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Review on recent advancement in semen additives for improving cryopreservation of bull semen

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Abstract
Artificial insemination (AI) and semen cryopreservation are the most widely used assisted reproductive technologies (ART) in livestock farming. Bovine reproduction, particularly in developing countries, relies heavily on assisted reproduction using AI with cryopreserved semen. During cryopreservation, the sperm cells face plenty of cold-induced insults. Especially oxidative stress due to excessive Reactive Oxygen Species (ROS) production, the sperm plasma membranes suffer a lot of damage. It leads to the reduction in the quality of spermatozoa in terms of its motility and fertility. Additives are various supplements added in semen extender to improve the freeze thaw sperm recovery and fertility. Majority of the supplements have antioxidant property hence scavenging free radicals and preventing lipid peroxidation. Semen additives are classified based on their chemical composition and their mechanism of action. This review article will discuss about various semen additives used over the past decade for cryopreservation of bovine semen.

Keywords: Additives, bovine, bull, cryopreservation, semen

Introduction
In developing countries, assisted reproductive technologies are still in an infantile stage; artificial insemination and frozen semen technology play a significant role in the field of bovine reproduction. But to exploit the existing superior male germplasm using frozen semen technology there are various hurdles to be dealt with. One such important issue that arises during the cryopreservation process of bull semen is the reduction in quality and fertility of semen due to the various physiological, mechanical stresses, and structural changes the sperm cells have to encounter. The quality of a substantial fraction of sperm cells is compromised during the freeze-thaw cycle even after following the controlled semen extension and freezing protocols. Sperm cells are envisaged to face plenty of insults during preservation such as fluctuations in pH, osmotic changes, energy depletion, cold shock, and cryodamages. Cryopreservation leads to excessive production of reactive oxygen species (ROS) due to the various stresses it has to endure such as atmospheric oxygen exposure, thermal shock, removal of seminal plasma [16].

The plasma membrane of the sperm cell is the primary site of damage during cryopreservation. The ROS attacks the phospholipid and cholesterol of the plasma membrane causing lipid peroxidation and cholesterol efflux during cryopreservation leading to disturbances in the cholesterol and phospholipid ratio. Successive insults faced by sperm cells in the form of lipid peroxidation, ice crystallization, changes in pH, and osmotic pressure will lead to debilitated motility and membrane integrity, DNA damage, and cell death. All these factors will eventually lead to cell death and compromise the fertilization capacity of sperm [160].

So, the question arises as to what and how the semen additives will be useful in the scenario to overcome the damages to sperm cells during freezing and thawing? Semen additives are supplements added to semen extender to enhance the semen keeping quality. Additives generally possess antioxidative properties, thus scavenging and neutralizing free radicals and protecting the sperm cells from lipid peroxidative damages. Apart from free radical scavenging activity, some additives improve sperm motility, stability, and fertility. Several new additives have been researched and designed in recent years. These additives can protect male gametes from the harmful effects of cryopreservation [9].

Different classes of semen additives

Several components possessing a protective effect on sperm cells during cryopreservation are added regularly to the long list of existing semen additives. Thus for ease, additives are classified according to their chemical composition or their mechanism of action.

1. Antioxidant: Resveratrol, Vitamin-E, Vitamin C.
2. Antioxidant Preservatives: Butylated hydroxy anisole (BHA), Butylated hydroxy toluene (BHT), Tert-butyl hydroquinone.
3. Methylxanthine: Pentoxifylline (PTX), Theophylline, Caffeine.
4. Trace Elements: Magnesium, Zinc, Copper, Selenium.
5. Enzymes/ Co-factor: Glutathione peroxidase (GPx), Superoxide desmutase (SOD), Catalase.
6. Amino acids/Proteins: Methionine, Cysteine, Taurine, Glutamine, Hypotaurine, Bovine serum albumin.
7. Sugar/Polysaccharides: Raffinose, Hyaluronic acid, Trehalose.
8. Cell membrane stabilizers: DHA (Docosahexaenoic acid), CLC (Cholesterol loaded cyclodextrins).
9. Miscellaneous: Antifreeze proteins, Iodixanol, Oxyrase.

Below listed are the semen additives used for cryopreservation of bull semen (Table 1).

Table 1: List of semen additives used for cryopreservation of bull semen.

Sr. no.	Additive	Extender	Mechanism of Action	Sr. no.	Additive	Extender	Mechanism of Action
1	1.5 mmol/l LYC (Lycopene) [161]	Triladyl®	Natural carotenoid quench singlet oxygen	30	Elamipretide TFA (5-10 µM) [97]	BIOXcell®	Restores energy production, reduces ROS and increases ATP synthesis
2	AX (Astaxanthin) supplementation (@2 µM) [144]	TEYCAF	Neutralizes free radicals or other oxidants	31	Relaxin (25 ng/ml) [55]	Bullxcell®	Improves motility and viability through specific sperm-surface receptors
3	Resveratrol (1 mM) [31]	Tris-based extender	Antioxidant activity: acts both in the initiation and propagation of the oxidative process	32	Trehalose 100mM [155]	TFYG	Protective role against osmotic effect-protects sperm plasma membrane
4	Cysteamine 7.5 Mm [137]	Tris-based extender	Increases the synthesis of both glutathione and other potent antioxidant enzymes	33	100 mM sucrose [58]	TCYF extender	Non-permeable sugar render hypertonic media decreasing intracellular freezable water
5	Cysteamine 2 mM and ALA (Alpha-Lipoic Acid)1 mM [72]	Tris-based extender	High reactivity towards free radicals , increase tissue levels of reduced glutathione	34	Raffinose 25mM plus glutamine 3mM [158]	Optydil® extender	Sugar decrease intracellular ice crystal formation, glutamine protect the structure of biological membranes of sperm
6	30 µM of CoQ10 (Coenzyme Q10) [133]	Tris -egg yolk extender	Scavenges free radicals and inhibits oxidation of lipid	35	Hyaluronan 0.25 mg ml^{-1} [137]	Tris-based extender	Increase in phosphorylation and ATP levels-improved flagellar function and motility
7	5 mM sodium pyruvate (Pyr) [94]	Triladyl® extender	Acts as a H_2O_2 scavenger	36	Dithioerythritol at 0.5 mm [32]	Tris-based extender	Prevents the oxidation of sulphydryl groups
8	1 mM of GSH (reduced glutathione) [116]	TFYG	Neutralizing ROS by reduction of peroxide	37	3 ng/mL of DHA [84]	BioXcell®	Component of lipid bilayer of the sperm plasma membrane
9	2.5 mM, l-ascorbic acid+ 2 mM reduced glutathione [53]	Tris-based extender	Potent antioxidant activity	38	10 ng/ml DHA+0.02 mmol Vit E [117]	BioXcell®	Component of lipid bilayer of the sperm plasma membrane
10	100 IU/mL of SOD (Superoxide dismutase) [116]	TFYG	Catalyzes dismutation of superoxide radical	39	5 ng/ml ALA [85]	BioXcell®	Improving plasma membrane fluidity and integrity
11	Catalase 20 IU/mL [11]	Triladyl®	Removes or minimizes both intracellular andextracellular H_2O_2	40	1.5% Soy Lecithin in 2% Virgin Coconut Oil [153]	Tris-based extender	Saturated and unsaturated fatty acids , antioxidants: maintain the structure of sperm plasma membrane
12	Melatonin 2mM [155]	TFYG	Potent scavenging effect on NO and Reactive Oxygen Species (ROS)	41	2 mg of CLC/120 x10^6 sperm [114]	TCA	Cholesterol strengthens and protects membrane structures
13	EDTA (0.1%) [122]	TFYG	Prevents conversion of superoxide anion into hydrogen peroxide by	42	0.9 mM Mn+2 [52]	Tris-based extender	Cofactor of mitochondrial superoxide

			inhibiting divalent-ion-dependant SOD.				dismutase, an antioxidant enzyme which scavenges oxygen free radicals
14	1 mg/ml Vit E [25]	Tris-based extender	Scavenges oxygen radicals , intercepts lipid peroxyl radicals	43	Se-NPs 1.0 mg/ml (Selenium Nano particles) [89]	Tris-yolk fructose (TYF)	Antioxidant defense , enhances ATP-utilizing and ATP-regenerating pathways
15	2.50 mg/ ml vitamin B12 [77]	Tris-based extender	Decrease the amount of ROS from oxidative stress	44	1 µg/ml (AFPI)Antifreeze protein I [125]	Eqcell-sire®; IMV, L'Aigle, France	Inhibit ice recrystallization : protecting membranes against freezing injuries
16	2.5 mM, l-ascorbic acid + catalase 100 IU/ml [53]	Tris-based extender	Potent antioxidant activity	45	Iodixanol (2.5 %) [39]	Tris -egg yolk	Non-penetrating cryoprotectant (altering the ice crystal formation by removal of water from the solution at lower temperatures)
17	50 IU/mL SOD + 0.5 mM GSH [116]	TFYG	Counteraction of ROS	46	Oxyrase (0.125 IU/ml) [119]	Tris-egg yolk extender	Reduce ROS production by reducing O_2 levels
18	0.5mM butylated hydroxytoluene [10]	Tris-citric acid extender	Decrease in oxidative stress and ROS production	47	(Honey)10% [40]	Tris-citric acid-fructose egg yolk	Antioxidant Property
19	Tert-butyl hydroquinone (tBHQ) 5 µM [79]	Optydil® extender	Reduces reactive oxygen species	48	4 µg/mL *Spirulina maxima* extract [113]	BIOXcell® extender	Antioxidant potential
20	Sodium nitroprusside (SNP) 100 nmol/ml [91]	Bioxcell®	Prohibition of lipid per oxidation is associated with its ability in reaction with alkoxy lipid radicals (LX), lipid peroxyl (LOO) and chain-breaking oxidation	49	10% Pomegranate Juice extract [57]	Tris-citric acid-egg yolk-fructose	Antioxidant property of polyphenols
21	PTX (Pentoxifylline) (3.6 mM) [25]	Tris-based extender	Neutralization of reactive oxygen species (ROS), Phosphodiesterase inhibitor (↑cAMP)	50	Catechin 50 µg/mL (CT), green tea extract [81]	Tris-based extender	Antioxidant property of polyphenols
22	Caffeine (0.5%) [122]	TFYG	Stimulatory effect on kinetic activity (↑cAMP) and respiration of spermatozoa	51	*Diospyros kaki* (Persimmon fruit) (1-6%) [172]	Tris-Citrate-Fructose egg yolk	Antioxidant property of flavonoids
23	Glutamine 3 mM [158]	Optydil® extender	Main components of glutathione: prevent Lipid peroxidation	52	10 g L^{-1} of rosemary extract [43]	TEY extender	Antioxidative components: Carnosol, carnosic, rosmanic acids
24	Fetuin10 mg ml^{-1} [137]	Tris-based extender	Antioxidant property	53	Curcumin (50 µmol/l [159]	Triladyl®	ROS scavenger and a powerful LPO inhibitor
25	BSA-Bovine Serum Albumin (1 g/100 mL) [12]	Citrate-egg yolk extender	Eliminate free radicals generated by oxidative stress	54	Silymarin (*Silybum marianum*) (0.18 & 0.36 mg/ml) [56]	Tris-Citrate-Fructose egg yolk	Antioxidant property
26	Cysteine (5 mM) [124]	EYTG	Precursors of Glutathione biosynthesis	55	*Aloe vera* extract (5l/µml) [142]	TEYCAFG	Acts as an antioxidant
27	50 mM Taurine [36]	Tris-based egg yolk extender	Inhibits lipid peroxidation, protects the cells against the accumulation of reactive oxygen species	56	*Avena sativa* seeds aqueous extract (AEASS) (1-1.5%) [6]	Tris-extender	Polyphenols present provides antioxidant property
28	Arginine 0.005 M/ml [5]	Tris-yolk-fructose	Production of nitric oxide (NO) mechanism -protects structural and functional integrity	57	*Eurycoma longifolia* aqueous extract (5 mg/ml) [15]	Tris-egg yolk extender	Bioactive components (quassinoids) acts against oxidative stress
29	Buserelin® (2µg/ml) [98]	BIOXcell®	It protects the sensitive DNA structures and cell membrane of sperm				

Antioxidants

Antioxidants are agents that can neutralize or reduce the formation of free radicals, breakdown the substrates of oxidation, and decrease the risk of injuries to spermatozoa during cryopreservation [132]. Antioxidants may be a preventive antioxidant viz. catalase, EDTA, and, transferrin, which prevents the formation of ROS, or scavenging antioxidants such as ascorbic acid and vitamin E, which removes the existing ROS [101]. Antioxidants can also be categorized into enzymatic antioxidants viz. GSH, catalase, SOD and non-enzymatic antioxidants viz. carotenoids, vitamins C and E, taurine, albumin, cysteines [21]. Some of the primary antioxidants naturally present in mammalian semen are glutathione, reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase Vit E and C, melatonin, etc [123]. These antioxidants protect the sperm cells from harmful lipid peroxidation and maintain its integrity and viability. However, there is a significant reduction in the level of intracellular antioxidants following a freeze/thaw cycle [29]. Thus, antioxidant supplementation is beneficial to defend the sperm cells from oxidative stress during the cryopreservation process.

Enzymatic antioxidants neutralize free radicals by converting dangerous free radicals into hydrogen peroxide (H_2O_2) and then to water. The enzymatic antioxidants that take part in the neutralization of free radicals are superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase. SOD catalyzes the dismutation process of superoxide (O_2^-) into oxygen (O_2) and hydrogen peroxide (H_2O_2). It prevents cholesterol efflux and the production of lipid peroxidation end-products such as malondialdehyde or MDA). Thus it suppresses the premature hyperactivation, capacitation, and the acrosomal reaction of sperm cells. Moreover, it can maintain the acrosomal, plasma, and membrane integrity, the cytoskeletal structure of flagella, and the motility of the sperm.

Glutathione is a tripeptide that is naturally present in semen but its concentration decreases following cryopreservation due to removal and dilution of seminal plasma [29]. Glutathione is mainly present in reduced form (GSH) and oxidized form (GSSG), their ratio being an indicator of cellular oxidative stress. The glutathione antioxidant system consists of reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione peroxidase (GPx), glutathione reductase (GRX), and glutathione-s-transferase. GPx catalyzes the conversion of toxic H_2O_2 into water and hydroperoxides by GSH [28]. GRX stimulates the reduction of GSSG back to GSH to complete the cycle. Thus, GSH and GPx are added in semen extenders as additives to prevent the lipid peroxidation induced by toxic H_2O_2. Catalase is an enzymatic antioxidant present both in the cytoplasm as well as in the seminal plasma. It is the first line of defense against ROS and free radicals. However, the concentrations are significantly lower in bovine semen; hence its supplementation in the extender can help sperm cells to defend against lipid peroxidation [64]. It catalyzes both intracellular and extracellular H_2O_2 present into water (H_2O) and oxygen (O_2) [18]. One catalase molecule can dissociate 2 million molecules of H_2O_2 per minute and suppresses the NADPH oxidase enzyme system to reduce production as well as neutralize superoxide radicals, inhibiting the chain reaction that leads to LPO [1].

Non- enzymatic antioxidants are diverse groups of compounds that functions as an antioxidant but doesn't belong to enzyme group chemically. This includes vitamins, carotenoids, melatonin, thiol groups, etc. Vitamins such as vitamin E, B, and C are capable of scavenging ROS and can prevent lipid peroxidation of the sperm membrane. Vitamin C or ascorbic acid is a water-soluble antioxidant that can scavenge various radicals such as superoxide, hydroxyl, and hydrogen peroxide radicals [62]. About 65% of the seminal antioxidant capacity is in the form of vitamin C which prevents the formation of harmful hydroperoxide products (malondialdehyde, 4-hydroxy-2-hexanal, 4-hydroxy-2-nonenal) by preventing the formation of peroxide radicals [82]. Vitamin B12 or Cyanocobalamin (stable form), a water-soluble vitamin, is added into semen extenders as it can scavenge ROS and prevent membrane lipid peroxidation. It indirectly stimulates ROS scavenging by the preservation of glutathione, reduces oxidative stress induced by homocysteine and glycation end products [162]. Vitamin B12 functions as a coenzyme in various biochemical processes related to energy and amino acid metabolism [83]. Vitamin E or alpha-tocopherol is one of the vital components of the spermatozoal antioxidant system and is a potent ROS scavenger, thus prevents lipid peroxidation of the cell membrane [20]. It plays a role in halting the propagation of lipid peroxidation chain reaction by intercepting lipid peroxyl radicals that are important for the propagation of lipid peroxidation. It also breaks the covalent links formed between ROS and fatty acid side chains in the sperm membrane lipids protecting the membrane from the harmful effects of lipid peroxidation [171].

Carotenoids are a group of natural pigments found in plants and animals having antioxidant properties. Astaxanthin (AX) is a carotenoid pigment present in aquatic creatures such as salmon, trout, shrimp, and lobster, etc. It can easily penetrate the sperm cellular membrane thus protects the sperm both internally and externally from lipid peroxidation [70]. It also neutralizes the free radicals by either accepting or donating an electron without being destructed or becoming a pro-oxidant during the process. Lycopene (ψ, ψ-carotene; LYC) is another natural carotenoid pigment found in red fruits and vegetables such as tomatoes, watermelons, carrots, papayas, etc. Lycopene possesses natural ROS –quenching ability, especially for singlet oxygen ($^1O_2$) and trapping of peroxyl radicals. It is a highly unsaturated hydrocarbon consisting of 13 double bonds, out of which 11 are unconjugated and thus can donate numerous electrons for ROS neutralization [13]. During singlet oxygen ($^1O_2$) quenching, energy gets transferred from $^1O_2$ to the lycopene, thus trapping ROS before they can initiate the lipid peroxidative chain reactions [37]. Lycopene is twice as efficient as β-carotene in quenching singlet oxygen and ten times faster in comparison to vitamin E (α-tocopherol) [129]. Another important non-carotenoid polyphenolic antioxidant present in plants is Resveratrol (3, 5, 40-trihydroxystilbene, RES). It is a natural phytoalexin polyphenolic compound produced by various plants such as berries and grapes [67] in response to injury, fungal attack, or UV irradiation. Phytoalexins are antimicrobial and antioxidants that can inhibit the growth of pathogens at the site of infection. Resveratrol is more efficient as an antioxidant in comparison to vitamin C and E and also elicit minimum toxicity [141]. It can permeate the peroxidised rigid membranes and increase the membrane fluidity, thus resulting in better interaction with the free radicals present in the disordered membrane lipid bilayer [30]. Resveratrol can also intercept both the initiation and propagation process of lipid peroxidation [141] and improve the viability of spermatozoa via stimulating the mitochondrial functions of the cell [99].

Another group of non-enzymatic antioxidants is thiols containing –SH functional groups. Cysteamine is a thiol antioxidant and can stimulate glutathione synthesis by reacting with cysteine [45], thus can protect the sperm cell against ROS-induced damages during cryopreservation [111]. Alpha-Lipoic acid (ALA; 1, 2-dithiolane-3-pentanoic acid) is an endogenous thiol group characterized by high antioxidative property against free radicals and increases the amount of reduced glutathione level in tissues, thus reduces lipid peroxidation level [26, 54]. ALA can permeate biological membranes easily due to small size and high lipophilicity, thus quenching free radical efficiently in both lipid and aqueous environments [150]. It is distributed and accumulated rapidly in the body and gets converted to its more potent antioxidant form, dihydrolipoic acid [120].

Various sodium salts are used as semen additives. Sodium pyruvate is a salt that can neutralize peroxynitrite (ONOOA-) and hydrogen peroxide (H_2O_2). Pyruvate metabolism will increase the formation of NADPH, which reduces glutathione disulfide (GSSG) to glutathione (GSH), therefore improving the antioxidant activity [105]. Another compound, sodium nitroprusside (SNP), is a medication used for lowering blood pressure. It can increase the nitric oxide (NO) levels in the blood, which prohibits lipid peroxidation by reacting with lipid peroxyl (LOO), alkoxy lipid radicals (LX), lipid peroxyl (LOO), breaking the chain reaction of oxidation [166].

Melatonin or N-acetyl-5-methoxy tryptamine is a neurohormone derived from indole, synthesized and released by the pineal gland. Melatonin is an intracellular antioxidant [87] that can pass through membranes easily and gets distributed uniformly across all the cellular components due to its smaller size. Apart from ROS scavenging property, it can improve the mobility and velocity of sperm cells either via stimulation of cAMP [169] or Ca^{2+} ions inside the cell [78]. Melatonin supplementation can be beneficial because of the presence of melatonin receptors (MT1 and MT2) on the sperm itself, where melatonin can bind to and affect sperm function. Other important antioxidants used for bull semen cryopreservation are Dithioerythritol [32], Ethylenediaminetetraacetic acid or EDTA [122], Coenzyme Q10 or CoQ10 [133], etc.

Antioxidant preservatives

Antioxidant preservatives are compounds used to terminate auto-oxidation of unsaturated fatty acids present in oils and lipids. They are used to decelerate the process of rancidity in oils and fats, thus extending the shelf life. Oxygen preferentially reacts with antioxidant preservatives instead of oxidizing fats and oils, thus protecting them from rancidity and spoilage. Examples of antioxidant preservatives used in semen cryopreservation include butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and tert-butyl hydroquinone. Butylated hydroxytoluene (BHT) is an organic compound derived from phenol and can modify the functions of the sperm membrane's lipid bilayers [73]. BHT serves as a ROS scavenger and reduces the damage to sperm membranes and motility apparatus during the freeze-thaw process [92]. Anderson *et al*. (1994) [8], using spin labels and electron spin resonance techniques, suggested that BHT increases membrane fluidity and presents them as less susceptible to cold shock. Also, BHT has antiviral properties and may improve the fertility of bull semen by reducing the risk of transmitting diseases caused by viruses to cows via AI.

Methylxanthines

Methylxanthines are a group of compounds produced naturally and is present in various common foodstuffs such as tea, coffee, and chocolate. They inhibit cyclic nucleotide phosphodiesterase (PDE) and prevent the inactivation of secondary messengers like cyclic adenosine monophosphate (cAMP). It also helps sperm cells to maintain their motility as cAMP dependant protein kinase A (PKA) signalling pathway is one of the significant signaling pathways of sperm motility. Two important examples are Pentoxifylline (PTX) and caffeine that stimulates the motility of sperm by inhibiting PDE and having an additional effect on ROS neutralization, cellular metabolism, etc.

Caffeine is having a stimulatory effect on sperm respiration and kinetic activity. Caffeine inhibits cyclic nucleotide phosphodiesterase, an enzyme that hydrolyzes cAMP thereby increasing the cAMP level [66]. It stimulates spermatozoan glycolysis and also breaks down glycogen into glucose [143]. Caffeine may also affect the cellular metabolism of sperm cells and induce hyper-activation [74].

Pentoxifylline (PTX) is a methylxanthine that inhibits cyclic nucleotide phosphodiesterase (PDE), thereby increasing intracellular cAMP level [168]. It can neutralize reactive oxygen species (ROS) and reduce the effect of lipid peroxidation [24, 60]. It also inhibits tumor-necrosis factor-alpha (TNF-alpha) that is accountable for DNA fragmentation and apoptosis or programmed cell death [109, 115].

Amino acids/Proteins

Amino acids (AAs) are one of the constituents of seminal plasma and can prevent oxidative damage during cryopreservation. Amino acid is added in the semen extender as it preserves the structure of the sperm plasma membrane during the freeze-thaw process. It also maintains the lipids of the sperm membrane that are susceptible to peroxidative damage [42]. Amino acids function as non-enzymatic antioxidants or enhance enzymatic antioxidant synthesis inhibiting lipid peroxidation. They either inhibit lipid peroxidation or modulate osmotic mechanism. Examples of amino acids used as cryoprotective agents are glycine, cysteine, taurine, methionine, glutamine, and proline. Amino acids such as glutamine, cysteine, and glycine are necessary for the synthesis of glutathione (GSH) [157]. Glutamine, a tripeptide improves sperm motility and freezing ability after the free-thawing process [138, 90]. Cysteine can penetrate the cell membrane easily. It not only enhances the intracellular GSH biosynthesis but also individually acts as a nonenzymatic antioxidant due to the presence of thiol group (-SH) which directly reacts with electrophiles including reactive radicals [71]. Taurine (2-aminoethanesulfonic acid) and its precursor, hypotaurine (2-aminoethanesulfunic acid) are the end products of cysteine metabolism [80]. They act as a non-enzymatic scavenger of ROS and thus provide protection against LPO, thereby improving sperm membrane integrity and motility. Taurine also enhances the level and functions of other enzymatic antioxidants like GPx, SOD, and CAT [107]. It prevents cholesterol efflux from the membrane, decreases the MDA production, and protects DNA from fragmentation during oxidative stress. Arginine, the precursor of nitric oxide (NO) maintains the motility and metabolism of sperm [106]. Nitric oxide (NO) acts as a ROS scavenger, thus protecting the lipid bilayer membrane from peroxidation [146]. NO, also improves the motility of sperm, as it increases the rate of glycolysis, which subsequently increases the level of Adenosine-5'-triphosphate (ATP) [121].

Apart from simple amino acids, polypeptides and proteins have been incorporated in semen dilutors as semen additives. Polypeptides being simple polymer of a defined side chain of amino acids, whereas proteins are a complex molecule of folded polypeptides. Buserelin acetate, a synthetic peptide GnRH analogue, is used as a treatment for female infertility, hormone-responsive cancers, uterine diseases, etc. This synthetic peptide reduces the percentage of DNA fragmentation and thereby improves the quality of frozen-thawed bovine semen [98]. Elamipretide (Bendavia, MTP-131, SS-31), an aromatic-cationic tetrapeptide, can easily pass cellular membranes and get localized in the inner mitochondrial membrane transiently. In the mitochondrial inner membrane, it associates itself with cardiolipin, an essential component of mitochondrial energy metabolism. Through this, Elamipretide can reduce ROS production and increase ATP synthesis in affected cells by restoring energy production [151, 152].

Bovine Serum Albumin (BSA) and Fetuin are carrier proteins found in the blood that has antioxidant property. Their antioxidant properties are associated with the reductive capacity of chemical groups present in their amino acid constituents [63]. Relaxin, a regulatory peptide of the insulin family, is a vital component of seminal plasma, and bovine semen contains the highest concentration of it. Relaxin influences sperm motility and viability through specific sperm- surface receptors [93]. Relaxin supplementation to bull semen as an additive in cryopreservation media has improved the motility, viability, and fertility of sperm [55].

Sugar/polysaccharides

Sugars are generally classified into simple or complex sugars. Simple sugars, especially "disaccharides" can stabilize plasma membrane lipid bilayers [3]. These sugars can interact with phospholipids of the sperm plasma membrane to increase the sperm survivability post-freeze-thawing [4]. Disaccharides like sucrose, lactose, trehalose, raffinose, and dextrans are non-permeable through the plasma membrane. It creates osmotic pressure and leads to cell dehydration, thus decreasing the amount of intracellular freezable water and subsequently lowering the incidence of intracellular ice crystal formation. Disaccharides like sucrose and trehalose can serve as a cryoprotectant [135], as it aids cell dehydration, preventing ice crystal injury of sperm cells during cryopreservation [4]. Trehalose can create a non-hygroscopic glass-like state and protect the components of the sperm plasma membrane during the cryopreservation process. It can interact and modify the membrane phospholipids resulting in improved flexibility of plasma membrane that can tolerate cryo-injuries [4]. Furthermore, it improves the antioxidant activity by increasing the glutathione level and reducing the lipid peroxide level [3].

Raffinose is a trisaccharide that stimulates cellular osmotic dehydration by interacting with the plasma membrane. It forms a metastable glass-like state and reduces the risk of intracellular ice crystallization [147]. It also has an indirect positive effect on specific ROS scavenger production [163].

Hyaluronan is a disaccharide polymer and an essential element of the extracellular matrix [59]. It mediates various sperm functions such as motility [69] and capacitation [149]. Hyaluronan receptors are located in the head, mid-piece, and tail region of spermatozoa and can stabilize motility in frozen-thawed semen [95]. Supplementation of hyaluronan may increase the rate of phosphorylation and ATP levels, improving the flagellar motility and function [138].

Cell membrane stabilizers

Cell membrane stabilizers are the constituents of the cell plasma membrane and function as semen additives for providing stability and strength to the sperm membrane. The plasma membrane of mammalian spermatozoa is composed of the lipid bilayer of phospholipids. The phospholipid composition and cholesterol: phospholipid ratio of the sperm plasma membrane determines the sensitivity of the sperm to “cold shock” [75]. Phospholipids are composed of fatty acids, usually omega-3 fatty acids. Lipid peroxidation during cryopreservation can cause damage to the fatty acids, particularly polyunsaturated fatty acids (PUFAs) [96].

Docosahexaenoic acid (DHA) is one of the main components of bovine spermatozoa and sum up to 55-60% of the total PUFAs content [88]. DHA can improve the viability and quality of cryopreserved bull semen. DHA gets successfully incorporated into the sperm plasma membrane before cryopreservation and protects the cell membrane by increasing the membrane resistance to ice crystal damage. Alpha-Linolenic acid (ALA) is an omega-3 fatty acid present in the sperm plasma membrane that decreases in concentration during the freeze-thaw cycle [110]. ALA provides energy and also regulates the cellular membrane proteins [140]. It can also act as a cell membrane stabilizer and can maintain and regulates the sperm cell’s membrane function sustaining its quality and fertility during the cryopreservation process.

Virgin coconut oil (VCO) is rich in both saturated and unsaturated fatty acid content [108]. It also possesses natural antioxidant capacity due to the presence of antioxidants such as polyphenols, tocotrienol, and tocopherols [118]. VCO are hydrophobic in nature, thus needs to be emulsified with Soybean Lecithin (SL), an excellent emulsifier [49]. The composition of SL is similar as that of egg yolk and can prevent semen from the adverse effect of cold shock during the freeze-thaw cycle. VCO with SL can replenish the phospholipid content of the sperm membrane and preserve the structure and function of spermatozoa [156].

An integral component of the sperm cell membrane is Cholesterol which protects and strengthens the structures of plasma membrane even below the phase transition temperature [127]. Cholesterol is removed from the sperm plasma membrane during cryopreservation causing premature capacitation [17]. Cyclodextrins are oligosaccharides in nature, consisting of a hydrophobic interior core and a hydrophilic exterior. Cholesterol is hydrophobic and thus can be incorporated into the sperm plasma membrane using cyclodextrins [38]. Cholesterol loaded cyclodextrins (CLC) thus can prevent cryocapacitation and apoptosis-like changes induced by cryopreservation.

Trace elements

Trace elements are minerals that are present in the living tissues at minute concentrations. Trace minerals are necessary for various functioning of enzymes and proteins associated with male reproduction and fertility. Trace elements take part in multiple pathways and may function as a cofactor, enzyme activator, or secondary molecular structure stabilizer. Trace elements such as Zinc, Copper, Manganese, Selenium, Iron, Cobalt, Iodine used as semen additive can improve the quality of sperm upon cryopreservative processes.

Manganese supplementation can reduce the leakage of lipids and other sperm contents during oxidative stress conditions [22]. Manganese can easily penetrate the sperm cell and help in maintenance of optimum ionic balance thus minimize the suffering of sperm cells during the freeze-thaw cycle [102]. Manganese is a cofactor of enzymatic antioxidant superoxide dismutase [2], and may increase the amount of glutathione cycle enzymes such as thiols, reduced and oxidized glutathione [22]. Manganese stabilizes the sperm plasma membrane [27] and may form complexes with unsaturated lipid components making them more resistant to free radical attacks [34]. Manganese stimulates sperm cell motility, as it potentiates adenylate cyclase activity and increases the concentrations of adenosine monophosphate (cAMP) [102]. Selenium is another essential trace element that is essential for numerous physiological processes, especially for antioxidant defense [76]. Selenium is a vital component of glutathione peroxidase, an enzyme that protects the cell's internal structures against free radicals and is an antioxidant for cellular membrane lipids [134]. Selenium supplementation improves enzymatic rates of ATP-utilizing and ATP-regenerating pathways of the sperm, which are evaluated by motility and oxygen consumption of the sperm [126].

Antifreeze proteins

In nature, there exist several unique species which can survive low temperature because of a specific group of proteins that can bind to ice. These proteins are called antifreeze proteins (AFPs) and can prevent ice crystal growth in cells. These proteins are present in a wide variety of organisms ranging from bacteria, fungi, insects to plants, fish, etc. These proteins play a specific role in the survival of the animals at natural freezing temperatures. They act by depressing the freezing point of water, preventing ice crystal growth and ice recrystallization. They interact with lipid components of the plasma membrane, blocks ion channels, and stabilize the transmembrane electrolyte gradients at low temperatures. It reduces the calcium influx and stabilizes the membrane [164]. In bovines, AFPs increase osmotic resistance and decreases mechanical damage to the sperm [125]. AFPs prevent the ice crystal growth between the melting point or colligative freezing point and hysteretic freezing point or the temperature of ice crystal growth [48]. The difference between these two temperatures is known as thermal hysteresis and is an outcome of the concentration and specific activity of AFPs. AFP's binds to the surface of ice crystals and prevents water molecules from joining the ice lattice and grow in size [130]. It causes the ice to grow as convex surface fronts between two adjacent adsorbed AFPs, thus lowering the temperature of ice crystal growth. The range of thermal hysteresis produced by different AFPs varies, highest in freeze-tolerant insects (3-6°c), marine fishes (0.7-1.5 ºC), and a small fraction in other cryotolerant organisms [51, 47].

Iodixanol

Iodixanol is an iodinated radio-contrast agent used as an active ingredient in various 'cushion' products during stallion semen centrifugation. It protects the plasma membrane and preserves the motility of the sperm during the process of freezing and thawing. Saragusty *et al*. (2009) [136] first demonstrated the effect of iodixanol in bull semen and observed that it raises the glass transition temperature and alters the ice crystal structure formation by removing water from the solution during cryopreservation. Iodixanol interacts with spermatozoa to protect against intracellular ice formation, reduce recrystallization, and forms a protective outer coating layer helping the spermatozoa to maintain its integrity during semen cryopreservation. It acts similarly to cryoprotectants such as ethylene glycol, sugar, and glycerol.

Oxyrase

There exist two strategies to reduce oxidative stress during cryopreservation; the first is through the reduction of generated ROS by using antioxidants, and the second is by reducing sources of ROS production [7]. Oxyrase, an *Escherichia coli* membrane derivative, reduces ROS production by reducing the O_2 level in semen extender. A part of bacteria's electron transport system, oxyrase can reduce O_2 concentrations in solutions to low levels in the presence of hydrogen donor substrate [50]. It has no cytotoxic effect on the spermatozoa and has been used successfully in semen extenders to improve post-thaw semen qualities [44].

Natural extracts

Natural or plant extracts have been used for ages as folk medicine in both human as well as ethnoveterinary practices. Natural or plant extracts are being incorporated in various pharmaceutical and consumable food products to fortify the nutritive and antioxidant value. There has been an increase in terms of utilizing natural/plant extracts as semen additives recently as they are laden with various helpful compounds like polyphenols, flavonoids, and carotenoids. These phytochemicals found in natural extracts like flavonoids and other phenolic compounds possess much higher antioxidant activity than vitamins E and C [33].The presence of these compounds helps in neutralizing free radicals in the semen during processing and preservation, thereby improving the quality of the semen. Listed below are some natural or herbal extract used as semen additives for cryopreservation of bull semen. Honey is a natural product produced by honey bees that is mainly a combination of sugars, proteins, enzymes, vitamins, minerals, flavonoids, and phenolic acids. It contains a rich amount of various sugars serving as an energy source for the spermatozoa's survival and motility as well as can act as a non-penetrating cryoprotectant during cryopreservation [65]. Honey also contains a minute quantity of various compounds that may serve as antioxidants such as pinobanksin, chrysin, catalase, vitamin C, and pinocembrin [61].

Diospyros kaki or persimmon is an edible fruit possessing a high amount of biologically active substances [46] represented by antioxidants like carotenoids [170], polyphenols and flavonoids [167], dietary fiber, vitamins and organic acids [86], carbohydrates [19], triterpenoids and minerals [112]. Flavonoids are potent free radical scavengers, and their antioxidant capacity is a function of the number of hydroxyl groups present on the phenyl ring of its molecular structure [103]. Persimmon possesses a high amount of flavonoids that are potent antioxidants and can suppress the production of nitric oxide and malondialdehyde, a marker for oxidative stress [148]. Pomegranate (*Punica granatum*) is another fruit that is consumed worldwide and is abundant in antioxidants such as vitamin C and polyphenols (anthocyanins, punicalagin, ellagic and gallic acid) [154]. Due to the combined activity of various phytochemicals present in pomegranate Juice extract, it acts as potent antioxidant [100, 139]. Oats (*Avena sativa L.*) is a cereal crop consumed worldwide and popular due to its health benefits. It is rich in fiber content, minerals, vitamins, and a plethora of polyphenols [41] such as ferulic acid, caffeic acid, vanillic acid, p- hydroxybenzoic acid, etc [35]. Polyphenols contribute to the antioxidative property of oats by scavenging oxygen and nitrogen radical species [35].

Turmeric, a rhizomatous plant used as a culinary spice and in folk medicine, is a rich source of a natural antioxidant called curcumin (CUR) [68]. Curcumin is known to be potent against various reactive oxygen species like hydroxyl radicals, superoxide anion radicals, nitrogen dioxide radicals [145]. Oxidative stress due to semen cryopreservation can cause protein oxidation, fragmentation, and proteolysis leading to the generation of an excessive quantity of protein carbonyls [128]. Curcumin was found effective in reducing the number of oxidized proteins in bull spermatozoa due to its reactive oxygen species trapping property and also the prevention of residual oxygen species to alter the molecular structure of proteins found in spermatozoa. Rosemary (*Rosmarinus officinalis*) is a perennial herb that contains potent antioxidative compounds like carnosol, carnosic, and rosmanic acids having a protective antioxidant role against free radicals and lipid peroxidation (LPO) [104]. Another potent extract known for its antioxidant properties is silymarin, a flavonolignan extracted from the milk thistle plant (*Silybum marianum*). As an antioxidant, it reacts rapidly with free radicals inhibiting lipid peroxidation and stimulates the activity of enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase [165]. Various other natural extract that have been used for bull semen cryopreservation are green tea extract [81], *Spirulina maxima* extract [113], rosemary extract [43], *Aloe vera* extract [142], *Eurycoma longifolia* extract [15], etc.

Conclusion

Semen additives are useful not in terms of single but multiple parameters for improving the cryo preservability of sperm cells such as motility, viability, membrane integrity, antioxidant property, and subsequently exerting a positive effect on fertility. Incorporation of various semen additives in semen extenders can significantly improve post-thaw sperm quality parameters. Advancement of new sperm assessment technology such as Flow cytometry and CASA, also aids in understanding the mechanism and function of additives on spermatozoa. Thus, semen additives at correct doses are a reliable component of semen extenders and can be used at frozen semen production facilities for improving the cryo preservability of frozen bull semen.

References

1. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reproductive biology and endocrinology 2005;3(1):28.
2. Aguirre JD, Culotta VC. Battles with iron: manganese in oxidative stress protection. Journal of Biological Chemistry 2012;287(17):13541-8.
3. Aisen E, Quintana M, Medina V, Morello H, Venturino A. Ultramicroscopic and biochemical changes in ram spermatozoa cryopreserved with trehalose-based hypertonic extenders. Cryobiology 2005;50(3):239-49.
4. Aisen EG, Medina VH, Venturino A. Cryopreservation and post-thawed fertility of ram semen frozen in different trehalose concentrations. Theriogenology 2002; 57(7):1801-8.
5. AL-Ebady AS, Hussain SO, AL-Badry KI, Abd Rajab B. Effect of adding arginine in different concentrations on some physical properties of poor motile bull sperms during different months. Journal of Veterinary Medicine and Animal Health 2012;4(9):130-5.
6. Ali MM, Banana HJ. Effect of adding n-acetylcystiene and avena sativa extract to tris extender on post-cryopreservative semen characteristics of holstein bulls. Plant Archives 2020;20(1):1209-16.
7. Amidi F, Pazhohan A, Nashtaei MS, Khodarahmian M, Nekoonam S. The role of antioxidants in sperm freezing: a review. Cell and tissue banking 2016;17(4):745-56.
8. Anderson S, Harkness W, Akin Y, Kaproth M, Killian G. Categorical Data Analysis of the Effect on Bull Fertility, of Butylated Hydroxytoluene Addition to Semen, Extenders Prior to Freezing. Journal of dairy science. 1994;77(8):2302-7.
9. Andreea A, Stela Z. Role of antioxidant additives in the protection of the cryopreserved semen against free radicals. Romanian Biotechnological Letters 2010; 15(3):33-41.
10. Ansari MS, Rakha BA, Akhter S. Effect of butylated hydroxytoluene supplementation in extender on motility, plasmalemma and viability of Sahiwal bull spermatozoa. Pak J Zool 2011;43(2):311-4.
11. Arslan HO, Herrera C, Malama E, Siuda M, Leiding C, Bollwein H. Effect of the addition of different catalase concentrations to a TRIS-egg yolk extender on quality and in vitro fertilization rate of frozen-thawed bull sperm'. Cryobiology 2019;91:40-52.
12. Ashrafi I, Kohram H, Nasrabadi HT. Antioxidant effects of bovine serum albumin on kinetics, microscopic and oxidative characters of cryopreserved bull spermatozoa. Spanish Journal of Agricultural Research 2013;(3):695-701.
13. Atasoy N. Biochemistry of lycopene. Journal of Animal and Veterinary Advances 2012;11(15):2605-10.
14. BAHS. Basic Animal Husbandry and Fisheries Statistics. Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Krishi Bhawan, New Delhi 2019.
15. Baiee FH, Wahid H, Rosnina Y, Ariff O, Yimer N, Jeber Z *et al*. Impact of Eurycoma longifolia extract on DNA integrity, lipid peroxidation, and functional parameters in chilled and cryopreserved bull sperm. Cryobiology. 2018; 80:43-50.
16. Bailey J, Morrier A, Cormier N. Semen cryopreservation: Successes and persistent problems in farm species. Canadian journal of animal science 2003;83(3):393-401.
17. Bailey JL, Bilodeau JF, Cormier NA. Semen cryopreservation in domestic animals: a damaging and capacitating phenomenon. Journal of andrology 2000; 21(1):1-7.
18. Baker HG, Brindle J, Irvine DS, Aitken RJ. Protective effect of antioxidants on the impairment of sperm motility by activated polymorphonuclear leukocytes. Fertility and Sterility 1996;65(2):411-9.
19. Baltacioğlu H, Artik N. Study of postharvest changes in the chemical composition of persimmon by HPLC. Turkish Journal of Agriculture and Forestry 2013; 37(5):568-74.
20. Bansal AK, Bilaspuri GS. Antioxidant effect of vitamin E on motility, viability and lipid peroxidation of cattle spermatozoa under oxidative stress. Animal science papers and reports 2009;27(1):5-14.
21. Bansal AK, Bilaspuri GS. Impacts of oxidative stress and

antioxidants on semen functions. Veterinary medicine international 2011;1:1-7.
22. Bansal AK, Kaur AR. Cooperative functions of manganese and thiol redox system against oxidative stress in human spermatozoa. Journal of human reproductive sciences 2009;2(2):76.
23. Bansal AK. Effect of Antioxidants on Crossbred Cattle Bull Spermatozoa under Oxidative Stress (Doctoral dissertation, Punjab Agricultural University; Ludhiana) 2006.
24. Bell M, Wang RU, Hellstrom WJ, Sikka SC. Effect of cryoprotective additives and cryopreservation protocol on sperm membrane lipid peroxidation and recovery of motile human sperm. Journal of andrology 1993; 14(6):472-8.
25. Bhakat M, Mohanty TK, Raina VS, Gupta AK, Pankaj PK, Mahapatra RK *et al*. Study on suitable semen additives incorporation into the extender stored at refrigerated temperature. Asian-Australasian Journal of Animal Sciences 2011;24(10):1348-57.
26. Biewenga GP, Haenen GR, Bast A. The pharmacology of the antioxidant lipoic acid. General Pharmacology: The Vascular System 1997;29(3):315-31.
27. Bilaspuri GS, Bansal AK. Mn2+: A potent antioxidant and stimulator of sperm capacitation and acrosome reaction in crossbred cattle bulls. Archives Animal Breeding 2008;51(2):149-58.
28. Bilodeau JF, Blanchette S, Gagnon C, Sirard MA. Thiols prevent H2O2-mediated loss of sperm motility in cryopreserved bull semen. Theriogenology 2001; 56(2):275-86.
29. Bilodeau JF, Chatterjee S, Sirard MA, Gagnon C. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Molecular Reproduction and Development: Incorporating Gamete Research 2000;55(3):282-8.
30. Brittes J, Lucio M, Nunes C, Lima JL, Reis Y. Effects of resveratrol on membrane biophysical properties: relevance for its pharmacological effects. Chemistry and physics of lipids 2010;163(8):747-54.
31. Bucak MN, Ataman MB, Başpınar N, Uysal O, Taşpınar M, Bilgili A *et al*. Lycopene and resveratrol improve post-thaw bull sperm parameters: sperm motility, mitochondrial activity and DNA integrity. Andrologia 2015;47(5):545-52.
32. Bucak MN, Başpınar N, Tuncer PB, Coyan K, Sarıözkan S, Akalın PP *et al*. Effects of curcumin and dithioerythritol on frozen-thawed bovine semen. Andrologia 2012;44:102-9.
33. Cao G, Booth SL, Sadowski JA, Prior RL. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. The American journal of clinical nutrition 1998;68(5):1081-7.
34. Cavallini L, Valente M, Bindoli A. On the mechanism of inhibition of lipid peroxidation by manganese. InorganicaChimica Acta 1984;91(2):117-120.
35. Chen CY, Milbury PE, Kwak HK, Collins FW, Samuel P, Blumberg JB. Avenanthramides and phenolic acids from oats are bioavailable and act synergistically with vitamin C to enhance hamster and human LDL resistance to oxidation. The Journal of nutrition 2004;134(6):1459-66.
36. Chhillar S, Singh VK, Kumar R, Atreja SK. Effects of Taurine or Trehalose supplementation on functional competence of cryopreserved Karan Fries semen. Animal reproduction science 2012;135(1-4):1-7.
37. Choi SK, Seo JS. Lycopene supplementation suppresses oxidative stress induced by a high fat diet in gerbils. Nutrition research and practice 2013;7(1):26-33.
38. Choi YH, Toyoda Y. Cyclodextrin removes cholesterol from mouse sperm and induces capacitation in a protein-free medium. Biology of reproduction 1998;59(6):1328-33.
39. Chuawongboon P, Sirisathien S, Pongpeng J, Sakhong D, Nagai T, Vongpralub T. Effects of supplementation of iodixanol to semen extender on quality and fertilization ability of frozen–thawed Thai native bull sperm. Animal Science Journal 2017;88(9):1311-20.
40. Chung EL, Nayan N, Nasir NS, Hing PS, Ramli S, Rahman MH *et al*. Effect of honey as an additive for cryopreservation on bull semen quality from different cattle breeds under tropical condition. J. Anim. Health Prod 2019;7(4):171-8.
41. Clemens R, van Klinken BJ. The future of oats in the food and health continuum. British journal of nutrition 2014;112(S2):S75-9.
42. Curi R, Lagranha CJ, Doi SQ, Sellitti DF, Procópio J, Pithon-Curi TC *et al*. Molecular mechanisms of glutamine action. Journal of cellular physiology 2005;204(2):392-401.
43. Daghigh-Kia H, Olfati-Karaji R, Hoseinkhani A, Ashrafi I. Effect of rosemary (Rosmarinus officinalis) extracts and glutathione antioxidants on bull semen quality after cryopreservation. Spanish journal of agricultural research 2014;(1):98-105.
44. Darr C, Martorana K, Scanlan T, Meyers S. The effect of low oxygen during the early phases of sperm freezing in stallions with low progressive motility: can we improve post-thaw motility of stallion sperm?. Journal of Equine Veterinary Science 2016;42:44-51.
45. De Matos DG, Gasparrini B, Pasqualini SR, Thompson JG. Effect of glutathione synthesis stimulation during in vitro maturation of ovine oocytes on embryo development and intracellular peroxide content. Theriogenology 2002;57(5):1443-51.
46. Dembitsky VM, Poovarodom S, Leontowicz H, Leontowicz M, Vearasilp S, Trakhtenberg S *et al*. The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. Food research international 2011;44(7):1671-701.
47. DeVries AL, Cheng CH. The role of antifreeze glycopeptides and peptides in the survival of cold-water fishes. In Water and Life 1992, 301-315.
48. DeVries AL. Antifreeze glycopeptides and peptides: Interactions with ice and water. Methods in enzymology 1986;127:293-303.
49. Dickinson E. Towards more natural emulsifiers. Trends in Food Science & Technology 1993;4(10):330-4.
50. Dong Q, Correa LM, VandeVoort CA. Rhesus monkey sperm cryopreservation with TEST-yolk extender in the absence of permeable cryoprotectant. Cryobiolog 2009; 58(1):20-7.
51. Duman JG. Antifreeze and ice nucleator proteins in terrestrial arthropods. Annual Review of Physiology 2001;63(1):327-357.
52. Eidan SM, Abdulkareem TA, Sultan OA. Influence of adding manganese to Tris extender on some post-cryopreservation semen attributes of Holstein bulls.

IJAAS 2015;1:26-30.
53. Eidan SM. Effect on post-cryopreserved semen characteristics of Holstein bulls of adding combinations of vitamin C and either catalase or reduced glutathione to Tris extender. Animal reproduction science 2016;167:1-7.
54. El-Beshbishy HA, Bahashwan SA, Aly HA, Fakher HA. Abrogation of cisplatin-induced nephrotoxicity in mice by alpha lipoic acid through ameliorating oxidative stress and enhancing gene expression of antioxidant enzymes. European journal of pharmacology. 2011;668(1-2):278-84.
55. Elkhawagah AR, Martino NA, Ricci A, Storti V, Rumbolo F, Lange-Consiglio A *et al*. Effect of relaxin on cryopreserved beef bull semen characteristics. Cryobiology 2020;95:51-9.
56. El-Sheshtawy RI, El-Nattat WS. Impact of Silymarin enriched semen extender on bull sperm preservability. Asian Pacific Journal of Reproduction 2017;6(2):81.
57. El-Sheshtawy RI, Gamal A, El-Nattat WS. Effects of pomegranate juice in Tris-based extender on cattle semen quality after chilling and cryopreservation. Asian Pacific Journal of Reproduction 2016;5(4):335-9
58. El-Sheshtawy RI, Sisy GA, El-Nattat WS. Effects of different concentrations of sucrose or trehalose on the post-thawing quality of cattle bull semen. Asian Pacific Journal of Reproduction 2015;4(1):26-31.
59. Erlinger R. Glycosaminoglycans in porcine lung: an ultrastructural study using cupromeronic blue. Cell and tissue research 1995;281(3):473-83.
60. Esteves SC, Spaine DM, Cedenho AP. Effects of pentoxifylline treatment before freezing on motility, viability and acrosome status of poor quality human spermatozoa cryopreserved by the liquid nitrogen vapor method. Brazilian Journal of Medical and Biological Research 2007;40(7):985-92.
61. Fakhrildin MB, Alsaadi RA. Honey Supplementation to semen-freezing medium improves human sperm parameters post-thawing. Journal of family & reproductive health 2014;8(1):27.
62. Fraga CG, Motchnik PA, Shigenaga MK, Helbock HJ, Jacob RA, Ames BN. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proceedings of the National Academy of Sciences 1991;88(24):11003-6.
63. Fonseca DP, Khalil NM, Mainardes RM. Bovine serum albumin-based nanoparticles containing resveratrol: Characterization and antioxidant activity. Journal of Drug Delivery Science and Technolog 2017;39:147-55.
64. Foote RH. Catalase content of rabbit, ram, bull and boar semen. Journal of Animal Science 1962;21(4):966-8.
65. Fuller BJ. Cryoprotectants: the essential antifreezes to protect life in the frozen state. CryoLetters 2004; 25(6):375-88.
66. Funahashi H, Nagai T. Regulation of in vitro penetration of frozen–thawed boar spermatozoa by caffeine and adenosine. Molecular Reproduction and Development: Incorporating Gamete Research 2001;58(4):424-31.
67. Gadani B, Bucci D, Spinaci M, Tamanini C, Galeati G. Resveratrol and Epigallocatechin-3-gallate addition to thawed boar sperm improves in vitro fertilization. Theriogenology 2017;90:88-93.
68. Gao X, Kuo J, Jiang H, Deeb D, Liu Y, Divine G *et al*. Immunomodulatory activity of curcumin: suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production in vitro. Biochemical pharmacology 2004;68(1):51-61.
69. Ghosh I, Bharadwaj A, Datta K. Reduction in the level of hyaluronan binding protein 1 (HABP1) is associated with loss of sperm motility. Journal of reproductive immunology. 2002; 53(1-2):45-54.
70. Goto S, Kogure K, Abe K, Kimata Y, Kitahama K, Yamashita E *et al*. Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin. Biochimicaetbiophysicaacta (BBA)-biomembranes 2001;1512(2):251-8.
71. Gouveia MJ, Brindley PJ, Gärtner F, Costa JM, Vale N. Drug repurposing for schistosomiasis: combinations of drugs or biomolecules. Pharmaceuticals 2018;11(1):15.
72. Güngör Ş, Aksoy A, Deniz YE, Avdatek F, Öztürk C, Ataman MB *et al*. Combination of cysteamine and lipoic acid improves the post-thawed bull sperm parameters. KocatepeVeterinerDergisi 2016;9(2):88-96.
73. Hammerstedt RH, Keith AD, Snipes W, Amann RP, Arruda D, Griel Jr LC. Use of spin labels to evaluate effects of cold shock and osmolality on sperm. Biology of reproduction 1978;18(4):686-96.
74. Ho HC, Suarez SS. An inositol 1, 4, 5-trisphosphate receptor-gated intracellular Ca2+ store is involved in regulating sperm hyperactivated motility. Biology of reproduction 2001;65(5):1606-15.
75. Holt WV. Fundamental aspects of sperm cryobiology: the importance of species and individual differences. Theriogenology 2000;53(1):47-58.
76. Hosnedlova B, Kepinska M, Skalickova S, Fernandez C, Ruttkay-Nedecky B, Malevu TD *et al*. A summary of new findings on the biological effects of selenium in selected animal species-a critical review. International journal of molecular sciences. 2017;18(10):2209.
77. Hu JH, Tian WQ, Zhao XL, Zan LS, Xin YP, Li QW. The cryoprotective effects of vitamin B12 supplementation on bovine semen quality. Reproduction in domestic animals. 2011; 46(1):66-73.
78. Huerto-Delgadillo L, Antón-Tay F, Benitez-King G. Effects of melatonin on microtubule assembly depend on hormone concentration: role of melatonin as a calmodulin antagonist. Journal of pineal research. 1994;17(2):55-62.
79. Hussaini SM, Zhandi M, Shahneh AZ, Sharafi M, Nejati-Javaremi A, Yousefi A *et al*. Effect of tert-butyl hydroquinone on bull semen cryopreservation. CryoLetters. 2017;38(5):372-8.
80. Huxtable RJ. Physiological actions of taurine. Physiological reviews. 1992;72(1):101-63.
81. Inanç ME, Çil B, Yeni D, Avdatek F, Orakçi D, Tuncer PB *et al*. The effect of green tea extract supplementation in bull semen cryopreservation. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 2019;25(5):703-8.
82. Jialal I, Vega GL, Grundy SM. Physiologic levels of ascorbate inhibit the oxidative modification of low density lipoprotein. Atherosclerosis 1990;82(3):185-91.
83. Juanchi X, Albarran G, Negron-Mendoza A. Radiolysis of cyanocobalamin (vitamin B12). Radiation Physics and Chemistry 2000;57(3-6):337-9.
84. Kaka A, Haron W, Yusoff R, Yimer N, Khumran AM, Sarsaifi K *et al*. Effect of docosahexanoic acid on quality of frozen–thawed bull semen in BioXcell extender. Reproduction, fertility and development 2017;29(3):490-

5.
85. Kaka A, Wahid H, Rosnina Y, Yimer N, Khumran AM, Sarsaifi K *et al*. α-Linolenic acid supplementation in BioXcell® extender can improve the quality of post-cooling and frozen-thawed bovine sperm. Animal reproduction science 2015;153:1-7.
86. Karakasova L, Babanovska-Milenkovska F, Lazov M, Karakasov B, Stojanova M. Quality properties of solar dried persimmon (Diospyros kaki). Journal of Hygienic Engineering and Design 2013;4(1):54-9.
87. Karimfar MH, Niazvand F, Haghani K, Ghafourian S, Shirazi R, Bakhtiyari S. The protective effects of melatonin against cryopreservation-induced oxidative stress in human sperm. International journal of immunopathology and pharmacology 2015;28(1):69-76.
88. Kelso KA, Redpath A, Noble RC, Speake BK. Lipid and antioxidant changes in spermatozoa and seminal plasma throughout the reproductive period of bulls. Reproduction 1997;109(1):1-6.
89. Khalil WA, El-Harairy MA, Zeidan AE, Hassan MA. Impact of selenium nano-particles in semen extender on bull sperm quality after cryopreservation. Theriogenology 2019;126:121-7.
90. Khlifaoui M, Battut I, Bruyas JF, Chatagnon G, Trimeche A, Tainturier D. Effects of glutamine on post-thaw motility of stallion spermatozoa: an approach of the mechanism of action at spermatozoa level. Theriogenology 2005;63(1):138-49.
91. Khodaei H, Chamani M, Mahdavi B, Akhondi AA. Effects of adding sodium nitroprusside to semen diluents on motility, viability and lipid peroxidation of sperm in holstein bulls. International Journal of Fertility & Sterility 2016;9(4):521.
92. Killian G, Honadel T, McNutt T, Henault M, Wegner C, Dunlap D. Evaluation of butylated hydroxytoluene as a cryopreservative added to whole or skim milk diluent for bull semen. Journal of Dairy Science 1989;72(5):1291-5.
93. Kohsaka T, Hamano K, Sasada H, Watanabe S, Ogine T, Suzuki E *et al*. Seminal immunoreactiverelaxin in domestic animals and its relationship to sperm motility as a possible index for predicting the fertilizing ability of sires. International journal of andrology 2003;26(2):115-20.
94. Korkmaz F, Malama E, Siuda M, Leiding C, Bollwein H. Effects of sodium pyruvate on viability, synthesis of reactive oxygen species, lipid peroxidation and DNA integrity of cryopreserved bovine sperm. Animal reproduction science 2017;185:18-27.
95. Kornovski BS, McCoshen J, Kredentser J, Turley E. The regulation of sperm motility by a novel hyaluronan receptor. Fertility and sterility 1994;61(5):935-40.
96. Kothari, Shiva, Thompson, Aaron, Agarwal, Ashok *et al*. Free radicals: Their beneficial and detrimental effects on sperm function. Indian journal of experimental biology. 2010;4:425-35.
97. Kowalczyk A, Ewa CP. Antioxidant Effect of Elamipretide on Bull's Sperm Cells During Freezing/Thawing Process 2020.
98. Kowalczyk A, Kuczaj M, SzulA, Czerniawska-Piątkowska E. Buserelin Acetate Reduces Mortality and DNA Defragmentation of Bovine Sperm Cells Exposed to Oxidative Stress. Pakistan Journal of Zoology 2020; 52(5):1795-9.
99. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F *et al*. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006;127:1109-22.
100. Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. The American journal of clinical nutrition 1999; 70(3):475s-90s.
101. Lampiao F. Free radicals generation in an in vitro fertilization setting and how to minimize them. World J Obstet Gynecol 2012;1(3):29-34.
102. Lapointe S, Ahmad I, Buhr MM, Sirard MA. Modulation of postthaw motility, survival, calcium uptake, and fertility of bovine sperm by magnesium and manganese. Journal of dairy science 1996;79(12):2163-9
103. LeBlanc BW, Davis OK, Boue S, DeLucca A, Deeby T. Antioxidant activity of Sonoran Desert bee pollen. Food Chemistry 2009;115(4):1299-305.
104. Lo AH, Liang YC, Lin-Shiau SY, Ho CT, Lin JK. Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor-κB in mouse macrophages. Carcinogenesi 2002;23(6):983-91.
105. Mallet RT, Sun J, Knott EM, Sharma AB, Olivencia-Yurvati AH. Metabolic cardioprotection by pyruvate: recent progress. Experimental Biology and Medicine. 2005;230(7):435-43.
106. Mann T, Lutwak-Mann C. Male reproductive function and the composition of semen: general considerations. In Male Reproductive Function and Semen 1981,1-37.
107. Marcinkiewicz J, Kontny E. Taurine and inflammatory diseases. Amino acids 2014;46(1):7-20.
108. Marina AM, Che Man YB, Nazimah SA, Amin I. Antioxidant capacity and phenolic acids of virgin coconut oil. International Journal of Food Sciences and Nutrition. 2009;60(2):114-23.
109. Maxwell DT, Jacobson JD, King A, Chan PJ. Effect of pentoxifylline on tumor suppressor and proto-oncogene apoptosis in sperm. Journal of assisted reproduction and genetics 2002;19(6):279-83.
110. Medeiros CM, Forell F, Oliveira AT, Rodrigues JL. Current status of sperm cryopreservation: why isn't it better? .Theriogenology 2002;57(1):327-44.
111. Merton JS, Knijn HM, Flapper H, Dotinga F, Roelen BA, Vos PL *et al*. Cysteamine supplementation during in vitro maturation of slaughterhouse-and opu-derived bovine oocytes improves embryonic development without affecting cryotolerance, pregnancy rate, and calf characteristics. Theriogenology 2013;80(4):365-71.
112. Mir-Marqués A, Domingo A, Cervera ML, de la Guardia M. Mineral profile of kaki fruits (Diospyros kaki L.). Food chemistry 2015;172:291-7.
113. Mizera A, Kuczaj M, Szul A. Impact of the Spirulina maxima extract addition to semen extender on bovine sperm quality. Italian Journal of Animal Science 2019;18(1):601-7.
114. Moce E, Graham JK. Cholesterol-loaded cyclodextrins added to fresh bull ejaculates improve sperm cryosurvival. Journal of animal science 2006;84(4):826-33.
115. Mundle SD, Reza S, Ali A, Mativi BY, Shetty V, Venugopal P *et al*. Correlation of tumor necrosis factor α (TNFα) with high caspase 3-like activity in myelodysplastic syndromes. Cancer Letters 1999;140(1-

2):201-7.
116. Murtaza A, Ahmad M, Zubair M, Umar S, Mushtaq A, Gul AH *et al*. Comparative effects of addition of superoxide dismutase and reduced glutathione on cryopreservation of Sahiwal bull semen. Journal of the Hellenic Veterinary Medical Society 2018;69(4):1291-6.
117. Nasiri AH, Towhidi A, Zeinoaldini S. Combined effect of DHA and α-tocopherol supplementation during bull semen cryopreservation on sperm characteristics and fatty acid composition. Andrologia 2012;44:550-5.
118. Nevin KG, Rajamohan T. Virgin coconut oil supplemented diet increases the antioxidant status in rats. Food chemistry 2006;99(2):260-6.
119. Ngou AA, Ghosh SK, Prasad JK, Katiyar R, Kumar A, Rautela R *et al*. Exploring the role of E. coli derived enzyme, Oxyrase, as an oxygen scavenger to improve the cryotolerance of spermatozoa of Sahiwal bull. Cryobiology 2020.
120. Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. Free radical biology and medicine. 1995;19(2):227-50.
121. Patel AB, Srivastava S, Phadke RS, Govil G. Arginine activates glycolysis of goat epididymal spermatozoa: an NMR study. Biophysical journal 1998;75(3):1522-8.
122. Patel BR, Siddiquee GM. Effect of semen diluent additives on spermatozoal viability of Kankrej bull semen following cryopreservation. Wayamba J Anim Sci 2012, 377-80.
123. Perumal P, Vupru K, Khate K. Effect of addition of melatonin on the liquid storage (5 C) of mithun (bos frontalis) semen. International Journal of Zoology 2013.
124. Perumal P. Reduced glutathione and cysteine hydrochloride on crossbred bull semen. Nature Precedings 2012, 1-1.
125. Prathalingam NS, Holt WV, Revell SG, Mirczuk S, Fleck RA, Watson PF. Impact of antifreeze proteins and antifreeze glycoproteins on bovine sperm during freeze-thaw. Theriogenology 2006;66(8):1894-900.
126. Pratt WD, Murray FA, Conrad HR, Moxon AL, Kinder JE. Effects of selenium supplementation on bull sperm metabolism in vitro. Theriogenology 1980;13(5):369-79.
127. Quinn PJ. Principles of membrane stability and phase behavior under extreme conditions. Journal of bioenergetics and biomembranes 1989;21(1):3-19.
128. Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S *et al*. Oxidative stress, prooxidants, and antioxidants: the interplay. BioMed research international 2014.
129. Rao LG, Guns E, Rao AV. Lycopene: Its role in human health and disease. Agro Food 2003;7:25-30.
130. Raymond JA, Wilson P, DeVries AL. Inhibition of growth of nonbasal planes in ice by fish antifreezes. Proceedings of the National Academy of Sciences 1989; 86(3):881-5.
131. Renard P, Grizard G, Griveau JF, Sion B, Boucher D, Le Lannou D. Improvement of motility and fertilization potential of postthaw human sperm using glutamine. Cryobiology. 1996;33(3):311-9.
132. Roca J, Gil MA, Hernandez M, Parrilla I, Vazquez JM, Martinez EA. Survival and fertility of boar spermatozoa after freeze-thawing in extender supplemented with butylated hydroxytoluene. Journal of andrology 2004;25(3):397-405.
133. Saeed AM, El-Nagar HA, Wafa WM, Hussein YS. Effect of coenzyme Q10 as an antioxidant added to semen extender during cryopreservation of buffalo and cattle semen. Journal of Animal and Poultry Production 2016;7(11):403-8.
134. Sánchez-Gutiérrez M, García-Montalvo EA, Izquierdo-Vega JA, Del Razo LM. Effect of dietary selenium deficiency on the in vitro fertilizing ability of mice spermatozoa. Cell Biology and Toxicology 2008; 24(4):321-9.
135. Sanchez-Partida LG, Setchell BP, Maxwell WM. Effect of compatible solutes and diluent composition on the post-thaw motility of ram sperm. Reproduction, Fertility and Development 1998;10(4):347-58.
136. Saragusty J, Gacitua H, Rozenboim I, Arav A. Protective effects of iodixanol during bovine sperm cryopreservation. Theriogenology 2009;71(9):1425-1432.
137. Sarıözkan S, Tuncer PB, Büyükleblebici S, Bucak MN, Cantürk F, Eken A. Antioxidative effects of cysteamine, hyaluronan and fetuin on post-thaw semen quality, DNA integrity and oxidative stress parameters in the B rown S wiss bull. Andrologia 2015;47(2):138-47.
138. Sbracia M, Grasso J, Sayme N, Stronk J, Huszar G. Hyaluronic acid substantially increases the retention of motility in cryopreserved/thawed human spermatozoa. Human Reproduction (Oxford, England) 1997;12(9):1949-54.
139. Seeram NP, Aviram M, Zhang Y, Henning SM, Feng L, Dreher M *et al*. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. Journal of agricultural and food chemistry 2008;56(4):1415-22.
140. Shevchenko A, Simons K. Lipidomics: coming to grips with lipid diversity. Nature reviews Molecular cell biology 2010;11(8):593-8.
141. Silva EC, Cajueiro JF, Silva SV, Soares PC, Guerra MM. Effect of antioxidants resveratrol and quercetin on in vitro evaluation of frozen ram sperm. Theriogenology. 2012;77(8):1722-6.
142. Singh P, Agarwal S, Singh H, Verma PK, Pandey AK, Kumar S. Antioxidant Effects of Aloe vera as Semen Additive in Cryopreservation of Cattle Bull Semen. Int. J. Curr. Microbiol. App. Sci 2020;9(9):1625-35.
143. Singh P, Raina VS. Comparative effect of certain additives on preservation of buffalo bull semen at refrigeration temperature. Indian Journal of Animal Production and Management 1999;15(2):74-6.
144. Soren S, Singh SV, Kumar S. Effect of Astaxanthin Supplementation on Semen (Karan Fries Bulls) Storage at 5 C. International Journal of Current Microbiology and Applied Sciences 2017;6(5):23-8.
145. Sreejayan R, MN. Nitric oxide scavenging by curcuminoids. J. Pharm. Pharmacol 1997;49(1).
146. Srivastava S, Desai P, Coutinho E, Govil G. Protective effect of l-arginine against lipid peroxidation in goat epididymal spermatozoa. Physiological chemistry and physics and medical NMR 2000;32(2):127-35.
147. Storey BT, Noiles EE, Thompson KA. Comparison of glycerol, other polyols, trehalose, and raffinose to provide a defined cryoprotectant medium for mouse sperm cryopreservation. Cryobiology. 1998; 37(1):46-58.
148. Sun L, Zhang J, Fang K, Ding Y, Zhang L, Zhang Y. Flavonoids from persimmon (Diospyros kaki) leaves (FPL) attenuate H 2 O 2-induced apoptosis in MC3T3-E1 cells via the NF-κB pathway. Food & function

2014;5(3):471-9.
149. Suzuki K, Asano A, Eriksson B, Niwa K, Nagai T, Rodriguez-Martinez H. Capacitation status and in vitro fertility of boar spermatozoa: effects of seminal plasma, cumulus-oocyte-complexes-conditioned medium and hyaluronan. International journal of andrology 2002; 25(2):84-93.
150. Suzuki YJ, Tsuchiya M, Packer L. Thioctic acid and dihydrolipoic acid are novel antioxidants which interact with reactive oxygen species. Free radical research communications 1991;15(5):255-63.
151. Szeto HH, Birk AV. Serendipity and the discovery of novel compounds that restore mitochondrial plasticity. Clinical Pharmacology & Therapeutics 2014;96(6):672-83.
152. Szeto HH. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. British journal of pharmacology 2014; 171(8):2029-50.
153. Tarig AA, Wahid H, Rosnina Y, Yimer N, Goh YM, Baiee FH *et al*. Effect of different concentrations of soybean lecithin and virgin coconut oil in Tris-based extender on the quality of chilled and frozen-thawed bull semen. Veterinary World 2017;10(6):672.
154. Tezcan F, Gültekin-Özgüven M, Diken T, Özçelik B, Erim FB. Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food Chemistry 2009;115(3):873-7.
155. Thumar H, Patel M, Nakhashi, Haresh, Babulal, Suthar *et al*. Comparative efficacy of melatonin and trehalose additives on enzyme leakage during cryopreservation of kankrej bull semen. International Journal of Zoology. 2017;9:1-10.
156. Trimeche A, Anton M, Renard P, Gandemer G, Tainturier D. Quail egg yolk: a novel cryoprotectant for the freeze preservation of Poitou jackass sperm. Cryobiology 1997;34(4):385-93.
157. Tuncer PB, Bucak MN, Büyükleblebici S, Sarıözkan S, Yeni D, Eken A *et al* The effect of cysteine and glutathione on sperm and oxidative stress parameters of post-thawed bull semen. Cryobiology 2010;61(3):303-7.
158. Tuncer PB, Sarıözkan S, Bucak MN, Ulutaş PA, Akalın PP, Büyükleblebici S *et al*. Effect of glutamine and sugars after bull spermatozoa cryopreservation. Theriogenology 2011;75(8):1459-65.
159. Tvrdá E, Greifová H, Mackovich A, Hashim F, Lukáč N. Curcumin offers antioxidant protection to cryopreserved bovine semen. Czech Journal of Animal Science 2018; 63(7):247-55.
160. Tvrdá E, Kňažická Z, Bárdos L, Massányi P, Lukáč N. Impact of oxidative stress on male fertility—a review. Actaveterinariahungarica 2011;59(4):465-84.
161. Tvrda E, Mackovich A, Greifova H, Hashim F, Lukac N. Antioxidant effects of lycopene on bovine sperm survival and oxidative profile following cryopreservation. Veterinárnímedicína 2017;62(8):429-36.
162. Van de Lagemaat EE, De Groot LC, van den Heuvel EG. Vitamin B12 in relation to oxidative stress: a systematic review. Nutrients. 2019;11(2):482.
163. Van den Ende W, Valluru R. Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging?. Journal of experimental botany 2009;60(1):9-18.
164. Wang JH. A comprehensive evaluation of the effects and mechanisms of antifreeze proteins during low-temperature preservation. Cryobiology. 2000;41(1):1-9.
165. Wellington K, Jarvis B. Silymarin: a review of its clinical properties in the management of hepatic disorders. BioDrugs 2001;15(7):465-89.
166. Wendehenne D, Pugin A, Klessig DF, Durner J. Nitric oxide: comparative synthesis and signaling in animal and plant cells. Trends in plant science. 2001;6(4):177-83.
167. Xie C, Xie Z, Xu X, Yang D. Persimmon (Diospyros kaki L.) leaves: a review on traditional uses, phytochemistry and pharmacological properties. Journal of ethnopharmacology 2015;163:229-40.
168. Yovich JL. Pentoxifylline: actions and applications in assisted reproduction. Human reproduction 1993;8(11):1786-91.
169. Yung LY, Tsim ST, Wong YH. Stimulation of cAMP accumulation by the cloned Xenopus melatonin receptor through Gi and Gz proteins. FEBS letters 1995;372(1):99-102.
170. Zaghdoudi K, Pontvianne S, Framboisier X, Achard M, Kudaibergenova R, Ayadi-Trabelsi M *et al*. Accelerated solvent extraction of carotenoids from: Tunisian Kaki (Diospyros kaki L.), peach (Prunus persica L.) and apricot (Prunus armeniaca L.). Food chemistry. 2015;184:131-9.
171. Zhang JG, Nicholls-Grzemski FA, Tirmenstein MA, Fariss MW. Vitamin E succinate protects hepatocytes against the toxic effect of reactive oxygen species generated at mitochondrial complexes I and III by alkylating agents. Chemico-biological interactions 2001;138(3):267-84.
172. Zhou C, Sheng Y, Zhao D, Wang Z, Tao J. Variation of oleanolic and ursolic acid in the flesh of persimmon fruit among different cultivars. Molecules. 2010;15(9):6580-7