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Sperm lipid profile status of New Zealand rabbits during chilled storage for up to 72 hours concerning the addition of glutathione and taurine: An *in vitro* study

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Abstract

This study evaluated the effects of adding various doses of taurine and glutathione (GSH) to the rabbit semen extender at 24, 48, and 72 h after cooling at 5°C. Ejaculates with standard color, motility (> 85%), volume, and concentration of 400x106/mL were employed. These ejaculates were diluted with extenders supplemented with 0.5, 1, and 2 mM of GSH and 1, 5, and 10 mM of taurine and chilled at 5 °C. Semen samples were obtained from 20 New Zealand bucks. Non-supplemented samples were used as controls. The levels of total protein, triglycerides, and cholesterol in sperm were measured. The sperm's lipid profile was enhanced by GSH and taurine supplementation by reducing cooling-associated stress, which was determined by maintaining a constant triglyceride concentration. The total protein was maintained by GSH and taurine which was ascertained by keeping triglyceride concentration stable and maintaining the total protein levels in the extender and lowering the cholesterol levels. GSH and taurine supplementation to the extender had protective influences on the *in vitro* rabbit semen quality during chilled storage for up to 72 h, which were remarkable with increasing supplementation dose and cooling time at 5 °C. The continuity of vitality results from maintaining the vital properties of the cell wall structure by preserving the basic components of the cell wall such as triglyceride, cholesterol, and total protein.

Keywords: Glutathione (GSH); taurine; rabbit; sperm lipid profile.

Introduction

Rabbits are one of the wild animals spread all over the world of economic importance from nutritional and societal points of view. They are of high economic value for the producer and the consumer together as a result of the speed of their farms and for their financial return on their breeders.

There are numerous advantages of using rabbits as a model in this kind of experiment. Unlike mice, it is not necessary to sacrifice the rabbits to obtain sperm samples. Meanwhile, the size of the animals is relevant where small animals are simple to handle. In addition to the accessibility with which samples can be achieved, the speed with which results can be

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obtained where a doe's pregnancy lasts for about one month. The capacity to perform the same experiment, using rabbit sperm as a laboratory model represents another factor that makes it so important to use this animal species.

Conservation of the fertilizing capacity of fresh semen for the longest possible time is essential in the practice of artificial insemination (AI) which becomes more favorable and most suitable for large commercial Rabbitries (Marco-Jimenez et al.,2006). A basic problem with semen preservation is the high unsaturated fatty acid content of the spermatozoal membrane which tends to bind oxygen resulting in the formation of numerous peroxide bonds. Lipid peroxidation induced by reactive oxygen species (ROS) directly damages the phospholipids components of the cell membrane (Cheeseman, 1993). Therefore, the purpose of this research was to determine whether there is any change in the lipid profile of semen with different concentrations additive from glutathione and taurine on the basic extender.

Individuals need a source of dietary protein and nutrition, which is a basic requirement due to the growing human population and limited resource base, especially in face of the global climatic changes (Wiebe et al., 2019). The production of more animals, breeds, and variations is expected to increase animal productivity, which is an essential objective.

Currently, fresh diluted semen collected within 6–18 hours is used for AI programs in rabbits, and bucks are often housed on the same farm (Johinke et al., 2015). With a longer time between semen collection and female insemination made possible by increased semen storage capacity, AI performance would increase in farms without males would be possible (Gogol, 2013 and Aziza, 2017). However, keeping rabbit sperm in storage for more than 24 to 48 hours reduces fertility (Rosato and Iaffaldano, 2011). Therefore, one of the main objectives is to freeze the semen or extend the period of liquid semen preservation beyond 48 hours. In the last decade, frozen semen has produced satisfactory results on sperm quality and fertilizing capacity *in vitro* (Di Iorio et al., 2020; Di Iorio et al., 2014), although the reduced sperm viability after freezing is a significant disadvantage for the common usage of frozen sperm in AI programs.

All sperm compartments affected by the intra- and extra-ice formation are damaged by sperm cryopreservation, which also results in an excess of ROS produced by damaged mitochondria that damages the sperm membrane (Rekha, 2016; Nishijima et al., 2021). These damages are responsible for the loss of sperm motility, viability, and acrosomal and DNA integrity decreasing the fertilizing capacity of frozen-thawed sperm (Saha et al., 2022). Spermatozoa are rich in polyunsaturated fatty acids with low antioxidant levels within the cytoplasm (El-Gindy, and Zeweil, 2017; Ismail et al., 2020). The processes involved in diluting and chilling or incubating semen create several challenges due to semen's sensitivity to hypertonic solutions (Castellini, 1996) and its susceptibility to oxidative damage, which causes sperm motility and sperm capacitation deficiencies as a result of sperm metabolism's overproduction of ROS (De Lamirande et al., 1997). In light of this view, chilling semen between 24-48 hours is still essential and is applicable, as this technique is simple and inexpensive, requiring no special equipment. In most rabbit farms, fresh diluted semen (Campaniello et al., 2010; Fadl et al., 2020) or chilled semen is used, due to it is low cost and high survival rate compared to cryopreservation (Di Iorio et al., 2014). Sperm will lose its function if the polyunsaturated fatty acids in the sperm membrane are oxidized. Under these conditions, it was stated that supplementation with antioxidants reduces the impact of oxidative stress by ROS, generally, during the process of semen preservation *in vitro*. The lipid composition of sperm has a recognized importance as a structural and functional component. In particular, phospholipids (PL) of spermatic cells are characterized by very high proportions of long-chain polyunsaturated fatty acids (PUFA). Relationships are evident, in a broad general male population,

between various lipid profiles and semen quality. High amounts of PUFA make cells more vulnerable to the peroxidative damage caused by free radicals, which is thought to be a major factor in male infertility (Gliozzi et al., 2009). The high degree of unsaturation found in sperm is believed to contribute actively to the regulation of cell movement and lipid metabolism; it also confers sufficient fluidity to the sperm plasma membrane for the fusion events that characterize fertilization (Dutta, et al., 2019). Cholesterol is a steroid lipid found in the cell membranes. It is an essential component of mammalian plasma membranes (PM) where it is required to establish proper membrane permeability and fluidity (Yeagle 1985).

Meanwhile, proteins are involved in sperm capacitation, acrosome reaction, and fertilization. The most studied role of **Binder of Sperm Proteins** (BSPs) is their ability to bind and remove phospholipids and cholesterol from the sperm membrane, an initial event of capacitation (Thérien et al., 1999).

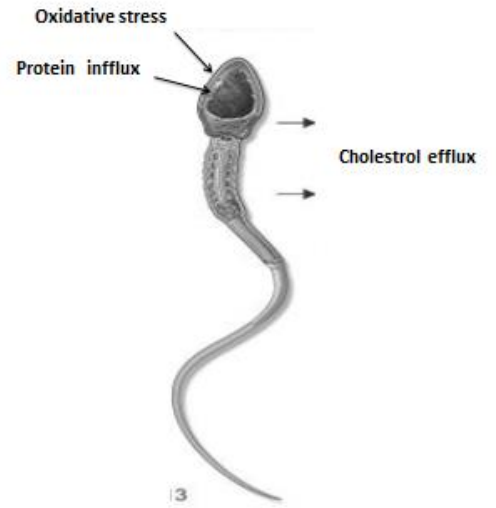

Figure (1): The continuous oxidative stress increases the exit of cholesterol from the cell membrane into the seminal fluid as a result of its binding to the protein.

Accordingly, more attempts are needed to improve semen extenders and storage conditions to prolong the time stored semen can maintain its functional status. The supplementation of antioxidant substances such as glutathione (GSH), taurine, lycopene, cysteine, and glutamine directly to the animals or in semen extenders to increase the antioxidant abilities and lower the effects of oxidative stress is just one method that has been used to preserve and improve semen quality and sperm viability (El-Khawagah et al., 2020). The addition of antioxidants such as taurine to ovine sperm (Bucak and Tekin, 2007), feline sperm (Baran et al., 2009), and rabbit sperm (Alvarez and Storey, 1983) has been shown to protect sperm against the harmful effects of ROS and improves sperm motility and membrane integrity during sperm liquid storage. Taurine is a sulfonic amino acid and is a nonenzymatic scavenger that plays an important role in the protection of spermatozoa against ROS, in case of exposure to aerobic conditions and storage at 4◦C under a refrigerator (Bucak and Tekin, 2007;

Alvarez and Storey, 1983). In recent years, taurine has been used as anti-oxidant in semen extenders and has been used in the cryopreservation of boar (Funahashi and Sano, 2005), bull (Uysal et al., 2007), human (Lopes et al., 1998), ram (Bucak et al., 2007), and goat sperm (Atessahin et al., 2008) to improve motility, viability, membrane integrity, and fertility of spermatozoa by inhibiting lipid peroxidation and protecting cells against accumulation of ROS (Chen et al., 1993). Further, a perusal of the literature revealed no information on the effect of the addition of taurine on the maintenance of sperm viability during low-temperature liquid storage of rabbit semen for a long period. The most common antioxidants, GSH, and taurine, have significantly enhanced the antioxidant properties of semen extenders. GSH improves the nuclear and plasma membrane integrity of sperm, maintaining the quality of the semen, and enhances spermatozoa's capacity to fertilize after the freezing-thawing process (Bucak and Tekin, 2007). Due to GSH's ability to act as an electron donor and lower the formation of H2O2 as well as its oxidation to glutathione disulfide (GSSG), it has anti-inflammatory effects. Additionally, the spermatozoa's plasma membrane is penetrated by the amino acid cysteine in GSH, boosting its intracellular biosynthesis. This protects the sperm's DNA, proteins, and membrane lipids by scavenging ROS (Zhu et al., 2017). Taurine, a sulfonic amino acid, is a non-enzymatic scavenger that also defends spermatozoa by preventing lipid peroxidation and shielding sperm cells from an accumulation of ROS (Estrada et al., 2017). Though, to the best of our knowledge, only a single study has investigated the effect of exogenous antioxidants supplementation on rabbit semen quality during long-term chilling (24h, 48h, and 72 h post-chilling) at 5 °C (Ahmad et al., 2021), while taurine has been considered only for *in vitro* incubation at 37 or 39 °C for 240 or 270 min (Paal et al., 2017). So, the antioxidant addition to a basic extender to maintain the effective operation of spermatozoa is crucial to guarantee a crucial balance between lipids, ROS, and the various elements of the cell's antioxidant system (Gliozzi et al., 2009). Therefore, the current study aimed to test the effect of supplementing different concentrations of GSH and taurine on the cholesterol, triglyceride, and total protein concentrations of semen extender after different chilling times.

Materials and Methods

Animal Management and Semen Collection

The current study was conducted following the ethical and regulation procedures of the Department of Animal Wealth Development, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt. This study used 20 rabbit bucks (n = 20) of the New Zealand white rabbit breed (about 2-3 kg body weight, and 1-1.5-year-old). Bucks were individually labeled and caged with free access to food and water. Animals were housed in well-ventilated rooms with a natural daylight supply. Bucks were trained for two weeks for semen collection using an artificial vagina and a mature female as a mount stimulator. Then, ten ejaculates from each buck were collected (one ejaculate/week), during the ten-week trials, for a total of 200 ejaculates. In each collection, fresh semen samples were microscopically examined and evaluated after removing the gel clot. The spermatozoa activity was microscopically evaluated at 100× (to evaluate mass motility) and 400× (for individual motility) using a phase-contrast microscope (Micros Austria, Micros St. Veit Hunnenbrunn – Gewerbezone, Veit/Glan, Austria) (Ciribe et al., 2018). A Neubauer hemocytometer was used to measure sperm concentration. Only ejaculates with a standard color, more than 85% motility, about 0.5 mL volume, and ~400 × 10^6/mL concentration were used for the study. During each time of collection, ejaculates were pooled (n = 20 with an average volume of ejaculates ~ 8.75 ± 1.165mL) to avoid individual variations and get sufficient semen volume for different treatments (Ahmad et al. 2021).

Extender preparation and semen processing and evaluation

The pooled semen samples were diluted in a 1:2 basic extender (Ahmad et al., 2021) containing 20mM Tris. 57mM citric acid anhydrous, 5mM fructose, 22mM glucose, and 4.6mM streptomycin along with 10 ml egg yolk and completed to 100 mL with distilled water with pH and osmatic pressure were kept at pH 6.9, 300 mOsm. The diluted pooled semen samples were divided equally into 7 fractions (1 fraction/treatment). The diluted semen samples were then supplemented with 0 (control with diluent only without additives), 0.5, 1, and 2 mM glutathione (GSH; cat.no. Y0000517, Merck KGaA, Darmstadt, Germany) and 1, 5, and 10 mM taurine (cat.no. T0625, Merck KGaA, Darmstadt, Germany). The pH was adjusted to 6.8-7 with a dilution factor of 1:2 (semen: prepared extenders), and the diluted semen was kept at 5 °C. Then, changes in semen cholesterol, triglyceride, and total protein were evaluated after 24, 48, and 72 h post-chilling. All the evaluations were repeated ten times throughout the trial (ten weeks).

Assessing the concentrations of cholesterol, triglyceride, and total protein in seminal plasma

Semen samples at 24, 48, and 72 post-chilling were centrifuged at 1,000 × g for 20 min, and the supernatant, composed of semen extender and seminal fluid, was used to assess the concentration of cholesterol, triglyceride, and total protein. Commercial kits were used to estimate the triglyceride concentration (mg/dl) (Bio-diagnostic - TR 20 30, Egypt), cholesterol concentration (mg/dl) (Bio-diagnostic - CH 12 20, Egypt) and total protein concentration (g/dl) (Bio-Diagnostic CAT. NO. TP 20 20) according to manufacturer's instructions.

Statistical Analysis

Results of the current study were statistically analyzed using Two-way ANOVA to test the effect of GSH and taurine supplementation, chilling time, and their interactions on semen lipid profile, and total protein followed by Tukey's multiple comparison test using GraphPad Prism (GraphPrism© Software, La Jolla, CA, USA) (Cerbo et al., 2017). Results were expressed as means ± SEM and considered significantly different at $P < 0.05$.

Results

Impact of GSH and taurine supplementation on semen total protein concentration at different post-chilling times

Figure 1 displays the post-chilling on semen total protein conc supplemented with GSH and taurine at different concentrations (0.5-2 mM GSH and 1-10 mM taurine). The addition of GSH and taurine improved the total protein level, especially with increasing chilling time. At 24 h post-chilling, adding GSH and taurine at different ratios was significantly correlated with increasing total protein concentration in semen samples preserved at 5 °C specifically for G2, T5, and T10. After 48 h of chilling at 5 °C, all supplemented groups had the highest protein content than after 24 h post chilling ($P < 0.05$), especially the T5 supplemented group increased. and 72 h post-chilling, there was a slight decrease in supplemented groups and the control had the lowest level of total protein compared to the other treatments ($P < 0.05$), however, there was a noticeable and continuous reduction in total protein concentration in non-treated semen samples along the preservation time. this is suggested as a result of the influx of protein into the cell with increased oxidative stress related to post-chilled preservation to bind with cholesterol and increase its efflux out of the cell to seminal plasma An interesting improvement of cell membrane structure was reported for treated semen samples with GSH and taurine ($P < 0.05$).

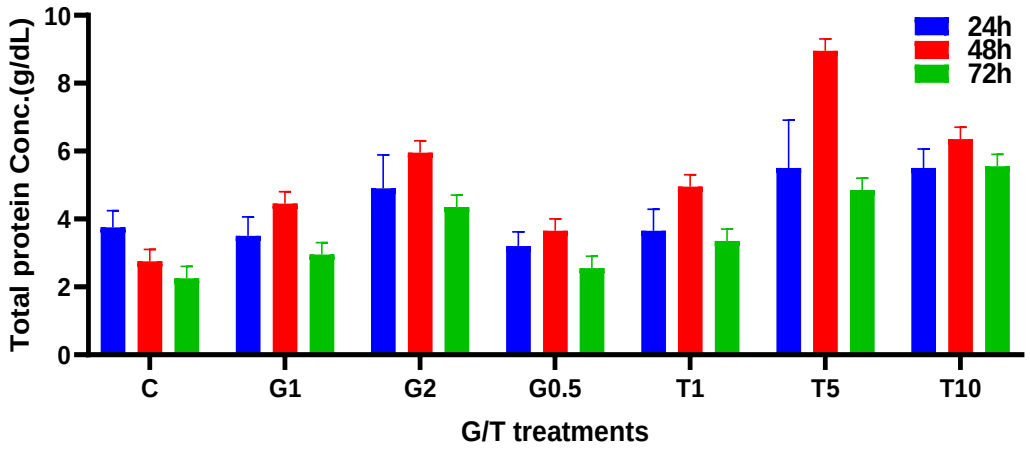

Figure 2. Effect of different concentrations of glutathione and taurine supplementation (0.5, 1, and 2 mM GSH, and 1, 5, and 10 mM taurine) on rabbit semen cholesterol level during 24, 48, and 72 h storage at 5 °C. Uppercase and lowercase letters indicate statistical significance at $P < 0.05$ between chilling times and extender supplements (GSH and taurine). C represents the control samples (no treatment), and G1, G2, and G0.5 represent 1, 2, and 0.5 mM of GSH, respectively. T1, T5, and T10 denote 1, 5, and 10 mM of taurine.

GSH and taurine supplementation improved the antioxidant properties of semen extender by altering the total cholesterol content.

Figure 2 illustrates the total cholesterol content in semen samples following GSH and taurine supplementation after different post-chilling times. After 24h all the treatments had similar levels. At 48h post-chilling at 5 °C. GSH and taurine supplementation at different concentrations was significantly correlated with lowering cholesterol concentration in semen samples preserved at 5 °C especially the supplemented G2, T1, and T10 compared to the other treated groups which had similar concentrations, after 72 h ($P < 0.05$) control group. There were significant effects of exposure time and different supplementations on the cholesterol levels all samples had less cholesterol content than the control ($P < 0.05$). However, increasing the chilling time to 72 h was significantly associated with increasing cholesterol content in the control confirmed by increasing the oxidation process followed by increasing cholesterol efflux from the cell membrane and increasing its level in seminal plasma, compared to GSH and taurine supplementation lowers oxidative stress in chilled semen samples, up to 72 h by reducing cholesterol oxidation. Therefore, increasing exposure time to 48 and 72 h altered cholesterol levels. After 48h and 72h, increasing the GSH and taurine doses was linked with a lowering of the cholesterol levels.

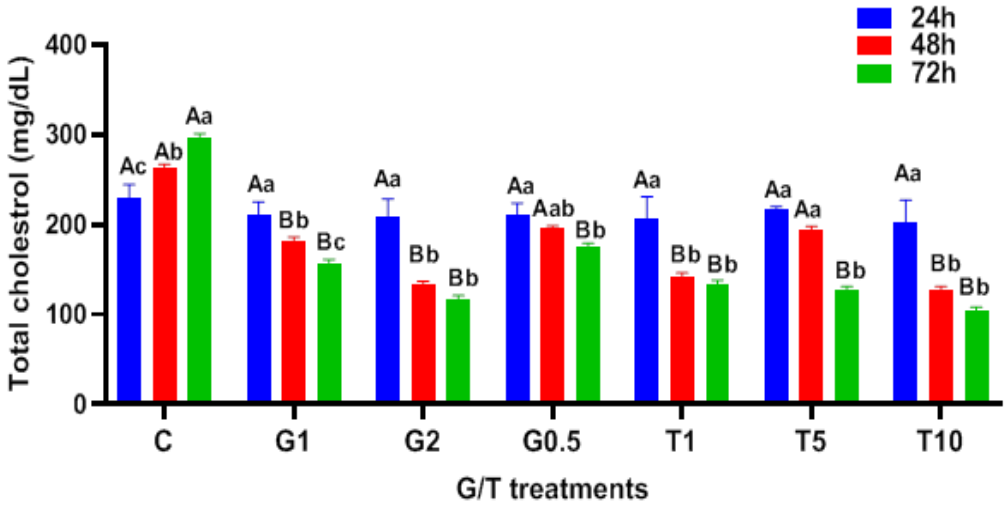

Figure 3. Effect of different concentrations of glutathione and taurine supplementation (0.5, 1, and 2 mM GSH, and 1, 5, and 10 mM taurine) on rabbit semen cholesterol level during 24, 48, and 72 h storage at 5 °C. Uppercase and lowercase letters indicate statistical significance at $P < 0.05$ between chilling times and extender supplements (GSH and taurine). C represents the control samples (no treatment), and G1, G2, and G0.5 represent 1, 2, and 0.5 mM of GSH, respectively. T1, T5, and T10 denote 1, 5, and 10 mM of taurine. Different uppercase and lowercase letters indicate statistical significance at $P < 0.05$ between chilling times and extender supplements (GSH and taurine), respectively.

GSH and taurine supplementation improved the antioxidant properties of semen extender by altering the total triglyceride content.

Figure 4 displays the alterations in triglyceride concentration during different chilling times and GSH and taurine supplementation. Both chilling time and GSH and taurine supplementation markedly modified triglyceride levels in semen with distinct interaction ($P < 0.05$). After 24 h post-chilling, higher concentrations of GSH and taurine significantly maintain stability triglyceride levels in semen samples, compared to the highest concentration of non-supplemented semen ($P < 0.05$). Increasing the chilling time to 48 and 72 h was associated with continuous persistence of triglyceride levels in all GSH and taurine-supplemented samples with light increasing with T5 supplemented group in contrast the non-supplemented semen samples ($P < 0.05$) significantly modified triglyceride levels which were prominent increasing with increasing chilling time ($P < 0.05$). This was due to insufficient antioxidants in diluted liquid and increasing oxidative stress leading to cell membrane lipid oxidation with triglyceride flow out of the cell membrane to the seminal plasma with increasing cooling time. So GSH and taurine supplementation in semen extender were associated with maintaining triglyceride concentration with increased chilling time.

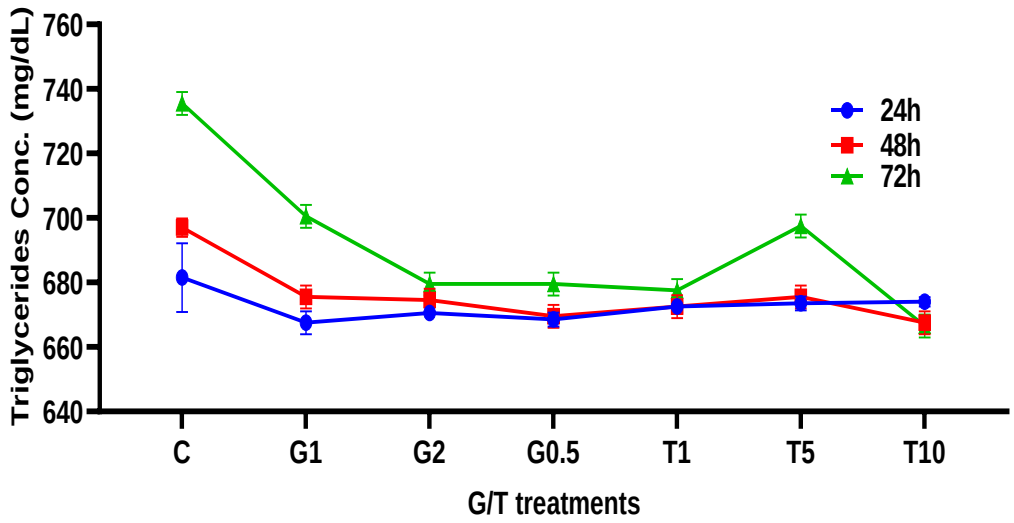

Figure 3. Triglyceride in response to different concentrations of GSH and taurine, where C represents the control samples (no treatment), G1, G2, and G0.5 represent 1, 2, and 0.5 mM of GSH, respectively. T1, T5, and T10 denote 1, 5, and 10 mM of taurine supplementation after different post-chilling times (24, 48, and 72 h) at 5 °C.

Discussion

Cell membrane fluidity is a prerequisite for normal cell functions and the fluidity and flexibility of cell membranes are mainly dependent on their lipid constitution (Lenzi1 et al, 1996). The lipid composition of sperm has a recognized importance as a structural and functional component. Relationships are quite evident, in a broad general male population, between various lipid profiles and semen quality. High amounts of PUFA make cells more vulnerable to the peroxidative damage caused by free radicals, which is thought to be a major factor in male infertility (Gliozzi et al 2009). ROS plays a regulating role in particular sperm functions, such as capacitation and acrosomal reaction, at low and controlled concentrations (Thompson et al 2013).

Procedures for cooling and freezing/thawing semen significantly alter the quality of preserved sperm. Because of the excessive generation of ROS like superoxide anion radicals (O2), hydrogen peroxide (H2O2), and lipid hydroperoxides, prolonged cooling reduces sperm quality and, as a result, its capacity to fertilize (Johinke, et al, 2014, Bansal, and Bilaspuri, 2010 and Palmieri, et al, 2014).

Moreover, semen dilution during chilled storage reduces the normal physiological antioxidants included in the semen. The maintenance of the fertilizing capacity of rabbit semen for longer than 48 to 72 h remains an important target for rabbit production because the farming system is based almost exclusively on AI programs (Saez Lancellotti et al. 2010). The high degree of unsaturation found in sperm is believed to contribute actively to regulation of cell movement and lipid metabolism; it also, confers sufficient fluidity to the sperm plasma membrane for the fusion events that characterize fertilization (Gliozzi, 2009). Cholesterol is a steroid lipid found in the cell membranes; it is an essential component of mammalian plasma membranes (PM) where it is required to establish proper membrane permeability and fluidity (Yeagle 1985). Also, it has been implicated in cell signaling processes (Simons Ehehalt, 2002). Changes in the organization of membrane lipids can have profound consequences on cellular functions such as signal transduction and

membrane trafficking (Visconti et al., 1999, Eyster, 2007). Liu and his colleagues, (2017) demonstrated that the total cholesterol level correlated with total sperm motility and progressive motility. So, supplementing the extender with antioxidant substances such as GSH and taurine improved the semen quality which is confirmed by the significant increases in the percentages of live spermatozoa, motile sperms, and acrosome reactions in this work, as was, also, presented in a previous part of our study (Bayomy et al., 2023). However, at 24 h post-chilling, GSH and taurine did not modify these values compared to the control. This effect is most likely connected to the sperm's inherent antioxidant capacity to scavenge ROS generation. The role of supplementation doses on these vital signs became apparent as the cooling duration increased (at 48 and 72 hours after cooling). This response could be attributed to the antioxidant capacities of both GSH and taurine, which protect the sperm membrane from oxidative stress caused by cooling and the excessive creation of ROS (Bansal, and Bilaspuri, 2010). Spermatozoa have antioxidant defenses against ROS formation, but the chilling process and dilution of the semen extender reduce this antioxidant power. Thus, exogenous antioxidant supplements like GSH and taurine are required for playing a defensive role against lipid peroxidation and maintaining membrane function through the preservation of cholesterol efflux that is necessary for the capacitation process which is related to the stability of total protein content (Petruska, 2014). The results obtained in this study agreed well with the literature on GSH and taurine supplementation in rabbits (Johinke, 2014; Ahmad et al 2021 and Maya-Soriano et al.,2015), stallions (Zhandi, and Ghadimi, 2014), and goat buck (Sarangi et al., 2017). Our study demonstrated that the total cholesterol level was correlated with GSH and taurine supplementation, with a statistical significance that goes in line with the results of Liu and his associates published in 2017. Various studies have focused on the associations between lipid profiles and semen quality. However, studies in this field have reported inconsistent conclusions and results. For example, Ergun et al., (2007) declared that high serum total triglyceride levels were statistically correlated with low sperm motility in a study of 18 infertile men. However, a study conducted by Hagiuda et al, (2014) concluded that the serum triglyceride level is positively associated with sperm morphological traits and has no significant relationship with sperm concentration or motility in contrast for seminal plasma, decreasing cholesterol level related to stability and fine structure of membrane. The variations in results between different studies might be due to variations in the reduced levels of normal sperm antioxidant power (Del Prete et al., 2018), *pH*, and osmolality (Oliveira, et al., 2014). In addition to the supplementing dose, species differences exist in the ability to face the stress associated with cooling and cryopreservation, and also in thawing-freezing issues (Rodriguez-Martinez, and Barth, 2007).

In this study, improvements observed in sperm quality may be attributed to prevent excessive generation of free radicals, produced by spermatozoa themselves, utilizing the antioxidant property of taurine which is in agreement with the results of Perumal (2013) who concluded that the possible protective effects of taurine supplementation represented in enhancing the antioxidant enzyme content and prevent efflux of cholesterol and phospholipids from cell membrane and malondialdehyde (MDA) production. Thus, it may protect the spermatozoa during preservation and enhance the fertility of this species. Besides, the current findings agreed with the results of Witte and Sch, (2007) who reported that cholesterol plays a special role in the sperm membrane because of its release from the sperm membrane that initiates the key step in the process of capacitation and acrosome reaction which is crucial for fertilization. Taurine prevents efflux of cholesterol from the sperm membrane and MDA production in diluents which indicates that it prevents premature capacitation and acrosomal reaction. So, it acts as an antioxidant. In the present study, our results indicated that the

efflux of cholesterol was decreased in the treated group as compared to the control untreated group which corroborate the result of other researchers (Chhillar, 2011, Sari¨ozkan, 2009, Saleh and HCLD, 2002). This finding is possibly related to lowering the extracellular level of triglyceride, which is a good indicator of sperm quality, measuring sperm membrane stability (Dogan, 2009). Moreover, GSH and taurine were correlated with maintaining triglyceride levels (all times) relatively Increasing in a control group, triglyceride levels with increasing cooling time in non-supplemented extenders might be linked with a lowering antioxidative scavengers compared to the supplementing extenders. Slightly decreasing in total protein level with continuous Cooling processes in contrast control group's total protein decreased continuously with the cooling process which impaired the sperm's functions by either overproduction of ROS or reducing the antioxidant defense system related to the influx of protein to the cell membrane that clings to cholesterol stimulating it to efflux to seminal plasma. This finding demonstrates that semen extenders that contain exogenous GSH and taurine protect sperm from excessive ROS formation during cooling by maintaining a balance between oxidation and antioxidant levels. (Bansal, and Bilaspuri, 2011). This result agrees with other studies that stated an improvement in membrane activities following GSH supplementation to semen extender as it is a cofactor of the enzymatic antioxidant systems during cooling (Ahmad et al., 2021). So, the semen samples treated with taurine and glutathione will have a higher chilling resistance than an untreated control group. In the present study, it was observed that sperm membrane stability through decreasing semen cholesterol, triglyceride efflux, and total protein influx that was noticed at T5, T10mM of taurine, and 2mM of glutathione was significantly lower than those of the other and control group. It was concluded that the possible protective effects of taurine and glutathione supplementation reside in enhancing the antioxidant enzyme content and preventing the efflux of cholesterol and phospholipids from the cell membrane, and influx of proteins to the cell membrane which adhesived to cholesterol. The improving effects of both GSH and taurine may also be due to the prevention of lipid peroxidation, as confirmed by lowering the MDA concentrations in GSH and taurine-treated semen compared to non-treated semen (Bayomy et al. 2023).

Conclusions

Exogenous supplementation of the extender with GSH and taurine improved the quality of rabbit semen that cooled at 5 °C by decreasing cholesterol, and triglyceride concentration in semen and increasing total protein level, as confirmed by maintaining triglyceride level in extracellular fluid. Moreover, both GSH and taurine reduced cooling-associated oxidative stress by lowering triglyceride concentration along with upregulating the permeability and fluidity of the sperm membrane. These improving effects increased with increasing supplementation dose (2 mM for GSH and 5 mM, 10 mM taurine) and the cooling time to 72 h.

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Institutional Review Board Statement: The animal study was reviewed and approved by the Institutional Ethics Committee of Kafrelsheikh University, Egypt (Approval number: KFS-2021/05).

Informed Consent Statement: Not applicable.

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