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Antioxidant Enzymes Activities, Semen Characteristics and Fertility of Local Cocks in Relationship with Glutathione Levels and Storage Period

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THIS STUDY was carried out to evaluate the effect of glutathione (GSH) supplementation at levels of 0.0 (control), 0.2, 0.4 and 0.6 mM to diluted semen stored for 0, 24, 48 and 72 hr at 5°C, on antioxidant enzymes activities in seminal plasma, semen characteristics and fertility rate (FR) of cocks Inshas strain. Supplementation of 0.6 mM GSH to the diluted cocks semen significantly increased ($P<0.05$) the concentration of alkaline phosphatase (ALP) (40.2 u/ml) compared to other treatments (33.0, 27.8 and 28.3 u/ml). Superoxide dismutase (SOD) concentration in seminal plasma was not significantly affected ($P<0.05$) by different levels of GSH, storage period or interaction between levels of GSH and storage period. Glutathione peroxidase (GSH-Px) concentration in seminal plasma was not affected by different levels of GSH ($P<0.05$). Total antioxidant capacity (TACO) in seminal plasma was significantly affected ($P<0.05$) by different levels of GSH, storage period or interaction between them. There was a positive correlation ($r=0.089$ and $r = 0.168$) between SOD concentration and (GSH-Px and TAOC) concentration, but negative correlation ($r=-0.119$) with ALP concentration. Positive insignificant correlation between (ALP, SOD and TACO) concentration in seminal plasma with the progressive motility, live sperm, curled tail and fertility, while positive significant correlation between GSH-Px concentration with progressive motility, curled tail and fertility. It could be concluded that the addition of GSH to semen extender of cockerels especially at levels 0.2 mM improved the antioxidant enzymes activities, which play a vital role in lowering the deleterious effect of reactive oxygen spices (ROS) on semen quality.

Keyword: Semen, Glutathione, *In vitro* storage, Antioxidant enzymes activity, Chicken

Introduction

The increasing use of artificial insemination (AI) in poultry industry emphasizes the need for good quality sperm distribution. A proper evaluation of semen prior to AI or storage is very important to avoid losses in fertility. Because semen evaluation is extremely important for semen storage and AI programs, a rapid, economical, objective and fertility predictive method of semen evaluation would be beneficial for the poultry industry protocols. Determination of antioxidant enzymes concentration in semen has been practiced for a long time (Peters, 2008).

Lipids are a basic component of semen, contributing to the membrane structure of spermatozoa, the metabolism of the sperm cells, and their ability to capacitate and fertilize the female gamete. In birds, the lipid composition of spermatozoa has an influence on fertility (Ansah and Buckland, 1982). El-Saadany (2002) reported that increasing storage time of diluted semen from local chicken breed for 0, 24, 48 and 72 hr decreased significantly ($P<0.01$) the fertility rate (FR) by 5.12, 9.55 and 12.53%, respectively. Shamiah et al. (2017) reported that GSH supplementation in semen extender significantly ($P<0.05$) increased FR. Semen supplemented with 0.2 mM GSH (74.5%) showed higher FR than those supplemented with 0.6 mM GSH or free semen (59.9 and 59.2%, respectively).

Sperm cells have a high content of unsaturated

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fatty acids in their membranes and they lack a significant cytoplasmic component containing antioxidants (Storey, 1997). Exposure of sperm to reactive oxygen species (ROS) resulted in lipid peroxidation (LPO), membrane breakdown, decreasing motility, abnormal morphology and a lowered capacity for oocytes penetration (Potts, et al., 1999). Deleterious effects of lipid peroxidation on poultry sperm include morphological defects, reduced motility, and poor fertilizing ability (Long and Kramer, 2003).

Several antioxidants such as glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) act as a defense mechanism against the LPO and play a major role in maintaining sperm motility and viability (Bucak et al., 2008). One of the substances that is widely distributed in other types of cells is glutathione (GSH), where one of its functions is to protect cells against the destructive effect of reactive oxygen species (ROS, Meister, 1983). To counter the destructive effects of ROS, seminal plasma has antioxidant systems composed of SOD, CAT, GSH and GSH-Px (Aitken & Baker, 2004 and Gadea et al., 2004). However, sperm membrane has a large content of unsaturated fatty acids and lacks a significant cytoplasmic component containing antioxidants, and is particularly susceptible to LPO in the presence of ROS, leading to impaired cell function and decreased sperm motility (Bucak and Tekin, 2007).

Glutahione is a tripeptide L-γ-glutamyl-L-cysteinylglycine and ubiquitously distributed in organisms and plays a very important role in intracellular defence system against oxidative stress (Gadea et al., 2004). Glutathione could protect sperm due to its strong antioxidant activities by inhibiting the lipid peroxidation of red deer sperm during freezing-thawing processes. Glutahione was reported to be used as a cryoprotectant for the sperm of mouse, canine, boar, ovine, bull and red deer (Sławeta & Laskowska, 1987, Takeo & Nakagata 2011, Perez et al., 2012, Anel-López et al., 2015 and Lucio et al., 2016). Recently, Wang and Dong (2017) reported that glutathione addition to the extender improved sperm quality, including sperm motility, acrosomal integrity, plasma-membrane integrity and sperm morphology.

So, the aim of the present study was to evaluate of the effect of GSH supplementation at levels of 0, 0.2, 0.4 and 0.6 mM to diluted semen stored for 0, 24, 48 and 72 hr at 5°C, on antioxidant enzymes activities and its relationship with sperm characteristics and fertility rate of Inshas strain of chicken.

Material and Methods

This study was carried out at Sakha Experimental Research Station, affiliated to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

Experimental chickens

Ten mature cockerels of local chicken (Inshas strain), 9-10 months of age and 2.30 -2.53 kg live body weight were used in this experiment. All cocks were kept in an individual cages (60x50x75 cm), provided with laying mash diet containing 15.5% crude protein and 2700 kcl ME per kg diet. Water was available all daytime.

Semen collection

Semen was collected in the morning from each cockerel once weekly by an abdominal massage method according to Lake and Stewart (1978) for five consecutive weeks as a semen collection period. Care was taken to avoid any contamination of semen with cloacae products and yellow and abnormal ejaculates were systematically discarded.

Only ejaculates with mass motility ≥70% on the day of collection were pooled on each collection day. Semen was evaluated for sperm cell concentration using a Neubauer hemacytometer, then semen was extended by the modified Beltsville Poultry Semen Extender (mBPSE) at a rate of 1 semen: 2 extender and divided into four parts, the 1st part was extended with control extender without any supplementation (T1). However, the 2nd, 3rd and 4th parts were supplemented with 0.2 (T2), 0.4 (T3) and 0.6 (T4) mM GSH ($C_{10}H_{17}N_3O_6S$), respectively, (S.d fine-Chem. Limited, India).

Semen extender supplemented with different GSH levels was stored in refrigerator at 5°C and evaluated after 0, 24, 48 and 72 hr. Semen evaluation included PSM, SL, SA, acrosome status, and Hypo Osmotic Swelling Test (HOST) of spermatozoa.

Semen evaluation

The semen quality evaluation was performed for all treatment samples at different *in vitro* storage periods. The progressive motility percentage was assessed according to Tabatabaei et al. (2009). Sperm livability percentage was determined using eosin/nigrosin stain according to Lukaszewicz et al. (2008). Sperm abnormalities acrosome integrity was determined according to Al-Daraji (2001). Sperm membrane integrity was assessed by Hypo-osmotic swelling test (HOST) as described by Lagares et al. (2000). From each treatment at different storage time, 0.3 milliliters were taken and centrifuged at 3000 g for 10 min at 4°C and the supernatant was frozen and stored at -20°C at least 24 hr until analysis.

Antioxidant profiles assays

Semen TAOC, SOD, GSH-Px and ALP activities were measured by commercially available diagnostic kits, (MDSS GmbH Schiffgraben 41, 30175 Hannover, Germany).

Fertility trial

Artificial insemination was performed using 1.0 ml syringes for the deposition of the semen as described by Sadanand et al. (2004) to assess fertility rate. Hens from the same local bred were divided into 16 groups (7 hens for each treatment), including semen supplemented with 4 GSH level at 4 storage periods).A Total of 256 eggs, 16 from each group was collected after 1 day post-insemination for a week and incubated to evaluate the fertility rate (FR). Eggs were candled to identify the fertile eggs according to the method of Islam et al. (2002) as the following: FR = Fertilized eggs/ incubated eggs x100.

Statistical analyses

Data obtained were statistically analyzed as a factorial design (4 GSH levels x 4 storage periods) by analysis of variance ANOVA (SAS, 2001). The significant differences were set at 0.05 probability level and tested using the Duncan's procedure (Duncan, 1955).

Results and Discussion

Semen diluted with 0.2 mM GSH showed higher percentages of progressive motility, live sperm and curled tail, and lower percentages of abnormal sperm and acrosomal damage as compared with other groups (P<0.05). Semen without GSH (control) showed the poorest quality (Fig. 1). These results clearly indicate that addition of GSH to semen extender at a level of 0.2 mM had impact on all sperm characteristics studied, regardless storage period. Also, addition of GSH to semen extender at a level of 0.2 mM improved fertility rate.

Ansari et al. (2014) observed that the addition of 0.5mM glutathione in semen extender improved the motility, plasma membrane integrity, viability and acrosomal integrity of Sahiwal bull spermatozoa. These results are in agreement with Wang and Dong (2017) who reported that glutathione addition to the extender improved sperm quality, including sperm motility, acrosomal integrity, plasma-membrane integrity and sperm morphology. Similarly, Gadea et al. (2005) reported beneficial effect of GSH in cryopreserved boar spermatozoa in terms of acrosomal intactness and percentage of non-capacitated spermatozoa, corroborated the findings of this study. Post translational modification in protein−thiols may mediate reduction in intracellular GSH and thus this is replenished by the supplementation of GSH into the extender (Gadea et al., 2004). In contrast, several studies also reported non-significant effects of GSH supplementation for protecting the sperms from cryocapacitation like changes (Foote et al., 2002, Sariozkan et al., 2009 and Tuncer et al., 2010).

In turkey, Long and Conn (2012) found that addition of phosphatidylcholine as antioxidant to the semen reduced the harmful effects of lipid peroxidation during semen storage at 4◦C. Also, administration of vitamins C, E, catalase (Amini et al., 2015a & b) and oleic acid (Eslami et al., 2016) to the rooster semen decreased the levels of malondialdehyde in seminal plasma and post-thawed of sperm.

Results presented in Fig. 1 show that overall mean of fertility rate (FR) of eggs after performing the artificial insemination of hens was affected by GSH level (P<0.05). GSH supplementation significantly (P<0.05) increased FR. Semen supplemented with 0.2 mM GSH (74.5%) showed higher FR than those supplemented with 6 mM GSH or free semen (59.9 and 59.2%, respectively). However, FR of semen extended with 0.4 mM GSH did not differ significantly from other GSH levels or from the control semen. It is known that semen quality and fertility reduced by advancing storage period (Shamiah et al., 2017). Results of

the present study indicated that addition of GSH to semen extender improved fertility rate. Moreover, FR was higher for semen supplemented with 0.2 mM GSH as compared with other semen treatments, being the highest for fresh semen 0.2 and 0.4 mM GSH (74.45 and 68.36%) (Fig. 1).

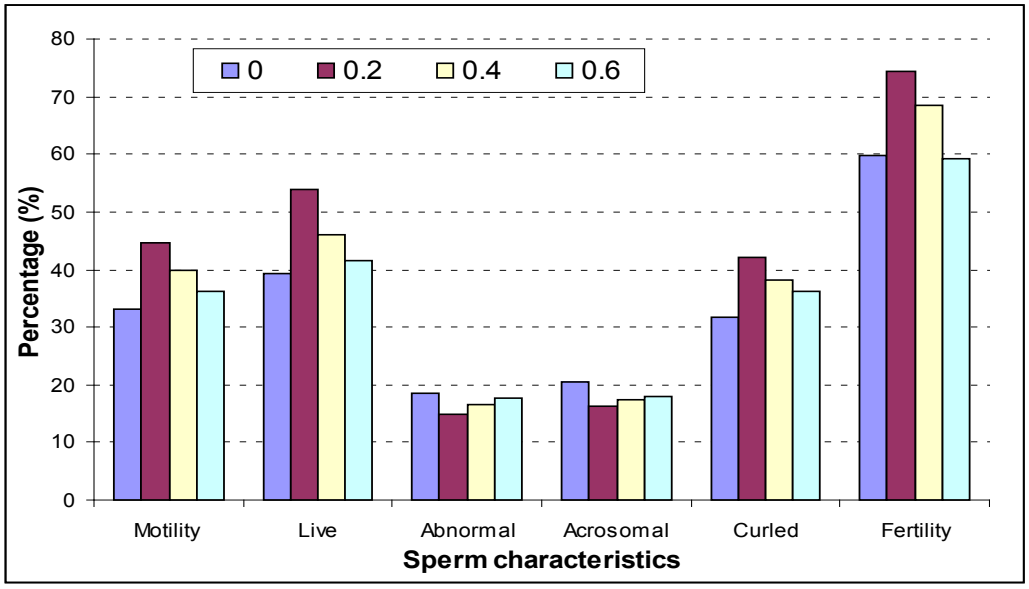

Fig. 1. Sperm characteristics of cockerel and Fertility rate (%) as affected by glutathione supplementation to semen extender stored at 5°C

The alkaline phosphatase (ALP) activity in seminal plasma of cockerel's was not significantly affected ($P<0.05$) by storage time, while the general trend decreased by increasing storage period (Table 1). Analysis of variance indicated that the overall mean of ALP enzyme activity was highly significantly ($P<0.001$) increased with increasing level of GSH, the ALP activity was significantly ($P<0.001$) higher (40.2 U/ml) with level 0.6 mM of GSH than other treatments (33.0, 27.8 and 28.3 U/ml). In the present study,the ALP activity insignificantly decreased by increasing storage period. In contrast, El-Saadany (2002) found that the ALP activity in seminal plasma in two local strains of coclerel's was increased significantly ($P<0.05$) by increasing storage period. While El-Saadany (2002) found that the ALP activity increased significantly ($P<0.05$) by increasing level of the antioxidant. Reduced GSH is synthesized in a controlled manner intra-cellularly in all mammalian cells and is involved in detoxification, antioxidant defense, and modulation of cell proliferation (Lu, 2009). It directly reacts with free radicals and catalyses the reduction of toxic H2O2 into water and hydro - peroxides (Bilodeau et al., 2001). Both removal as well as dilution of seminal plasma during semen freezing is reported to cause reduction in GSH content. This results in reduced GSH availability to spermatozoa as they fail to synthesize GSH by their own (Gadea et al., 2011). Further, in a previous study it is also shown that supplementation of GSH in the extenders prior to freezing minimized the oxidative damage caused by free radical generation (Gadea et al., 2005).

Effect of interaction between different levels of GSH and storage time on ALP concentration in diluted avian semen was not significant (Table 1). Mohan et al. (2013) found that no significant difference was noticed in ALP activity between fresh and preserved seminal plasma of guinea fowl. Also, Mahapatra et al. (1994) noted that ALP activity remained constant in fresh vs preserved semen in broiler breeders chicken.

As shown in Table 1, GSH-Px activity in seminal plasma was not significantly ($P<0.05$) affected by different levels of GSH, while the nearest value of GSH-Px concentration with levels (0.2 and 0.4 mM, 150.5 and 150.2 µ/g protein) was higher than (0.0 and 0.6 mM GSH, 144.2 and 144.9 mU/m). The overall mean of GSH-Px concentration in seminal plasma was significantly

(P<0.05) higher in time (0 hr) than in time (72hr), but did not differ from that in time (24 and 48hr). Also, no significant differces were observed among storage period (24, 48 and 72hr). Results of the present study are in the line with El-Saadany (2002) who found that GSH-Px activity increased significantly (P<0.05) by advantages storage period. Previous studies indicated that, seminal plasma possesses free radical-trapping activity and expresses SOD and GSH-Px activities (Surai et al., 1998). The free radical trapping activity of the seminal plasma was twice that of blood plasma and did not change after plasma boiling or storage during 24 hf at 20°C (Surai et al., 1998a). On the other hand, Thananurak et al. (2015) reported that membrane of avian spermatozoa is rich in polyunsaturated fatty acid (PUFAs) and can easily undergo LPO in the presence of ROS.

TABLE 1. Enzymatic activity in seminal plasma of cockerel as affected by glutathione supplementation to semen extender stored at 5°C

Item	Alkaline phosphatase	Glutathione peroxidase	Superoxide disumtase	Total antioxidant capacity
GSH (mM)				
0	28.3^{b}	144.2	159.3	0.97^{c}
0.2	27.8^{b}	150.5	162.2	1.32^{a}
0.4	33.0^{b}	150.2	161.4	1.15^{b}
0.6	40.2^{a}	144.9	158.4	1.08^{bc}
Storage period (h)				
0	36.4	163.0^{a}	165.3	1.04^{bc}
24	32.3	152.1^{ab}	167.5	1.37^{a}
48	30.8	145.8^{ab}	161.7	1.18^{b}
72	29.9	128.9^{b}	147.8	0.92^{c}
GSH × Storage period				
0×0	29.36	156.6	164.2	0.81
0×24	29.03	147.6	165.7	1.07
0×48	27.42	145.4	162.4	1.28
0×72	27.48	127.1	144.7	0.71
0.2×0	29.03	168.4	166.9	1.02
0.2×24	28.19	155.7	170.1	1.86
0.2×48	27.07	148.3	163.4	1.39
0.2×72	26.99	129.7	148.5	1.02
0.4×0	39.49	169.1	165.7	1.19
0.4×24	32.36	156.1	168.7	1.34
0.4×48	31.18	146.2	162.9	1.04
0.4×72	29.04	129.4	148.3	1.01
0.6×0	47.66	158.0	164.2	1.15
0.6×24	39.69	149.1	165.6	1.20
0.6×48	37.40	143.1	158.2	1.01
0.6×72	36.05	129.3	145.5	0.95
SEM	±4.31	±19.87	±16.56	±0.1
Significance				
GSH	***	NS	NS	***
Storage period	NS	NS	NS	***
GSH ×Storage period	NS	NS	NS	**

a and b: Means denoted within the same column with different superscripts for each effect are significantly different.
NS= non significantly, **= significantly at P<0.01 and ***= significantly at P<0.001

The overall mean of SOD concentration in seminal plasma was not affected significantly (P<0.05) by the different levels of GSH, storage period or interaction between them (Table 1). In general, SOD concentration in seminal plasma showed trend decreased insignificantly by advancing of storage period, while at levels of GSH, slightly increased up to 0.2 mM and slightly decreased up to 0.6 mM of GSH. The seminal plasma is well endowed with an array of antioxidants that act as free radical scavengers to protect spermatozoa against oxidative stress (Zini et al., 2000, Agarwal et al., 2004 and Sanocka & Kurpisza, 2004). Seminal plasma contains a number of enzymatic antioxidants such as SOD and CAT (Peeker et al., 1997, Mruk et al., 2002 and Calamera et al., 2003). Moreover, Perumal et al. (2013) found that the concentration of SOD and CAT was higher in liquid storage (5°C) within semen treated with antioxidant (taurine) and say the SOD activity of sperm samples is a good predictor of their survival time. But normally, seminal plasma is a potent source of this antioxidant, SOD (Kobayashi et al., 1991). Superoxide dismutase is an antioxidant enzyme found in avian and mammalian semen (Froman and Thurston, 1998, Kalaiselvi and Panneerselvam, 1998, Surai et al., 1998a). Its purpose is to scavenge for biological oxidants like ROS and protect cells from peroxidative damage (Froman and Thurston, 1998). Superoxide dismutase scavenges both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane. SOD spontaneously dismutase (O_2^-) anion to form O_2 and H_2O_2 (De Lamirande and Gagnon 1995). In addition, it contains a variety of nonenzymatic antioxidants such as vitamin C, vitamin E, pyruvate, glutathione, and carnitine (Meucci et al., 2003).

In Table 1, the overall mean of TACO concentration in seminal plasma was significantly affected (P<0.001) by deferent levels of GSH, storage period or interaction between them. The overall mean of TACO concentration in seminal plasma was significantly (P<0.001) highest in time (24hr) than in other times. Also, TACO concentration was significantly (P<0.001) higher in time (48hr) than in time (72 h). But did not differ among storage period (0.0, 48 and 72 hr). The overall mean of TACO concentration in seminal plasma was significantly (P<0.001) highest with level (0.2mM) of GSH than in other treatments. Also, TACO concentration was significantly (P<0.05) higher with level (0.4mM) than with level (0.0 mM), but did not differ among levels of GSH (0.4, 0.6 and 0.0 mM) (Table 1). The interaction between levels of GSH and storage period was affected significantly (P<0.01), the highest concentration of TACO (1.86 mM/ml) with level of GSH (0.2 mM) at time 24 h and the lowest concentration (0.71 mM/ml) with level of GSH (0.0 mM) at time 72 h (Table 1).

Free radicals or ROS are deleterious to cell membranes. Exposure of cell membranes to ROS induces lipid peroxidation causing membrane breakdown and loss of function (Halliwell & Gutteridge, 1984 and Thananurak et al., 2015). Lipid peroxidation results when intracellular production of ROS overcomes the antioxidant defense mechanisms utilized by cells including sperm (Alvarez et al., 1987, Alvarez and Storey, 1989), and an immediate accumulation of lipid peroxides occurs in the plasma membrane. Unlike somatic cells, which depend on cytoplasmic enzymes such as SOD and GSH-Px to defend against peroxidation, spermatozoa release most of their cytoplasm immediately prior to sperm motion, and as a result, lose their enzymatic protection (Wang et al., 1997). Avian sperm cell membranes have a much greater concentration of polyunsaturated fatty acids than mammalian sperm cells (Cerolini et al., 1997) and are therefore more susceptible to lipid peroxidation during in vitro handling and storage of sperm, which is the primary cause of fertility dysfunction (Cecil and Bakst, 1993). Thus, determining the level of sperm lipid peroxidation can be utilized as a biochemical index of semen quality (Jones et al., 1978 and Alvarez et al., 1987).

Table (2) shows slightly positive correlation (r=0.089 and r=0.168) between SOD concentration and (GSHPx and TAOC) concentration, but negative correlation (r = -0.119) with ALP concentration in seminal plasma of corresponding samples. Positive correlation significantly were found between TAOC concentration and (GSHPx and ALP), Also between concentration of GSHPx and ALP (Table 2). The total antioxidant capacity of seminal plasma represents the sum of the potential anti-ROS enzymes, such as GSH-PX. (Mahfouz, et al., 2009) Furthermore, Contri et al., (2011) reported a positive correlation between sperm parameters and total antioxidant capacity in seminal plasma.

TABLE 2. Correlation between some of antioxidants enzymes concentration in cocks seminal plasma.

Item	Alkaline phosphatase (ALP)	Glutathione peroxidase (GSHpx)	Total antioxidant capacity (TACO)
Glutathione peroxidase (GSHpx)	0.362*	1	
Total antioxidant capacity (TACO)	0.498**	0.673**	1
Superoxide disumtase (SOD)	-0.119	0.089	0.168

The positive correlation between ALP, SOD and TACO concentration in seminal plasma with the progressive motility, live sperm, curled tail and fertility were not significant, while positive significant correlation between GSH-Px concentration with progressive motility, curled tail and fertility (Table 3). In rooster, supplementation of oleic to semen extender caused an improve in total antioxidant activity concentration of seminal plasma and spermatozoa stored at 4◦C for at 24 h and 48h compared with control group (Eslami et al., 2016).

TABLE 3. Correlation between some of antioxidant enzymes concentration in cocks seminal plasma and semen characteristics.

Parameter	Alkaline phosphatase (ALP)	Glutathione peroxidase (GSHpx)	Total antioxidant capacity (TACO)	Superoxide Dismutase (SOD)
Progressive motility	0.166	0.298*	0.117	0.106
Live sperm	0.128	0.260	0.127	0.268
Abnormal sperm	-0.116	-0.22	-0.041	-0.346*
Acrosomal damage	-0.333*	-0.524**	-0.394**	-0.283
Curled tail	0.226	0.314*	0.159	0.142
Fertility	0.178	0.357*	0.242	0.27

Slightly negative correlation between (ALP, GSHPx and TAOC) concentration in seminal plasma and abnormal sperm was insignificant, but moderate negative correlation between (ALP, GSHPx and TAOC) concentration and acrosomal damage was significant also, SOD concentration with abnormal sperm (Table 3). Mammalian sperm is equipped with a defense system that comprises of both enzymatic and non-enzymatic antioxidants which offer protection against the ROS. But this indigenous defensive mechanism of the sperm is reported to be insufficient to cope with the oxidative stress (Nichi et al. 2006). Moreover, antioxidant levels of semen decrease as a result of cryopreservation (Bilodeau et al. 2000). However, addition of antioxidants in the extender has been reported to improve the quality of preserved mammalian semen (Bilodeau et al. 2001).

Glutathione is a tripeptide naturally occurring in semen and providing intracellular defense to the sperm against the oxidative stress caused by an over-production of ROS during the freezing and thawing process. Freeze-thawing of the semen causes a significant reduction in the glutathione content of the boar (Gadea et al. 2004) and bull semen (Bilodeau et al. 2000). Moreover, supplementation of glutathione in the extender has been shown to provide a protective role in maintaining the quality of bovine (Gadea et al. 2007) and buck semen (Sinha et al. 1996).

Shamiah et al., (2017) reported that the semen supplemented with GSH was characterized by moderate resistance morphological defects, increases motility and livability of spermatozoa in preserved semen as well as fertility rates. Supplementation of GSH at a level of 0.2 mM to extender of cockerel semen stored at 5°C for up to 72 h is an appropriate level to improve the fertilizing ability of chicken spermatozoa. Also, in buffalo, El-Kon and Darwish (2011) indicating that addition of 0.50-1.00 mM of GSH to semen diluent improved quality of liquid semen stored up to 120 hours in terms of reducing DNA damage and improving the fertility.

Hydrogen peroxide is the primary toxic ROS for human spermatozoa that its high concentration induces lipid peroxidation and results in cell death. Therefore, the balance of the SOD and CAT activities in semen is important for maintaining sperm motility (Hsieh et al., 2002), and complement, that there is a positive but no significantly correlation between SOD activity and sperm motility. They interpreted that higher SOD activity may scavenge the generation of ROS, which may lower the cytotoxicity to spermatozoa.

Conclusions

Results in the present study clearly indicated that the addition of GSH to semen extender of cockerels especially at levels 0.2 mM improved the antioxidant enzymes activity and these enzymes have a vital role in lowering the deleterious effect of reactive oxygen spices (ROS) on semen quality.

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تأثير مستويات الجلوتاثيون ومدة التخزين على النشاط الانزيمى لمضادات الاكسده وعلاقتها بخصائص السائل المنوي والخصوبة للديكة المحلية

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تهدف هذه الدراسة الى تقييم تأثير اضافة الجلوتاثيون بمستويات صفر ,٠,٢ ,٠,٤ و ٠,٦ ملل مول لمخفف السائل المنوى المخزن لمدة صفر , ٢٤ , ٤٨ و ٧٢ ساعه على درجة ٥°م على نشاط الانزيمات المضاده للاكسده فى بلازما السائل المنوي وتحديد مدى ارتباطها مع خصائص الحيوانات المنوية ومعدل الخصوبة للديوك من سلالة انشاص. وقد اوضحت النتائج عدم وجود تأثير معنوى لانزيم الالكالين فوسفاتيز(ALP) بزيادة فترة التخزين على الرغم من أنه عند إضافة ٠,٦ ملل مول من الجلوتاثيون أدى إلى زيادة معنوية فى تركيز انزيم الالكالين فوسفاتيز بمقدار(٤٠,٢ u/ml) مقارنة بالمعاملات الأخرى (٣٣,٠ u/ml ، ٢٧,٨ ، ٢٨,٣). كما لوحظ عدم تأثر نشاط إنزيم سوبراكسيد ديسيمتيز (SOD) في بلازما السائل المنوي عند اضافة اى مستوى من الجلوتاثيون او عند اى فترة تخزين وكذا عند التفاعل بينهما. لم يتاثر معنويا تركيز الجلوتاثيون بيروكسيديز في بلازما السائل المنوي باختلاف مستويات الجلوتاثيون المضافه ، في حين اقتربت قيم GSH-Px بين المستويين ٠,٢ و ٠,٤ ملل مول جلوتاثيون حيث كانت ١٥٠,٥ و١٥٠,٢ ميكرون /جرام بروتين أعلى من المستويين الكنترول (بدون جلوتاثيون) و ٠,٦ ملل مول جلوتاثيون حيث كانت ١٤٤,٢ و ١٤٤,٩ ميكرون /جرام بروتين. كما اوضحت الدراسه وجود تأثير معنوي لاضافة المستويات المختلفة من الجلوتاثيون مع طول فترة التخزين والتفاعل بينهما على المتوسط العام لتركيز TACO في بلازما السائل المنوي.

وجد ارتباط موجب (r = ٠,٠٨٩، r = ٠,١٦٨) بين تركيز ALP، TAOC ، GSH-Px ، SOD، بينما وجد ارتباط سلبي (r = - ٠,١١٩) مع تركيز ALP في بلازما السائل المنوي. كما وجد ارتباط معنوي موجب بين تركيز الإنزيمات المضادة للأكسدة في بلازما السائل المنوي و الحركة الجماعية و الحيوية و نسبة الحى، كما كان الارتباط ضعيفا بين تركيز الإنزيمات المضادة للأكسدة في البلازما المنوية مع الخصوبة، بينما وجد ارتباط سلبى كبير بين تركيز GSH-Px والخصوبة

الخلاصة :

النتائج فى هذه الدراسه تشير بوضوح الى ان إضافة الجلوتاثيون (GSH) الى مخفف السائل المنوى خاصة عند مستوى ٠,٢ ملل مول تحسن من نشاط الانزيمات المضاده للاكسده وان هذه الانزيمات لها دور فى تقليص الاثر الضار للشوارد (ROS) الحره غلى جودة السائل المنوي لديوك الدجاج.