Journal of Applied Veterinary Sciences, 7 (2): 13 – 24 (April, 2022).
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

A Review on Various Antioxidants Utilized in Bovine Semen Extenders

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ABSTRACT

High-quality cryopreserved bovine semen is vital for successful artificial insemination (AI) program, which is the most inexpensive and the quickest mode of genetic improvement. Cryopreservation of sperm is an effective method in infertility management; however, it can also affect spermatozoa in post-thawed semen. Many researchers showed that supplementation of extenders with antioxidants provides a cryoprotective effect on sperm quality by minimizing the harmful impact of reactive oxygen species (ROS) and enhancing post-thaw spermatozoa. This review sheds light on the protective effects of various antioxidants considered to reduce the oxidative stress following freeze-thawing of bull semen in AI programs.

Keywords: Antioxidants, Artificial Insemination, Bovine Semen Extenders, Cryopreservation, ROS.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2022.115377.1120

Received :09 January, 2022.
Accepted :26 February, 2022.
Published in April, 2022.

J. Appl. Vet. Sci., 7(2): 13-24.

INTRODUCTION

In mammals, oxidative stress (OS) is the major limiting factor which affect the quality and fertility preserved semen **(Izquierdo *et al.*, 2020).** The Reactive oxygen species (ROS) increase the lipid peroxidation (LPO) levels of unsaturated fatty acids in the sperm membrane (**Kadirvel *et al.*, 2014**, **Al-Mutary, 2021).** Energy production during sperm metabolism and the presence of dead or damaged sperm cells in semen are major sources of ROS generation. In buffaloes, during the semen preservation, OS increased ROS production, resulting in increased LPO levels in the membrane of spermatozoa (**Kadirvel *et al.*, 2014**, **Silvestre *et al.*, 2021**). Undergoing OS conditions, dysfunction of sperm mitochondria and deleterious effects on motility, vitality, plasmalemma integrity, and sperm morphology had occurred (**Garg *et al.*, 2009**, **Izquierdo *et al.*, 2020).** Therefore, the addition of exogenous protectants antioxidants is needed to control the ROS-mediated damages. (**Yeung *et al.*, 2019, Al-Mutary, 2021, Silvestre *et al.*, 2021**).

Glutathione, catalase, superoxide dismutase, and vitamins (C and E) play important role, as natural antioxidants in semen of mammals, against ROS to protect the sperm cells from LPO and to maintain sperm integrity (**Andrabi, 2009; Akhter *et al.*, 2011, Shah and Andrabi, 2021).** During sperm cryopreservation, the level of antioxidants decreases by semen extension with the dilution and increasing the generation of ROS molecules. Therefore, addition of antioxidants in the semen extender was recommended to decrease the cryo-damage to sperm cells (**Andrabi, 2009, Yeung *et al.*, 2019, Al-Mutary, M.G. 2021, Silvestre *et al.*, 2021**).

Classification of Antioxidants:

1. Endogenous Antioxidant:
Super Oxide dismutase (SOD), Catalase (CAT), Glutathione.

2. Exogenous Antioxidants:

2.1. Amino acids, Organic acids, Fatty acids:
Cysteine, Glutamine, Carnitine, Methionine, Inositol, Taurine, Hypotaurine, Butylated Hydroxytoluene (BHT), Dithioerythritol, Alpha Lipoic acid, Cystamine, Bradykinin.

2.2. Proteins:
Bovine Serum Albumin (BSA), Hyaluronan, Fetuin, Lactoferrin.

2.3. Vitamins:

Vitamin E, Vitamin C, Vitamin A, Butylated Hydroxy Anisole (BHA).

2.4. Hormones:

Melatonin and Leptin

2.5. Herbal plants (Natural antioxidant):

Rosemary, Origanum vulgare, Curcumin, Lycopene, Green tea, Resveratrol, Strawberry, Diospyros kaki, Silymarin, Palm dates, pollen grains, thyme, moringa oleifera.

2.6. Trace elements:

Selenium, Manganese, Zinc.

2.7. Disaccharides:

Trehalose.

2.8. Methyl Xanthine:

Pentoxifylline (PTX), Theophylline (TPY), Theobromine (TBR).

2.9. Algea:

Spirulina maxima, Spirulina platensis extract

2.10. Chemical synthesis Antioxidant:

Propyl gallate (PG)

2.11. Flavonoids as Antioxidants:

Apigenin and Quercetin

2.12. Non–enzymatic antioxidant:

Co-enzyme Q10 (Co-Q10)

2.13. Non-classified Antioxidants:

1. Endogenous Antioxidants:

The semen is provided with a defense natural antioxidant system, consisting of enzymes like Superoxide Dismutase (SOD), Catalase (CAT), decreased Glutathione (GSH), Glutathione Peroxidase (GSH-Px). These safeguard the spermatozoa from ROS-mediated cryoinjuries (**Kadirvel *et al.*, 2014).**

1.1. Superoxide dismutase:

It is a key enzyme in the cellular defense mechanism against oxygen toxicity. It is detected in spermatozoa and the seminal plasma of mammals to be responsible for catalyzing the dismutation of superoxide anions to hydrogen peroxide and oxygen (**Perumal, 2014**). The addition of antioxidants such as SOD to bovine semen has been proven to defend sperm against the hazardous consequences of ROS and improve sperm motility (**El-Sisy *et al.*, 2008; Asadpour *et al.*, 2012; Perumal, 2014).**

1.2.Catalase:

The antioxidant catalase enzyme catalyzes the reaction to convert H_2O_2 into water (**Pisoschi and Pop, 2015**). Several studies reported that catalase supplementation after thawing could defend bull spermatozoa against oxidative stress **(Fernandez-Santos et al., 2009; Peruma *et al.*, 2013).** The addition of antioxidant catalase to bovine semen has been proven to enhance sperm motility **(Asadpour *et al.*, 2011a; Peruma *et al.*, 2013).**

1.3.Glutathione:

Glutathione is a tripeptide, has an essential function as an antioxidant in eliminating ROS molecules. It has been found that glutathione can stimulate the mammalian spermatozoa for fertilization which is inhibited by the ROS molecules from the damaged spermatozoa in cryopreserved semen **(Bath *et al.*, 2010).** Glutathione can protect the quality of semen exposed to artificial oxidative stress induced by hydrogen peroxide in frozen-thawed semen (**Uysal *et al.*, 2007; Peruma *et al.*, 2011).** The addition of glutathione in the thawing medium might compensate for the glutathione decrease during cryopreservation and preserve the semen quality by reducing ROS levels in frozen semen **(Ansari *et al.*, 2010; Ansari *et al.*, 2011a).**

2. Exogenous antioxidants:

2.1. Amino, organic, and fatty acids:

2.1.1. Glutamine:

It has been reported to be used against damage caused by freeze-thawing in the bulls' semen **(Amirat-Briand *et al.*, 2009),** and it can improve post-thaw bovine sperm parameters **(Sarıozkan *et al.*, 2014).**

2.1.2. Carnitine:

It is a vitamin-like compound biosynthesized from two essential amino acids (lysine and methionine) in the liver, kidneys, and brain **(Bucak *et al.*, 2010).** L-carnitine is found at high concentrations in the mammalian epididymis and spermatozoa. **Wafa *et al.*, (2021)** stated that adding antioxidant carnitine improved subjective sperm motility.

2.1.3. inositol:

Among compounds of the epididymal fluid, inositol exists at a high level. It performs a necessary function in maintaining the viability of epithelial and sperm cells in the epididymis as an essential growth factor **(Bucak *et al.*, 2010).** It was stated that the motility of frozen-thawed bull sperm could be increased using inositol in the extender **(Reyes-Moreno *et al.*, 2000; Bucak *et al.*, 2010)**.

2.1.4. methionine:

It acts as a precursor amino acid of glutathione for defending cells from oxidative damage, and plays a quintessential role in detoxification. In addition, the thiol group of methionine has been proven to chelate lead and remove it from tissues **(Patra *et al.*, 2001).** Generally, methionine improves post-thaw bovine sperm parameters especially, sperm acrosome damage and which types of abnormalities **(Sarıozkan *et al.*, 2014).**

2.1.5. Cystine:

It is an amino acid having the capacity to shield the cell from free radicals by scavenging them directly **(Bilodeau *et al.*, 2001).** Cysteine has the potential to preserve sperm motility in the presence of exogenous hydrogen peroxide in frozen-thawed bull semen **(Bilodeau *et al.*, 2001).** Cysteine was used in extenders for the cryopreservation of bull spermatozoa **(Sarıozkan *et al.*, 2009; Ansari *et al.*, 2011 b,d and Sarıozkan *et al.*, 2014).**

2.1.6. Taurine (TA):

It is an intracellular amino acid that maintains the stability of biomembranes and scavenges ROS **(Huxtable, 1992)**. **In addition, several authors** proved that the supplementation of taurine to the freezing extender of buffalo semen enhanced post-thaw motility **(Sariözkan *et al.*, 2009; Reddy *et al.*, 2010; Chhillar *et al.*, 2012)** and semen quality of bulls **Chikhaliya *et al.* (2018)**.

2.1.7. Hypotaurine:

It is a precursor of taurine which exists in mammalian spermatozoa and is essential for sperm motility **(Guerin *et al.*, 1995)** because it has antioxidant properties (**Hu *et al.*, 2010; Reddy *et al.*, 2010),** which may exert valuable effects on the quality of the frozen-thaw buffalo spermatozoa (**Badr *et al.*, 2014).**

2.1.8. Alpha-lipoic acid:

It is well known that alpha-lipoic acid (α- LA) is a short-chain fatty acid that acts as a co-factor of the enzyme involved in mitochondrial respiration **(Lovell *et al.*, 2003).** Alpha-lipoic acid was reported to increase bull spermatozoa motility **(Ibrahim *et al.*, 2011; Osman *et al.*, 2012**) and improves the post-thawed motility of cryopreserved buffalo spermatozoa **(Gohar *et al.*, 2014).**

2.1.9. Butylated hydroxytoluene (BHT):

Supplementation of BHT to semen extender was found to be effective for enhancing bull semen quality after cryopreservation **(Ball *et al.*, 2001; Roca *et al.*, 2004; Shoae and Zamiri, 2008),** in particular viability of bull spermatozoa (**Ansari *et al.*, 2011; Muzafer *et al.*, 2012)**.

2.1.10. Dithioerythritol:

It is recognized as a protamine disulfide bond-reducing agent. It prevents the oxidation of sulphydryl groups **(Watanabe and Fukui, 2006),** and has beneficial consequences on post-thawed sperm quality **(Bucak *et al.*, 2010; Coyan *et al.*, 2010)**.

2.1.11. Cysteamine:

Cysteamine is a strong antioxidant that has a crucial function in the protection mechanism against ROS **(Merton *et al.*, 2013).** Its addition to the freezing extender could provide cryoprotection of thawed bull semen (**Sarıözkan *et al.*, 2015; Gungor *et al.*, 2016).**

2.1.12. Bradykinin:

Incorporation of Bradykinin in Tris-based extender might be beneficial in enhancing the motility of frozen-thawed bovine **(Somlev and Subev, 1998),** mammalian **(Siems *et al.*, 2003),** and buffalo semen **(Shukla and Misram, 2007).**

2.2. Proteins:

2.2.1. Bovine serum albumin:

Bovine serum albumin (BSA), a relatively soluble protein naturally occurs in mammalian semen that can guard the cell against the dangerous effects of free radicals in oxidative stress **(Fukuzawa *et al.*, 2005; Roche *et al.*, 2008).** The viability of bovine spermatozoa was confirmed in semen cryopreserved in an extender containing BSA **(Uysal and Bucak, 2007; Nang *et al.*, 2011; Akhter *et al.*, 2014).**

2.2.2. Hyaluronan:

Hyaluronan, a non-sulphated glycos-aminoglycan, is a fundamental element of the extracellular matrix. It mediates sperm features such as sperm motility **(Ghosh *et al.*, 2002). Uysal *et al.*, (2007)** efficaciously used hyaluronandoses in bull semen as a freezing extender. **Sariözkan *et al.* (2015)** stated that hyaluronan addition produced a significant improvement in the motility of post-thaw bull spermatozoa.

2.2.3. Fetuin:

Fetuin is a main glycoprotein component of fetal calf serum (FCS), improving sperm motility **(Jaiswal *et al.*, 2010; Sariözkan *et al.*, 2015)**.

2.2.4. Lactoferrin:

Lactoferrin is an iron-binding protein; working as a protecting agent to the spermatozoa increases the percentage of sperm motility **(Martins *et al.*, 2018). Hussein *et al.*, (2019)** observed that lactoferrin improves the post-thawing motility and viability of cryopreserved buffalo bull spermatozoa.

2.3. Vitamins:

2.3.1. Vitamin C (Ascorbic acid) :

Vitamin C is an important antioxidant **(Asadpour *et al.*, 2011).** Several studies reported significant improvement in post-thaw sperm motility containing vitamin C **(Andrabi *et al.*, 2008; Swain *et al.*, 2009; Asadpour *et al.*, 2011).**

2.3.2. Vitamin E (Alpha-tocopherol):

Vitamin E is believed to be the fundamental component of the antioxidant system of spermatozoa against ROS and LPO (**Towhidi ad Parks, 2012).**

Vitamin E accelerates the total motility and viability of bull spermatozoa after freeze-thawing **(Beheshti *et al.*, 2011; Nasiri *et al.*, 2012; Muzafer *et al.*, 2012; Tvrda *et al.*, 2013).** The α-tocopherol in Bioxcell extender could be efficient for cryopreservation of bull spermatozoa **(Motemani *et al.*, 2017).**

2.3.3. Butylated Hydroxyanisole (BHA):

BHA is a synthetic analog of vitamin E and acts by lowering oxygen radicals (ROS). In vitro studies have proven that BHA is a free radical scavenger that protects the cell membrane against lipid peroxidation (**Beconi et al., 1993**). However, (Pankaj et al., 2009) said that BHA had not shown its motility enhancer role.

2.4. Hormones:

2.4.1. Melatonin:

Melatonin has the functionality of removing and neutralizing free radicals. Several studies have reported that melatonin protected animal spermatozoa from adverse effects of peroxidative agents **(Sonmez *et al.*, 2007; Rao and Gangadharan 2008; Succu *et al.*, 2011).** Melatonin increased viability and motility of post-thawed bull **(Ashrafi *et al.*, 2013)** and buffalo semen **(Abdel-Khalek *et al.*, 2016)**.

2.4.2. Leptin:

Several authors reported that leptin could preserve sperm motility and viability in cooled buffalo semen **(Lange Consiglio *et al.*, 2009; Khaki *et al.* 2013).** Also, leptin supplementation improved the semen quality of the cryopreserved buffalo semen **(Abdel-Khalek *et al.*, 2016)**.

2.5. Herbal Antioxidants:

2.5.1. Strawberry:

Strawberry fruit is an important antioxidant **(Asghari and Hasanlooe, 2015).** Strawberry (SB) juice has a useful effect in the cryopreservation of buffalo semen (**El-Sheshtawy *et al.*, 2016**) because it can improve bull semen characteristics after freezing (**El-Sheshtawy and El-Nattat, 2018**).

2.5.2. Diospyros kaki:

Persimmon (Diospyros kaki) fruit is a strong antioxidant and improves semen quality of preserved semen (**Aljady *et al.*, 2010**). **El-Sheshtawy *et al.*, (2014 a, b)** found that kaki improved local bull breeds semen preservability and quality post-freezing (**El-Sheshtawy and El-Nattat, 2017**).

2.5.3. Silymarin:

Silymarin is an extract from the seeds and fruits of the milk thistle Silybum marianum which is a strong antioxidant **(Luangpirom *et al.*, 2013). El-Sheshtawy and El-Nattat (2017a)** found that silymarin improved sperm preservability in frozen bull semen.

2.5.4. Palm dates pollen grains:

Palm dates pollen grains extract a potent antioxidant **(Mansouri *et al.*, 2005). (El-Sheshtawy *et al.*, 2016 a)** stated that the Date palm pollen grains improved the preserving capability of chilled and frozen bull semen.

2.5.5. Curcumin (diferuoyl methane):

Curcumin (diferuoyl methane), is a natural antioxidant supplemented to extenders increased the freezing ability of Holstein bull spermatozoa during cryopreservation **(Bucak *et al.*, 2012).** Also, **Shah *et al.*, (2016)** found that curcumin improved the freezability of water buffalo bull spermatozoa.

2.5.6. Green Tea Extract (Camellia sinensis):

Green tea (Camila sensis) is a strong antioxidant (**Khan *et al.*, 2017).** Green tea extract enhanced the semen characteristics of cryopreserved bull spermatozoa **(Khan *et al.*, 2017)**.

2.5.7.Lycopene:

Lycopene (LYC) has a potent antioxidant. **(Bucak *et al.*, 2014; Tvrdá *et al.*, 2016)** suggested that lycopene supplementation improved the preserving capability of frozen bull semen.

2.5.8.Resveratrol:

Resveratrol, a nonflavonoid polyphenol found mainly in grapes, consider an important antioxidant **(Collodel *et al.*, 2011).** It was suggested that the supplementation of the semen extender with resveratrol offers protection on sperm motility and DNA integrity **(Bucak *et al.*, 2014; Longobardi *et al.*, 2017; Ahmed *et al.*, 2020)**.

2.5.9. Rosemary (Rosmarinus officinalis) ROM:

Rosemary (Rosmarinus officinalis) is a medicinal plant considered an antioxidant. The addition of ROM significantly improved the sperm survival rate and motility of bull spermatozoa **(Daghigh-Kia *et al.*, 2014)**.

2.5.10. Origanum Vulgare:

Origanum vulgare is an aromatic plant that has antioxidative activities. The addition of Origanum vulgare extract to the semen extender improved the quality of frozen-thawed bull semen. **(Daghigh-Kia *et al.*, 2016)**.

2.5.11. Moringa Oleifera:

Moringa Oleifera is a good source of antioxidants. Researchers reported that Moringa is an efficient antioxidant in extenders of buffalo semen that

enhances semen characteristics **(Dowidar *et al.*, 2018; El-Nagar *et al.*, 2019)**.

2.6. Trace Elements:

2.6.1. Manganese:

Manganese (Mn^{2+}) is a chain-breaking antioxidant in biological systems **(Lapointe *et al.*, 1996)** said that supplementation with Mn2+ improved sperm motility in bull sperm. **(Bansal and Bilaspuri 2008)** stated that Mn^{2+} used to be a beneficial antioxidant, reducing oxidative stress (LPO) and enhancing sperm motility and viability.

2.6.2. Selenium:

Selenium (Se) is an antioxidant, **(Sanchez-Gutierrez *et al.*, 2008). Dorostkar *et al.*, (2012)** indicated that Selenium supplementation improved sperm motility the viability of water buffaloes semen.

2.6.3. Zinc(Zn) :

Zinc is an important trace element necessary for reproduction, in addition to antioxidant potential **(Mirnamniha *et al.*, 2019).** Zinc is essential for preserving the viability and fertility of buffalo spermatozoa **(Ahmed and El-Tohamy, 1997).** Adding zinc to the extender improved sperm quality preservation of buffalo **(Dorostkar *et al.*, 2014).**

2.7. Disaccharides:

2.7.1.Trehalose:

Trehalose has an indirect antioxidant impact via growing the level of glutathione and reducing the level of lipid peroxide **(Aisen *et al.*, 2005)** leading to improved post-thaw sperm motility (Reddy et al., 2010). The extender contained trehalose improved spermatozoa cryopreservation in buffalo bull **(Hu *et al.*, 2010; Tuncer *et al.*, 2011; Badr *et al.*, 2010). (Chhillar *et al.*, 2012)** reported that trehalose decreased H_2O_2 in frozen-thawed bull semen.

2.8. Methylxanthines:

Pentoxifylline (PTX) - Theophylline (TPY) - Theobromine (TBR):

Methylxanthines are commonly used as additives in sperm suspensions to enhance sperm characteristics. Among methylxanthines caffeine, Pentoxifylline, Theobromine and Theophylline have been used. Methylxanthine supplementation results in better seminal characteristics in fresh and cryopreserved spermatozoa **(Maxwell *et al.*, 2002; Pankaj *et al.*, 2009).**

Pentoxifylline improves sperm motility, capacitation and acrosome reaction **(Perreault and Roger, 1992).** Several studies said that treatment of sperm with pentoxifylline enhanced fresh and post-thaw sperm fertilizing ability **(Rizk *et al.*, 2005; Yunes *et al.*, 2005; Esteves *et al.*, 2007). Pankaj *et al.*, (2009)** found that BHT, PTX and TPY being oxygen radical scavengers, supported the preservation of the viability of spermatozoa, in agreement with other studies, for extension of Murrah bull semen.

2.9.Algea:

2.9.1. Spirulina maxima:

Spirulina maxima is a blue-green microalga and have antioxidant potential **(El-Tantawy 2016).** The supplementation of Spirulina maxima extract (SME) to the extender improved the post-thaw spermatozoa quality in bulls **(Granaci 2007; Mizera *et al.*, 2019).**

2.9.2. Spirulina platensis:

Spirulina is considered a strong natural antioxidant. The positive effect of Spirulina was previously reported in bovine semen **(Mizera *et al.*, 2019).** The addition of Spirulina platensis to the freezing extender improved the semen quality of the buffalo bull **(Badr *et al.*, 2021).**

2.10. Chemical synthesis Antioxidant:

10.1.Propyl gallate (PG) :

Propyl gallate (PG) is not a natural compound and can only be obtained via chemical synthesis **(Nguyen *et al.*, 2021)**. It is also an antioxidant. **Shukla and Misra (2005)** found that the addition of n-propyl gallate resulted in high post-thaw motility and viability of bull spermatozoa.

2.11. Flavonoids as Antioxidants (Apigenin, Quercetin):

2.11.1.Apigenin(AP):

Apigenin, a flavonoid, is extensively distributed in a plant **(Tang *et al.*, 2017).** Recent researchers have proven that adding Apigenin has a protective effect on the freezing of bovine semen **(Wang *et al.*, 2021).**

2.11.2. Quercetin (QUE)::

Quercetin is a flavonoid that scavenges reactive nitrogen species and ROS **(Boots *et al.*, 2008).** The addition of QUE enhanced the post-thaw motility and in vivo fertility of buffalo bull spermatozoa **(Ahmed *et al.*, 2019).**

2.12.Non–enzymatic antioxidant:

Coenzyme Q10 (Co-Q10) is a non–enzymatic antioxidant **(Dowidar *et al.*, 2018)** stated that supplementing semen extenders with Coenzyme Q10 enhanced semen characteristics of post-thawed bull spermatozoa and fertility.

2.13. Non-Classified Antioxidants:

2.13.1. Thioglycol:

Thioglycol, a low molecular weight thiol compound, researchers stated that thioglycol in extender improved the motility and semen characteristics of buffalo bull spermatozoa **(Ansari *et al.*, 2014).**

2.13.2. Iodixanol (Id):

Iodixanol exhibits antioxidant properties; researchers found that supplementation of the semen extender with Id improved progressive motility and viability of frozen bull spermatozoa **(Chuawongboon *et al.*, 2017; Marqui *et al.*, 2018).**

2.13.3. Astragalus polysaccharide (APS):

Astragalus polysaccharide can be considered an antioxidant **(Fu *et al.*, 2018)**. The addition of Astragalus polysaccharide (APS) has a protective impact on the freezing ability of bovine semen **(Wang *et al.*, 2021).**

2.13.4. Crocin :

Crocin, is a water-soluble carotenoid pigment of saffron (Crocus sativus L.), with antioxidant properties **(Singla and Giliyaru 2011). *Longobardi et al.*, (2021)** have proven a high-quality effect of crocin on frozen-thawed buffalo sperm.

CONCLUSION

Artificial insemination is the most approach devised for the genetic enhancement of animals. Cryopreservation is a technique through which spermatozoa from genetically superior elite bulls can be preserved at -196^{o}C in the liquid nitrogen, which allows safe and easy transportation of semen to the remotest parts of the world. Post-thaw survival of the sperm population is approximately 50%, even with the best available preservation technique. Many researchers recommended plant-derived antioxidants or herbs (economical, lower cytotoxicity and commonly available) as excellent sources of the natural antioxidant in preserving cattle semen. Future research should spotlight freezing protocols improvement, especially molecular mechanisms leading to the death of bovine spermatozoa during cryopreservation and finding the molecular markers of fertility to identify bulls of high breeding values in dairy and meat production.

Declaration of Conflicting Interests

The authors revealed that there is no potential conflicts of interest.

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How to cite this article:
Abdel-khalek A. E., Y.A. Dowidar, H.A. El-Nagar, W.M. Wafa, I. T. El-Ratel, and A.M. Mousbah, 2022. A Review on Various Antioxidants Utilized in Bovine Semen Extenders. Journal of Applied Veterinary Sciences, 7 (2): 13 – 24.
DOI:https://dx.doi.org/10.21608/javs.2022.115377.1120