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Effect and Strategies to Mitigate the Heat Stress on Buffalo Bull Reproduction

J. Arunpandian[1], Neeraj Srivastava[1*], Gynendra Singh[2], Shashikant Gupta[1], Anju Kujur[1], G. Aswini Sivan[1], and Amala Jackson[1]

[1]Division of Animal Reproduction, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh-243 122
[2]Division of Physiology and Climatology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh-243 122

ABSTRACT

Heat stress (HS) is hazardous to the physiological functioning of reproductive organs, as well as for the optimum production of spermatozoa quality and quantity. During the summer animals suffer from HS due to an elevated environmental temperature causing increased testicular temperature resulting in accelerated free radicals generation. The mammalian spermatozoa are more vulnerable to free radical damage primarily because the sperm membrane contains cholesterol, sterols and polyunsaturated fatty acids. Certain levels of reactive oxygen species (ROS) are required for variety of physiological processes such as acrosome reaction, capacitation and fertilization. On the other hand, when the ROS levels are elevated, pathological condition are produced with the formation of lipid peroxidase, affecting the sperm membrane integrity and leads to leakage of intracellular contents. This is followed by abolished structural integrity, sperm motility and viability. As a result, during the summer, the volume of semen produced is low, with poor seminal characteristics. There are several methods to mitigate the HS of the animal such as physically constructing a shade, sprinkler and cooling fan, which facilitates conducive environment around the animals and help to reduce the heat stress. Moreover, certain semen additives can also be added to minimize the detrimental effects of HS *post facto*. The present review elucidates mechanism of HS genesis, changes occurring in spermatozoa morphology and amelioration of damaging effects of HS by application of various protocols.
Key words: Spermatozoa, ROS, lipid per oxidation, heat stress, free radicals.

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INTRODUCTION

Recent data reports (DAHDF, Govt. of India, 2019-2020) India has 109.8 million buffaloes accounting for approximately 57.7 percent of global buffalo output. In India, buffalo contributes the half of the total milk production accounting for 91.82 million tons of milk in 2019. However, the effort to get maximum output from buffaloes is hampered by continuous climate change and resulting heat stress. Global warming and its consequences, such as climate change, are a universally acknowledged truth (Rahman *et al.,* 2011). The HS in turn affects genetic

*Corresponding author.
E-mail address: sangnee15@gmail.com (Neeraj Srivastava)
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potential, management and environment, influencing the bull's reproductive performance. Of all livestock species, buffaloes are particularly vulnerable to the HS due to various intrinsic constraints, such as lesser number of sweat glands and skin colour. Therefore, HS in buffaloes needs scientific management to attain optimum production efficiency. Heat stress is an outcome of exposure to elevated temperature and humidity interfering the oxidative metabolism of glucose, mitochondrial function and abundant formation of ROS in spermatic cells resulting in increased lipid per oxidation and spermatozoa abnormality (Nichi, 2006). In India during summer the temperature variation is 30 °C to 46°C whereas annual rainfall is 760 to 960 mm which mostly peak during the month of July and August, with the relative humidity ranging from 45-99 % (Bhakat *et al.,* 2015).

The bull fertility is assessed by employing tools such as conception rate (CR) by inseminating with viable spermatozoa in females Reports suggest that spermatozoa production in buffalo bulls is affected by heat stress. Thus, the present review elaborates the changes occur in male reproductive system such like as spermatogenesis, biophysical seminal characteristic, and fertility following heat stress, and various management tools and semen additives as a means to ameliorate the heat stress. Throughout the review, comparative studies with cattle bulls are quoted to drive home the points.

BUFFALO REPRODUCTIVE BIOLOGY

Buffalo is contemplated as a seasonal breeder with more efficient breeding activated in winter and autumn season as compared to other seasons. Due to certain natural restrictions, buffalo bull breeding capacity is limited as compared with cattle bulls, such as small testicular size, lowest spermatozoa output rate and low epididymal reserve. Moreover, the buffalo spermatozoa are more susceptible to HS and to free radical damage due to inadequate amount of cytoplasmic antioxidant granules and rich content of polyunsaturated fatty acid in their spermatozoa membrane. This is primarily due to low quantity of cholesterol and large head size compared to spermatozoa of other domestic animals (Kumar *et al.,* 2019).

EFFECT OF HEAT STRESS ON REPRODUCTION

The buffalo bulls exposed to high ambient temperature for several week show poor quality of semen and fertility (Collier *et al.,* 2017). Temperature and relative humidity are typically considered the causal agents for heat stress. Both hot-dry and hot-humid seasons have been reported to be unfavourable for both productivity and reproduction (Bhakat *et al.,* 2015). The air temperature, solar radiation, relative humidity, air flow and their interactions, are the climatic factors which often restrain the animal performance. Heat stress is the state at which the physiological mechanisms activated to maintain animal's body thermal balance, fails when exposed to elevated temperature. When HS is combined with excessive ambient humidity, the effects of HS are amplified (Marai *et al.,* 2008). The summer impact on bull fertility act multi-dimensionally through the diminished feed intake and inadequate release of hormones such as GnRH, FSH and LH required for reproduction. The temperature is raised during summer, thus testicular temperature is elevated resulting in hypoxia and subsequently production of ROS as metabolic by-products. Also, increased respiratory rate and depth to maintain the thermoregulatory system, resulting in a rise in alkalosis, insufficient oxygen supply and greatly increase ROS production is observed. During heat stress, activation of heat shock transcription factors (HSF1) takes place, which enhances the production of the heat shock protein with the objective of abolishing detrimental effects of heat overload (Collier *et al.,* 2008). Due to sustained prolonged heat stress, production of heat shock protein is altered and an amplified production of ROS is the result (Rhoads *et al.,* 2013). Such increased free radicals production results in lipid per oxidation and damaged DNA, establishing denaturation of proteins and subsequently enhanced apoptosis. Damage to spermatozoa DNA interferes with the protamination and impairs chromatin remodelling; both are linked with poor fertilization and embryo development. Moreover, due to sustained heat exposure the sperm antioxidant system has lesser than normal efficiency (Alam *et al.,* 2015).

The degree of oxidative damage of spermatozoa is mainly dependent on the environment and intensity of stress inducing factors, originating from either endogenous or exogenous sources. The levels of free radicals above physiological limits causes disruption of sperm membrane, which is already, predisposed to damage due to large number of sterols, phospholipids and poly unsaturated fatty acids. Overloaded oxidative stress has a deleterious impact on spermatogenesis, resulting in sperm membrane and DNA damage, as well as a decrease in the per cent hypo-osmotic positive spermatozoa (Sharma *et al.,* 2017). The ROS strikes the DNA bases (particularly guanine) and phosphodiester backbones that consequently destabilizes the molecule and create cellular conditions that ultimately result in DNA fragmentation. The seminal characters are altered by hot and humid environment, which has an impact not only on reproduction and libido, but also on the biophysical state of semen in the fresh and frozen stages (Vale, 2007).

Spermatogenesis

The spermatogenesis a continuous process occurring in seminiferous tubules and consist of two phases, spermatocytogenesis and spermiogenesis. In elevated temperature conditions, the pachytene and diplotene stage of spermatozoa and immature spermatid are particularly vulnerable in bovines (Rahman *et al.,* 2011). High temperatures generally have an inverse effect on spermatogenesis and sperm transformation, resulting in semen degeneration. Summer sterility is induced by seasonal variations, with spermatogenesis being restricted in the summer. The meiotic phase is most vulnerable phase to heat stress. Sustained elevated temperature leads to scrotal hyperthermia, resulting in production of great amount of reactive oxygen species affecting the mitochondrial formation and function, with negative impact on the sperm motility and viability in rams (Kastelic *et al.,* 2017) and bulls (Rahman *et al.,* 2011). Heat stress is thought to cause a misalignment of the mitochondrial sheath or a fracture in the spermatozoon tail's axonemal fibres during the growth and migration of spermatids elongated by rete testis (Luno *et al.,* 2020). Heat stress in the epididymal environment can cause spermatozoa to self-regulate their volume, resulting in changes in tail bending. The maximum expression of cytoplasmic droplets occurred between days 14 and 28 after heat stress, implying that Sertoli cells were similarly affected (Gonçalves *et al.,* 2021). These head and acrosome defects resulted from the action of hyperthermia in the basal layer of seminiferous tubules where spermatocytes were in meiosis II or spermatogonia were in mitotic division. Interruptions in acrosome biogenesis during spermatocyte meiosis and during spermatogonia mitosis may be related to the labelling of some methylated histones, therefore ensuing a stoppage in the development of the acrosome structure during spermatogenesis. The elevated environmental temperature besides long sunshine duration raise the testicular temperature which trigger the greatest amount of reactive oxygen species generation impairing the spermatogenesis and finally resulting in the lesser amount of sperm concentration (Silva *et al.,* 2018). Though different stages of spermatocytes are present during spermatogenesis, certain stages are susceptible to Heat stress producing abnormalities in spermatozoa.

Seminal plasma biochemical composition

The aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), amylase, total cholesterol and triglycerides present in seminal plasma are good indicators for sperm quality. The activities of ALT, AST, and ALP were shown to be much higher during the wet summer season as compared to other seasons. The transaminase enzyme like ALT and AST increased in summer months due to HS and are important factors for evaluating the sperm membrane and acrosome integrity. The elevated abnormal spermatozoa reported in wet summer are due to increased leakage of transaminase enzyme from damaged sperm membrane (Gundogan, 2006). The ALT is good indicator for evaluating the sperm functional status, membrane activity of spermatozoa and sperm membrane integrity. During HS the amount of ALP increased due to release of ACTH. The role of cholesterol is linked with structure of sperm membrane, spermatozoa metabolism, sperm capacitation and gamete fertilization. During summer months, it was reported that seminal plasma gets enriched with total cholesterol (Pandey *et al.,* 2017). Summer had lower levels of phosphorylcholine binding proteins than winter or rainy season, but there were no variations in heparin-binding proteins. At least in boars, the ratio of these two proteins is thought to be associated to fertility (Hansen *et al.,* 2013).

Sexual behavioural characters

The sexual behavioural characters of bull are temperament score, libido score, erection score, protrusion score, intensity of thrust, reaction time, total time taken to successful ejaculation and flehmen's reaction. In the summer months animal get physically exhausted due to HS thereby failing to show eagerness and libido with greater reaction time, with total time for successful ejaculation negatively impacting sperm production (Marai and Haeeb, 2010). The level of testosterone and thyroxine is reduced during the summer month's further affecting spermatogenesis (Mandal *et al.,* 2005).

Endocrine profile

The semen quality and quantity reflect the intensity of reproductive organs such like as testis, epididymis and accessory sex glands. Due to environmental changes in summer months the release of hormone GnRH is altered affecting the release of hormones controlling the spermatogenesis. During heat stress, the secretion of ACTH increases while the secretion of GnRH is reduced obstructing the release of the LH, which play crucial role in spermatogenesis (Bhakat *et al.,* 2015). Moreover, the HS alters the concentration of LH even in the absence of steroids. The level of thyroxin hormone decreases at HS with negative effect on metabolism and on appetite impairing the reproduction function. Also, the testosterone concentration is decreased during HS initially and remains low for two weeks but re-establishes later even in sustained heat stress.

TEMPERATURE HUMIDITY INDEX (THI)

The THI is a prominent indication of HS in animals to assess the performance of animals raised in tropical and subtropical climates. The environmental factors influence the sperm quality and quantity such as temperature and humidity. For optimum spermatogenesis the required air temperature is 13-18^{0}C and relative humidity of 55-65% with medium sunshine level (Table 1). Disruption in spermatogenesis occurs by increased testicular temperature either by raised environmental temperature or increased body temperature or both (Sharma *et al.,* 2017). Temperature, relative humidity (RH), sun radiation, air movement, and precipitation all contribute to heat stress (Fig 1). Temperature and relative humidity are employed in THI models to determine the degree of HS on an animal's reproductive features (Yousef, 1985).

Table 1: Classification of zone based on THI value (Amstrong, 1994)

S.no	THI (%)	Stress level	Symptoms in buffalo
1	<72	None	Normal in production
2	72-78	Mild	Respiratory and rectal temperature increased
3	79-88	Moderate	Water intake increased, food intake decreased, and rate of respiration significantly increased
4	89-98	severe	Panting and restlessness Rumination and urination decreased
5	>98	danger	Buffalo may die

TOOLS TO ALLEVIATE HEAT STRESS

The HS in animals adversely affects production which causes greatest economic losses for farmers. There are several methods to mitigate the HS in animal such as physical modification of environment, genetic development of heat tolerant breeds, nutritional management and semen additives to *post facto* mitigate the damage. Management measures to alleviate HS may be more or less effective depending on the environment, for example evaporative cooling is more effective at alleviating HS in dry condition than humid ones (Bohmanova *et al.,* 2007).

Physical modifications of environment

Commonly altering the environmental temperature comprises of building of shade and evaporating cooling techniques. The provision of shades which diminish heat stress, and prevent the solar radiation may either directly or indirectly minimize heat stress. Shades in the corrals must be between 3.6 and 4.2 meters high to ensure that solar radiation is reduced. It has been proven that shading reduces the incoming radiant heat load by 30% or more, and shading of the feed and water benefits animal productivity (Slimen *et al.,* 2014). The cooling systems reduce the heat burden on livestock by combining water misting and forced ventilation through the use of spray and fans and are widely used within free-stall barns or under shade in open area corrals. Fine droplets of water are spread into the air stream via fast evaporation and cool the surrounding environment in fogging and misting systems. The dairy fans and mist cooling along with rubber mat flooring can be utilized to ameliorate the HS in the buffalo bulls (Chikkagoudara *et al.,* 2020).

Genetic selection of heat tolerant breeds

Selection of the heat resistant animals is useful if selected stocks are able to maintain productivity and survivability following exposure to summer stress conditions. The cattle with longer hairs and darker colour of skin coat might be less adapted to hot environment. Conversely, cattle that has hair with greater diameter and short hair and light coat colour is more adapted to hot environment (Bernabucci *et al.,* 2010). The heat shock protein (HSP) genes have been used as markers for selection because of its association with thermo-tolerant capacity. The heat resistant genes in various breed include the HSF1 gene, HSP70 A1 gene and HSBP1 in Chinese Holstein cattle (Wang *et al.,* 2013) and HSP90AB1, in Thai native cattle (Deb *et al.,* 2014). Before considering the HSP as a marker for thermo-tolerant characteristic it requires further elucidative experiments.

Nutritional management

Environmental temperatures are highest in arid and semi-arid locations, where available feed resources are both low in quality and quantity, with negative impact on livestock reproductive success. Combating the metabolic effects

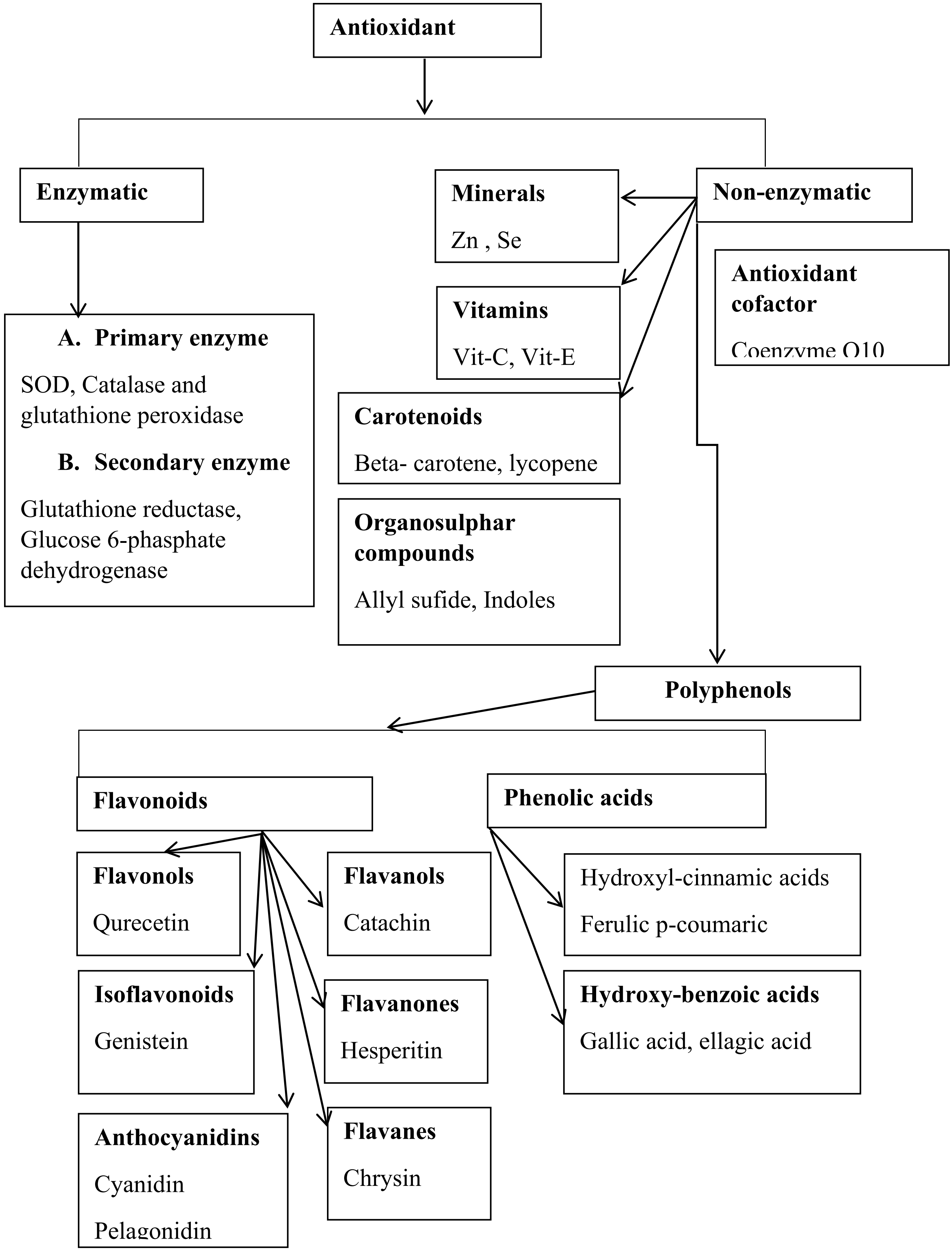

Fig. 1: Classification of antioxidants used as additives in semen

of HS is consequently critical, as animals suffering from mild to severe HS require an additional 7–25% maintenance requirement. The supplementation of selenium and vitamin E help by mitigating the detrimental effect of free radicals due to their antioxidant properties. The semen quality in bulls showed a positive effect by vitamin E injection under testicular HS conditions (Losano *et al.,* 2017). The supplementation of fatty acid in feed resulted in improvement in progressive motility percentage; the possible reason forwarded was that the fatty acids might have contributed towards requirement of energy during hot and humid environment (Butt *et al.,* 2019).

Additives in semen extender

Heat stress alters the normal physiology of reproductive system, and semen parameters such as semen volume, motility, concentration and abnormal spermatozoa and finally infertility. Heat stress alters spermatozoa morphology producing greater number of abnormal and dead spermatozoa. During the summer, the testicular temperature is elevated, increasing metabolic rate and requirement of oxygen (Gadea *et al.,* 2004). If intensified metabolism is not followed by enhanced blood flow, testicle tissue becomes hypoxic, resulting in excessive ROS production and lipid peroxidation (Sharma *et al.,* 2017).

The methods to reducing the ROS can be classified into two ways namely, 1) neutralization of ROS, 2) reduction in the production of ROS by removal of dead and damaged spermatozoa (Bisla *et al.,* 2020a; 2020b; Bisla *et al.*, 2021; Bisla *et al.*, 2022; Rautela *et al.*, 2022), 3) by targeted reduction in production of ROS using specific scavengers (Ngou *et al.*, 2020; Kumar *et al.*, 2021; 2022). The use of antioxidants and reductants coupled with (H_2 gassing) can neutralize the ROS (Kumar *et al.,* 2019). The classification of the methods to reduce the source of production of ROS are, 1) Through minimizing O_2 tension, 2) By methods of semen purification to remove the immature, dead and defective spermatozoa (Glass wool Sephadex (GSW), magnetic bead separation, filtration, Ficoll wash and nano particle separation) 3) Through reduction of mechanical stressors, white blood cell counts in semen and radiation exposure. They have been dealt with briefly as below

Antioxidants

Antioxidants neutralize the ROS and are generally used for their property of scavenging and suppressing the formation of ROS. (Sharma *et al.*, 2017) obtained high number of dead and abnormal spermatozoa during elevated THI (Temperature and Humidity Index). Dead spermatozoa play important role in generation of oxidative stress, and are prime source of ROS. Antioxidants are the agents which can reduce the oxidative stress by breaking the oxidative chain (Pande and Srivastava, 2017). Antioxidants can be classified into two groups: enzymatic and non-enzymatic antioxidants (Fig 2).

Enzymatic antioxidants

The enzymatic antioxidants are naturally occurring, neutralize the ROS and prevent the further injury of the cell structure of spermatozoa. Superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) are the enzymatic antioxidant. SOD is used for spontaneous oxygen toxicity and LPO.Supplementation with butylated hydroxytoluene, an antioxidant reduced the cold shock after cooling and freezing stage in boar spermatozoa (Bamba and Cran, 1988). Glutathione is one of the crucial antioxidant that reduces the oxidative damage to bull spermatozoa. The antioxidants such as glutathione and cysteine repair the spermatozoa damaged by hydrogen peroxide mediated radicals in frozen thawed bull semen (Bilodeau *et al.,* 2001).Since polyunsaturated fatty acid metabolic substances play an important role in the acrosome reaction of spermatozoa (Tripodi *et al.,* 2003). Supplementation of antioxidants seems to reduce the frequency of acrosomal defects during semen preservation (Sarlos *et al.,* 2002). The Semen purification method using nanotechnological approach is one of the most efficient methods to remove the dead spermatozoa from the ejaculates (Bisla *et al.,* 2020a; 2020b; Bisla *et al.*, 2021; Bisla *et al.*, 2022; Rautela *et al.*, 2022).

Non enzymatic antioxidants

Vitamin E is a powerful chain-breaking lipophilic antioxidant found on cell membranes that may break the covalent bonds generated by reactive oxygen species between fatty acid side chains in membrane lipids. It is one of the most important membrane protective agents against reactive oxygen species and lipid peroxidation without affecting ROS formation (Sharma and Agarwal 1996). Both *in vivo* and *in vitro*, a-tocopherol can protect cells from oxidative damage. Glutathione peroxidase (GSH-Px) converts hydrogen peroxide to water and lipoperoxides to alkyl alcohols by using GSH. The glutathione reductase (GSR), whose activity is induced by oxidative stress, may regenerate GSH from its oxidised state (GSSG) (Gadea *et al.,* 2004). Vitamin C is a water-soluble, high-potency chain-breaking antioxidant with minimal toxicity. It may scavenge oxygen radicals. It decreases lipid peroxidation by neutralising the effects of H_2O_2 on DNA (Srivastava and Pande, 2017), and recycling inactive vitamin E (Sierens *et al*., 2002). The extender supplemented with ascorbic acid improved bovine sperm motility, acrosome and membrane integrity and also decrease in GSH-Px in contrast to an increase in GSH (Hu *et al*., 2010) was observed. L-cysteine is a thiol-containing nonessential amino acid with a low molecular weight. Both *in vitro* and *in vivo*, it easily crosses the cell membrane and engage in intracellular GSH production. The detoxification of free radicals through activation of glutathione peroxidase, a critical enzyme in the defence against oxidative stress, and action of selenium as a cofactor of glutathione synthetase are two of selenium's functions in biological systems. Selenium in the form of selenite detoxifies the medium, preventing oxidative damage to cells (Zhang *et al*., 2006).

CONCLUSIONS

The livestock are adversely affected with HS due to climatic changes like increased temperature and relative humidity which has drastic impact on production as well as reproduction. The document elucidated the mechanism of HS and impact of HS upon male reproduction with changes occurs during HS in various semen parameters. The article summarizes the strategies commonly employed such as management, nutrition and supplementation of antioxidants to reduce the HS in the bull.

CONFLICT OF INTEREST

None.

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