endosperm Cocos Fresh comprises: 3% proteins and about10% carbohydrates, 50% water, Fatty acids nucifera L. The coconut endosperm comprises 35-40% of oil. The coconut oil is consists of saturated fatty acids The principle feature coconut oil is the high content of saturated medium-sized chain fatty acids and short 45-53,2% lauric acid (12:0) 6,8-21%, myristic acid (14:0) 7,5-10, , 4,6-10%, caprylic acid (8:0) 5-8%,2% palmitic acid (16:0) capric acid, (10:0) 2-4% stearic acid (18:0). Unsaturated fatty acids content of essential fatty acids (Codex Stan 210; 1999)5-10%, oleic acid (18:1), 1.0-2.5% and linoleic acid (18:2). There are near by 10% of carbohydrates in coconut endosperm. Some of them are D-galactose, galactomannan, D-galacturonic acid and L-rhamnose vitamin C. Other active principles organic

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acids such as minerals (magnesium, potassium, calcium, sodium, phosphorous, malic acid, citric acid and sitosterol's. The goal of this study was to assess effect of three Tris-extender types, of egg yolk, soy lecithin, and coconut milk, on sperm characteristics of Holstein bull semen at freezing stages.

MATERIALS AND METHODS

This research was managed at the International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

Semen collection

Artificial vagina device was used to collect the semen ejaculates from five sexually mature Holstein bulls. All bulls were clinically, healthy, and free from any internal and external parasites causes. The testicular tone was moved freely up, down and glandular inside the scrotum and nearly equal in size. Semen was collected from each bull and held immediately in a water bath at 37ºC before transporting to the lab. Semen ejaculates were pooled on each collection day and semen was collected for 11 weeks. On each collection day, semen was pooled and divided into 3 portions; the first was extended with egg yolk semen extender, Second was diluted with lecithin extender, and the third portion was extended with coconut extender.

Experimental extenders.

The Tris-base extender was Tris-citric acid-egg yolk (E1) was prepared from, 7.0% glycerol, 1.675 g citric acid, 3.025 g Tris– (amino methane -hydroxyl methyl) and 0.75 g glucose. In E1, 7% egg yolk was replace by 0.5% soy-lecithin (Sigma, St. Louis, MO, USA), or 15% coconut milk in E2 and E3, respectively Amounts of 0.005 g streptomycin and 0.25 g lincospectin were added to all extenders. All contents were carefully melted in bi-distilled water up to 100 ml.

Preparation of coconut milk extender.

The coconut milk diluent preparation followed by a simple nevertheless sterilized process. The meat of recently harvested coconut (*Cocos nucifera*) was carefully mixed and putted in a 250 ml conical flask instrument. The water which extracted from the coconut was added to the combination and the mixture allowed standing for about 1 h. Subsequently, the milk was extracted from the combination by enclosing in a heat sterilized white cloth and strongly squeezed. The milk was filtered over aseptic white clothes thrice to overcome all deposits, then the liquid extract was collected in an aseptic flask. The milk was kept in a refrigerator at 4°C until use (Ogbu *et al.*, 2014).

Semen freezing:

Pooled semen ejaculates were diluted at a rate of 1:15. The soy- lecithin and tris-egg yolk semen extenders were wormed in a water bath (37ºC) during semen extension and then cooled gradually at 5ºC for 4 hours in a refrigerator, as an equilibration period. At the end of time of equilibration stage, diluted semen was overloaded in (0.25) ml French straws contained 20 x 10^6 motile sperm to be filled by using a filling machine of semen. During filling in the straws, the extended semen was kept in ice water bath to keep its temperature at 5ºC. Straws were transported into liquid nitrogen container and located above the surface of liquid nitrogen horizontally in standing nitrogen vapor 4 cm for 10 minutes, then the straws were putted vertically in a metal container and captivated fully in container of liquid nitrogen at –196ºC for storage. . Frozen semen straws were dipping into a water bath for 30 seconds at 37ºC to be thawed.

Semen evaluation.

Percentage of livability, motility of spermatozoa, then sperm count was estimated by using a hot microscope stage in post-diluted, post-equilibrated and post-thawed semen adjusted at 37ºC. Research microscope with high power magnification (x400) and warmed stage (37°C) was used to estimate the percentage of progressive sperm motility (Amman and Hammerstedt,1980). Livability percentage of sperm cells was assessed by using eosin and nigrosin combination stain (Hackett and Macpherson, 1965). Dead sperm (stained ones) and live sperm (unstained ones), were calculated at field of 200 sperm cells. Percentage of intact acrosome was conducted as showmen by Watson (1975). Sperm morphology estimation has been described through a bright-field microscope by noticing stained semen ejaculates.

Assessment of acrosome integrity

Acrosome integrity was determined by using a Giemsa stain procedure as described by Watson, (1975). A drop of diluted semen was smeared on a pre-warmed slide and dried in a current of warm air. The smears were fixed by immersion in 10% buffered formal saline for 15 minutes, and then washed in running tape water. The smears were air dried and then immersed in buffered Giemsa solution (3 ml Giemsa stock solution was diluted with 2 ml of Sorensen's buffer, Ph 7, and 35 ml distilled water) in a coplin jar for 90 minutes after which they were rinsed briefly in distilled water and dried. The dried smears were studied under a light microscope at magnification of 100x using oil immersion lens. The percentage of normal acrosome was calculated for about 200 spermatozoa randomly selected from at least four microscopic fields. The acrosome was considered to be normal when the stain was clearly and evenly distributed over the spermatozoa anterior to equatorial segment.

Assessment of plasma membrane integrity

Hypoosmotic swelling-test (HOS-t) is used to estimate the plasma membrane integrity of sperm cells (Pérez-Llano *et al.*, 2003). Briefly, the straws were thawed in a water bath at 37°C for 45 s. The assay was achieved by mixing 1 ml of hypoosmotic solution (7.35 g sodium citrate·2·H2O and 13.51 g fructose in 1 l of distilled water) with 50 all of the semen. After incubation at 37°C for 30 min sperm swelling was evaluated by putting 15 all of well-mixed sample on a warm slide (37°C) which was enclosed with cover glass before being observed at 400 × magnification under light microscopy. After HOST Sperm with intact plasma membrane had coiled tails. Three hundred spermatozoa per slide were spotted. García-López *et al.* (1996), Vazquez *et al.* (1997) The sperms were classified as negative or positive based on the absence or presence of coiled tail.

Statistical analysis

Data of this study were statistically analyzed by SPSS (2013) using general model program one-way ANOVA. Duncan multiple variety tests were used to test the significant differences among means (Duncan, 1955). The percentage values were exposed to arcsine transformation before the analysis performance of modification after being recalculated from the transformed standards to ratios means were presented.

RESULTS AND DISCUSSION

Results

Sperm characteristics of diluted semen:

The effect of three different types of extenders on the sperm characteristics of Holstein bull spermatozoa after dilution stage are presented in Table 1. Sperm motility was slightly increased in tris egg yolk extender compared to soy lecithin and coconut milk extenders, but the differences were not significant. Sperm livability in post dilution stage was insignificantly higher in soy lecithin extender than egg yolk and coconut milk extenders. Sperm abnormalities were similar in all semen extenders after dilution stage. Plasma membrane integrity and intact acrosome were higher in soy lecithin extender than in egg yolk and coconut milk extenders, but the differences were not significant.

Table 1. Effect of type of extenders on sperm characteristics in diluted semen.

Sperm characteristics (%)	Semen extender		
	15% Egg yolk	0.5% Soy lecithin	15%coconut milk
Progressive motility	69.0 ± 0.86	67.5±2.62	67.7±0.92
Livability	82,9±. 36	83.2±0.94	81.8±1.70
Abnormalities	4.50 ±0.15	4.50±0.15	4.50±0.15
Plasma membrane integrity	80.1 ±1.01	81.5±1.13	80.6±1.11
Intact acrosome	80.9±0.89	82.3±1.13	81.3±0.79

Sperm characteristics of post-equilibrated semen:

The effect of different types of extenders on sperm characteristics after equilibration stage of cryopreservation are shown in Table 2. It was observed that soy lecithin extender showed the best impacts on all sperm characteristics compared with egg yolk extender and coconut milk extenders, but the differences were not significant.

Table 2. Effect of type of extenders on sperm characteristics in post-equilibrated semen.

Sperm characteristics (%)	Semen extender		
	15%Egg yolk	0.5%Soy lecithin	15%Coco nut milk
Progressive motility	60.5±1.14	62.5±1.23	59.2±1.37
Livability	73.3±1.40	74.9±1.47	70.9±1.57
Abnormalities	4.5±0.15	4.5±0.15	4.5±0.15
Plasma membrane integrity	75.3±1.1	75.8±1.3	75.2±1.1
Intact acrosome	76.3±0.93	77.1±1.34	76.0±1.01

Sperm characteristics of post-thawed semen:

The results present in Table 3 showed the effect of three different types of extenders on sperm characteristics after thawing stage of cryopreservation. It was observed that progressive motility and livability of sperm cells in post-thawed semen were significantly ($P<0.05$) the highest in soy lecithin, followed by egg yolk, and the lowest in coconut milk extenders. However, abnormality, plasma membrane integrity, and intact acrosome were not affected by type of extenders.

Table 3. Effect of types of extenders on sperm characteristics in post-thawed semen.

Sperm characteristics (%)	Semen extender		
	15% Egg yolk	0.5%Soy lecithin	15%Coco nut milk
Progressive motility	42.0±0.66^{b}	46.2±1.01^{a}	41.0±0.65^{c}
Livability	64.1±1.20^{b}	68.1±0.94 ^{a}	64.3±1.20^{c}
Abnormalities	4.5±0.15	4.5±0.15	4.5±0.15
Plasma membrane integrity	71.0±1.12	72.1±1.44	70.7±1.07
Intact acrosome	72.1±0.86	73.8±1.20	71.8±1.01

a, b and c mean denoted within the same row with different superscripts are significantly different at P<.0.05

Discussion

Sperm cryopreservation technique had a fundamental importance in the application of artificial insemination (AI). The cryopreservation and thawing have been shown to increase membrane integrity and motility (Prathalingam *et al.,* 2006). There is a tight association between intact plasma membrane, fertilizing ability, post-thaw sperm motility, and acrosome spermatozoa (Anzar and Garham, 1995). Lecithin (current in various cryoprotectants) keeps the plasma membrane by restoring phospholipid that is impaired because of heat, and protects the cell viability (Campbell and Farrel 2007). Soy-lecithin is the best alternative to phospholipids present in egg yolk for semen cryopreservation process. Egg yolk extender is normally used as a non-penetrable for tris-based extenders cryoprotectant, which increases the protection ability factors in contrast to thermal shock problem and keep acrosomal, and keeps sperm motility in addition to mitochondrial integrity (Moustacas *et al.,* 2011). Despite of egg yolk have cryoprotectant adversaries, HDLs and egg yolk grains that effects on sperm motility Ansari *et al.(* 2010).by the way, soybean lecithin is supposed as a better emulsifier was proven by Trotta *et al.* (2002) Rydhag and Wilton. (1981). Soybean lecithin as semen extender in freezing diluent might support cryoprotectants to distribute frequently and decrease the its partial concentration, which lead to mitigate the toxicity of cryoprotectants on boar sperm through the freeze-thawing procedure. The sperm movement features in terms of acrosome integrity and plasma membrane integrity were used as predictors for superiority of frozen-thawed boar sperm. In the current study, freezing diluent with 0.5% soybean lecithin could give the best cryoprotective action for spermatozoa during cryopreservation, in comparing with egg yolk and coconut milk extenders. This was the same to some studies on cryopreservation of sheep (Fukui *et al.,* 2008) and bovine ,Amirat *et al*., 2005; Gil *et al.,* 2000) semen. Many of scientists informed that the cryoprotective factors effect on soybean lecithin on freezing bovine sperm was the same to egg yolk extender (20%),Thun *et al.*.(2002), Aires *et al.*(2003). However, in the current study, the effect of soybean lecithin (0.5%) on improving the sperm movement features, acrosome integrity and plasma membrane integrity was greater than tris egg yolk semen extender. We evidenced that the causes were possibly as follows: firstly, egg yolk semen extender supplied deprived welfare for sperm through process of cryopreservation contrasting for bovine spermatozoa (Benson *et al*., 1967; Bathgate *et al*., 2006), so the defensive factors of soybean lecithin semen extender on frozen sperm was higher than those of egg yolk semen extender. Secondly, the attendance of particulate debris in diluents and higher viscosity were hypothetical as appreciated aspects erodes the spermatozoa fertilizing capability (Vishwanath and Shannon, 2000; Van Wagtendonk-de Leeuw *et al*., 2000). Therefore, soybean lecithin semen diluent might play as a supported role for sperm trough out process of semen cryopreservation because of its low viscosity and less debris cause. The Third reason was ideal concentration of soybean lecithin semen extender could play a defensive role for sperm membrane throughout the freeze-thawing procedure. Also, the spermatozoa should be capable of swim more simply in freezing diluents containing soybean lecithin extender than other diluents, which would lead to the best sperm movement. It was commonly recognized that cold shock and cryodamage could damage the biological role of sperm membrane because

of the difference of the lipid conformation (such as phospholipids, saturated hydrocarbon. etc) of its the fluidity of the plasma membrane and bilayer through the freeze-thawing method (De Leeuw *et al*., 1990; Pettitt and Buhr, 1998; Johnson *et al*., 2000). To our information , there are principally two hypotheses to show its defensive methods in the cryopreservation of sperm such as fundamental conformations of bio-membrane of spermatozoa of mammals, phospholipids were confirmed to play an principal function in adjusting the physiological role of bio-membranes and passing to the cell to reduce the freezing stage formation of crystal by substitution of plasmalogens to mitigate mechanical damage for spermatozoa bio-membrane, Waterhouse *et al.* (2006),Giraud *et al*.(2000) Graham and Foote (1987),. this view which supposed that soybean lecithin and lecithin of egg yolk could decrease the phospholipids / cholesterol ratio of spermatozoa cell membranes by permeating into the spermatozoa membrane was proven by few scientists, so capacitation as changes through the freezing method were controlled to increase the fertilizing capability of frozen-thawed sperm, Gamzu *et al*.(1997) ,Davis (1981). As well as, phospholipids could substitute many phospholipids of the spermatozoa membrane to preserve its formation Graham and Foote (1987) Trimeche *et al*.(1997). Though, additional theory was the same normally recognized by many academics. They thought that phospholipids extracted from soybean lecithin semen extender or egg yolk extender might not pass into spermatozoa membrane to modify the concentration of phospholipids in bio-membrane, nevertheless it could participate with spermatozoa membrane to make a defensive film in contrast to structure of lethal intracellular formation of ice crystal and keep the spermatozoa membrane from mechanical destroy through the freeze-thawing procedure, Quinn *et al*.(1980), Simpson *et al* (1987). soybean lecithin micro-elements were rather greater than spermatozoa below microscope. Thus, they assume that it was utmost unthinkable that soybean lecithin microparticles pass through spermatozoa membrane and stopped spermatozoa bio membrane contrary to cryodamage and we favored to approve with the previous view. By the way, soybean lecithin semen extender is also supposed a best emulsifier, Trotta *et al* . (2002), Rydhag and Wilton. (1981). It appears reasonable to assume that soybean lecithin semen extender in freezing diluent could inspire cryoprotectants factors to distribute regularly besides that it decrease the its partial concentration, which led to reduce the poisonousness of cryoprotectants on sperm of the boar during the freeze-thawing stage . Also, they hypothesize that soybean lecithin semen extender could keep the proportion of lipid structure formation of the spermatozoa membrane by relating by damaging units of seminal plasma which is the same as LDL, Bergeron and Manjunath (2006), Manjunath *et al.* (2007). Although, those suppositions must be more established. results discovered that soybean lecithin extender supplement might lead to a better cryoprotective capacity during freezing-thawing stage boar sperm. It importantly enhanced boar sperm acrosome integrity, motility membrane integrity, parameters. The characteristics of soybean lecithin semen extender on sperm superiority were importantly and the ideal concentration in bovine semen diluent. Another probability, also extensively accepted by lots of scientists, is that the soy lecithin semen extender or egg yolk extender phospholipids do not pass through the membrane to modify the concentration of phospholipids nevertheless may form a defensive film around the cell to stop the formation of intracellular ice crystals and to defend the sperm membrane from mechanical destroy through freezing Zhang *et al* (. 2009). The active part of egg yolk that supply with protection is thought to be a low-density lipoprotein Watson and Martin(1975). Lecithin in egg yolk and soybean saves sperm membrane phospholipids and supply with the tolerance of spermatozoa to freezing process Moussa *et al*.(2002). Although, there are numerous differences in the formation of lecithin obtained from egg yolk and those extracted from plants as soy-lecithin, Lekshmi Bhai *et al* .(2015). Also, there are clashing reports about the useful effect of lecithin Forouzanfar *et al.* (2010) Papa *et al* (2011). The rewards of lecithin semen-based extender over egg yolk concerning hygienic difficulties are un separate doubt. Nevertheless, the suggestion that cryoprotective volume of soy-lecithin semen extender is like that supported by egg yolk extender was not confirmed. semen cryopreservation characterizes the most commonly active generative method in present animal breeding. Not only does it allow the preservation of seldom genetics (germplasm preservation), nevertheless too enables for quickening genetic development by permitting the spreading of progeny confirmed bulls at a widespread rule with the consequent assistance of rising of efficiency and surge of appreciated economic genomic characters. Significantly, cryopreservation of sperm simplified the huge practice of artificial insemination in almost commercial cattle procedures ,Grotter *et al* (.2019). it must also be observed that Despite of cooling/thawing modifications have been showed the aim of sperm protection ,these differences have understood fairly reasonable achievement in relations of protection its applied integrity , lyophilization, viability and Vitrification are thought hopeful replacements with respect to protective sperm viability over all time. Though, the secretion source has not been be realized because of mainly to the change ability of the results, not only among various types but also in similar types. So, spermatozoa verification process, lyophilization applicability and free receipt resolve need to procedural modification and extra research to recover on their present to be near of success. The current results revealed higher ($P<0.05$) percentage sperm plasma membrane integrity in soy lecithin extender (0.5gm) compared to both of 15% egg yolk and 15% coconut milk extenders at post equilibration and post-thawing processes. Also, coconut milk consider as rich source of lipid Yong *et al*.(2009), a main constituent membrane of sperm that is elaborate in a sequences of functional and biochemical changes eventually essential for fertilization process Berque *et al.(* 2003). It holds great quantity of polyunsaturated fatty acids recognized to give to flexibility of membrane and resistance Wassal and Stillwell. (2009). Nevertheless, through process of cryopreservation, significant variations in phospholipids and lipid conformation less in seminal plasma occur ,Chakrabarty *et al.* (2007), Futino *et al*.(2010) Both 5% and 10% coconut milk diluents supply the best protection factors to acrosome integrity as sperm feature , possibly through a straight action throughout maintain or the exchange of acrosomal membrane phospholipids. Sakanaba *et al.* (2004) ,Young *et al.* (2006). concluded that the protection effect of coconut milk semen extender might be return to its main proteins which consist of main amino acids act avital part in membrane integrity of the cell. These results are similarly to that results which confirmed that spermatozoa verified with 15% and 20% of coconut milk had higher ($P<0.05$) sperm membrane integrity was founded by. Daramola *et al.* (2016).

CONCLUSION

In conclusion, soy-lecithin at a level of 0.5% (as lipoprotein plant source / the lipid) can be an alternative instead of both egg yolk and coconut semen extenders as an animal product in tris extender preparation for cryopreserved Holstein bull semen due to it improve sperm characteristics of cryopreserved Holstein bull semen.

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تاثير مراحل عمليه الحفظ بالتجميد على صفات السائل المنوى لطلائق الهولشتين باستخدام ثلاث انواع من مخففات السائل المنوى

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تهدف هذا الدراسه الى دراسه تاثير عمليه الحفظ بالتجميد على صفات السائل المنوى بإستخدام ثلاثه انواع مختلفه من مخففات السائل المنوى (مخفف ليسيسين فول الصويا (0.5%) - مخفف صفار البيض (15%)- مخفف لبن جوز الهند (15%). تم اخذ عينه ممثله للسائل المنوى لعدد خمسة طلائق هولشتين لاتقل الحيويه فيها عن 70% وتم اخذ عينه السائل المنوى تحت درجه الغرفه وبعد فتره الموازنه (بعد وضعها فى الثلاجه لمده اربع ساعات) ثم التجميد فى النتيروجين السائل على درجه حراره -196 لدراسه تاثير المخففات على الصفات الاتيه (حركه الحيوان - حيويه الحيوان المنوى - نسبه الشواذ- سلامه الغشاء البلازمى - سلامه الاكروسوم). تم تقدير الصفات السابقه فى السائل المنوى (بعد التخفيف - بعد فتره الموازنه - بعد الاساله) وقد أشارت النتائج الي عدم وجود فروق معنوية بعد التخفيف مباشرة وبعد فتره الموازنه بالنسبه لكل الصفات وزيادة معنوية في النسبة المئوية للحركه والحيويه بعد الاساله و عدم وجود فروق معنوية بالنسبه للحيوانات المنوية الحية ذات الأكروسوم السليم وسلامه الغشاء البلازمي للحيوان المنوى بعد الأسالة في المخفف المحتوي علي 0.5% ليسيثين الصويا مقارنة بالمخفف المحتوي علي صفار البيض 15% أومخفف لبن جوز الهند 15% لذلك توصى الدراسه باستبدال صفار البيض بالليسيثين في مخفف الترس عند مستوي (0.5%) لزيادة القدرة التجميدية للسائل المنوي لطلائق الهولشتين.