



## Global Warming: Impact, Adaptation and Ameliorative Measures of Semen Quality under Tropical Climatic Conditions in Crossbred Bulls

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### ABSTRACT

Provision of good semen quality is necessary throughout the year for sustainable dairy development. The aim of rearing crossbred cattle is to enhance the productivity. However, heat stress, which is common to tropical countries, influences their productivity. The semen quality of crossbred bulls declines under heat stress. The thermoregulatory mechanism of testes is affected under higher ambient temperature, which in turn enhances reactive oxygen species (ROS) production. ROS attack causes lipid peroxidation of sperm membrane, which results in reduced sperm motility. The spermatogenesis process and testosterone production are affected under heat stress. The quality of semen is affected significantly under stressful conditions, which affects the growth of dairy sector. Poor semen quality contributes to the failure of artificial insemination and conception. Some of the sperm defect cannot be evaluated through routine examination, *viz.*, the genetic/or molecular defects. The management of bulls under summer stress is necessary for production of good quality semen. This review aims to focus on examination of semen quality based on physical, biochemical and heat shock protein expression under tropical climatic conditions

**Keywords:** Semen quality, Crossbred bulls, Heat stress, ROS, HSPs.

Bulls are considered as the half of the herd and selected at an early age as future breeding bulls. However, a considerable number of male animals are culled at various stages of life due to limited growth and reproductive potentialities. The subfertility problems in bulls, like poor libido and poor seminal profile, may be accredited due to genetic, environmental and managerial causes. The reproductive performance of bulls is influenced significantly by environment (Mukhopadhyay *et al.*, 2010). The macro and microclimatic factors influence the bull's performances. Tropical climatic conditions (summer stress) exert comparatively more adverse effects on the overall quality of semen of crossbred bulls (Soren *et al.*, 2018). The heat stress triggers the release of corticosteroids, which hinders the release of LH, important for spermatogenesis (Breen *et al.*, 2007). Heat stress reduces the eagerness of bulls, increases the ejaculation time and declines the

quality of semen in crossbred bulls (Mandal *et al.*, 2000). The demand of the present scenario is food security. It strongly directs for improvement of dairy sector and its sustainable growth as well. The contribution towards milk production by crossbred dairy cattle is significant. However, global warming can limit the growth of dairy sector considerably in tropical countries. The failures in artificial insemination and conception rate during summer may not only contribute by female animals but male factor also. The quality of sperm compromised under hot and humid condition. Under elevated air temperature or hot climatic conditions, the thermoregulatory mechanism may be affected significantly. The suitable spermatogenesis requires a lower testicular temperature than body temperature in most of the mammals. The lower testicular temperature is physiologically maintained by counter-current mechanism as well as evaporation through scrotal

sweat gland. The quality of the semen plays a vital role in development of dairy sector.

### Global warming

The accumulation of greenhouse gases increases the trapping of heat and cause global warming. It is a fact (IPPC, 2007). The increase of temperature is one of the major threats to animals' productivity (Singh *et al.*, 2018). The high productive animals are very prone to heat stress due to their high metabolic heat. Animal's compromise their metabolism under high ambient temperature resulting in reduced productive performances. Global warming is likely to affect the food productivity and its sustainability. The increase of temperature by 0.2°C per decade and global average surface temperature would be between 1.8 and 4.0°C by 2100 (IPCC, 2007). Many parts of India reported negative impact of climate change on productivity of farm animals due to rise in temperature (Upadhyay *et al.*, 2008). The livestock sector is one of the major contributors in Indian agriculture. Livestock sector is the sufferer of global warming but also contributor of greenhouse gases (GHGs). It contributes up to 18% of the global greenhouse gas (GHG) emissions (Thornton and Herrero, 2010). About one third is reported to be due to land use change associated with livestock production, another one third is nitrous oxide from manure and slurry management, and roughly 25% is attributed to methane emissions from ruminant digestion (Thornton and Herrero, 2010). However, it plays a vital role in Indian economy contributing 40% to the agricultural GDP. Singh *et al.* (2018) reviewed the adverse effect of heat stress on sustainable development of dairy sector. Heat stress due to global warming can severely affect the milk production, growth and reproductive capability of dairy animals (Singh *et al.*, 2018).

### Testicular thermoregulatory mechanism

It is well known that the mammals maintain their testicular temperature below 2-6° C than their body temperature. It is necessary for suitable spermatogenesis. When the testicular thermoregulatory mechanism is disturbed, the spermatogenesis process is affected, resulting in increased sperm abnormalities. The pampiniform plexus, the network of small veins and arteries, maintains the testicular temperature through counter-current heat

exchange mechanism. The arteries which brings the blood from the body having high temperature (38.5-39.0 °C) than the blood (venous blood) coming from testes (32-34° C). At the network of veins and arteries, counter current heat exchange mechanism takes place where, the heat of arterial blood moves to venous blood and becomes cooler before entering to testes. The scrotum being rich in sweat glands; thus the evaporative mechanism is also active in regulating the testicular temperature.

### Heat stress estimation

The severity of heat stress can be measured by several formulas based on the environmental variables (ambient temperature, humidity, dry and wet bulb temperature). One of the widely used formula for estimating temperature humidity index (THI) is  $THI = 0.72(W + D) + 40.6$ , formula, where 'W' is wet bulb and 'D' is dry bulb temperature in °C (McDowell, 1972). When the THI reach above 90, this is considered as severe stress. The comfortable THI value for most farm animals ranges 65 to 72 (Upadhyay *et al.*, 2009). Values above these are an indicative alarm of heat stress to animal production.

### Physiological responses

Physiological responses to any adverse conditions are to adapt the animals to that particular environment. The elevation of rectal temperature, respiration, pulse rate, skin blood flow and other adaptive mechanisms are essential for maintaining the physiology of the animals. Singh *et al.* (2018) reviewed the compromised performances of crossbred cattle under heat stress. The reduction of dry matter intake (DMI) under heat stress is to reduce the internal heat production. Conrad (1985) recorded 10-35% reduction of feed intake at 35°C ambient temperature. The feed intake of cows can further decrease in higher ambient temperature. Mallonee *et al.* (1985) recorded reduction of 56% in outdoor cows during hot weather. The reduction of metabolic hormones i.e.,  $T_4$  and  $T_3$  causes decrease in metabolism. The level of the adrenaline and nor adrenaline increases significantly in higher temperature or any other stressful condition. Reduction of gastro intestinal motility, decrease in rate of ingesta passage etc. also occurs. Aldosterone is also affected due to thermal stress resulting in reduced sodium reabsorption followed by electrolytes imbalance. All these changes are necessary to make the animal adaptive to the challenged conditions.

**Stress indicator hormone (cortisol)**

The heat stress activate the hypothalamo-pituitary adrenal axis resulted in increase secretion of cortisol. The increased level of cortisol under stressful condition is a physiological adjustment to cope (Afsal *et al.*, 2018). Several studies revealed the rise of plasma cortisol significantly when animals were exposed to heat (Maibam *et al.*, 2014). The high level of cortisol suppresses the immune system of the body that makes animal prone to diseases.

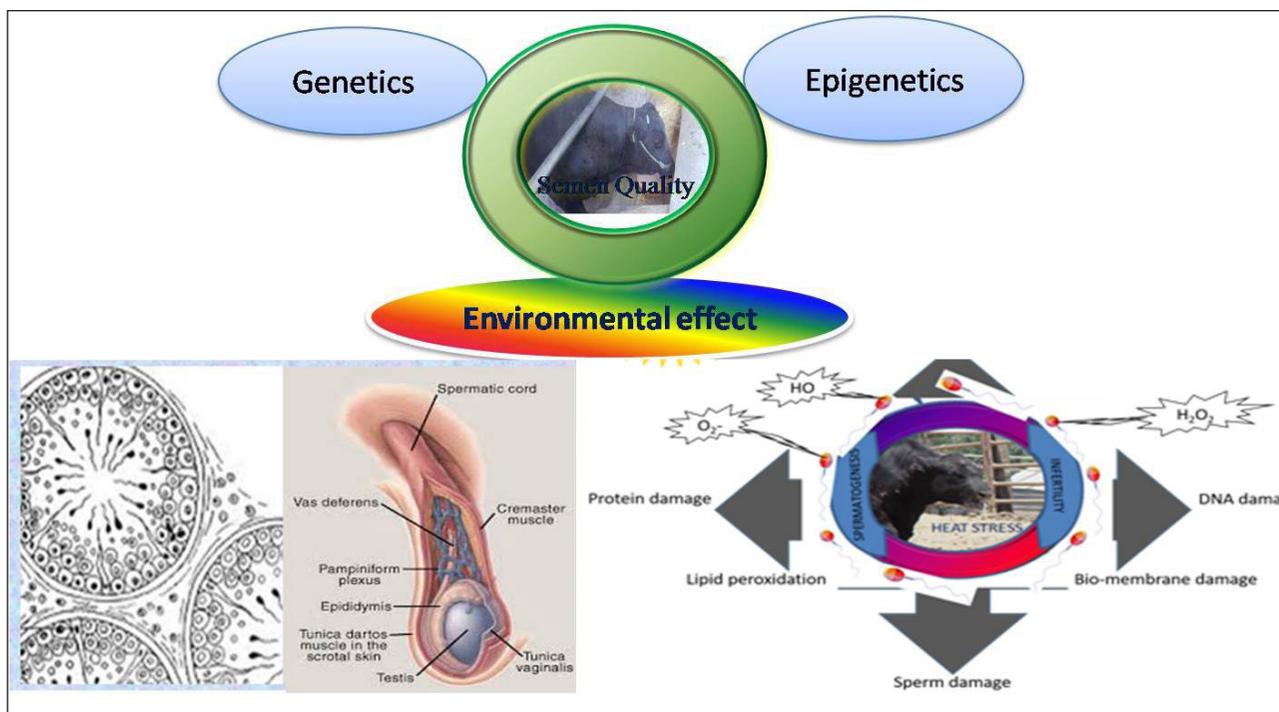
**Semen quality**

The quality of semen depends on the many factors mainly genetics, epigenetic and the environmental factors. Fig. 1 represents the possible effect of the environment (heat stress) on sperm production, thermoregulatory mechanism of testicle, adaptive mechanism and molecular events.

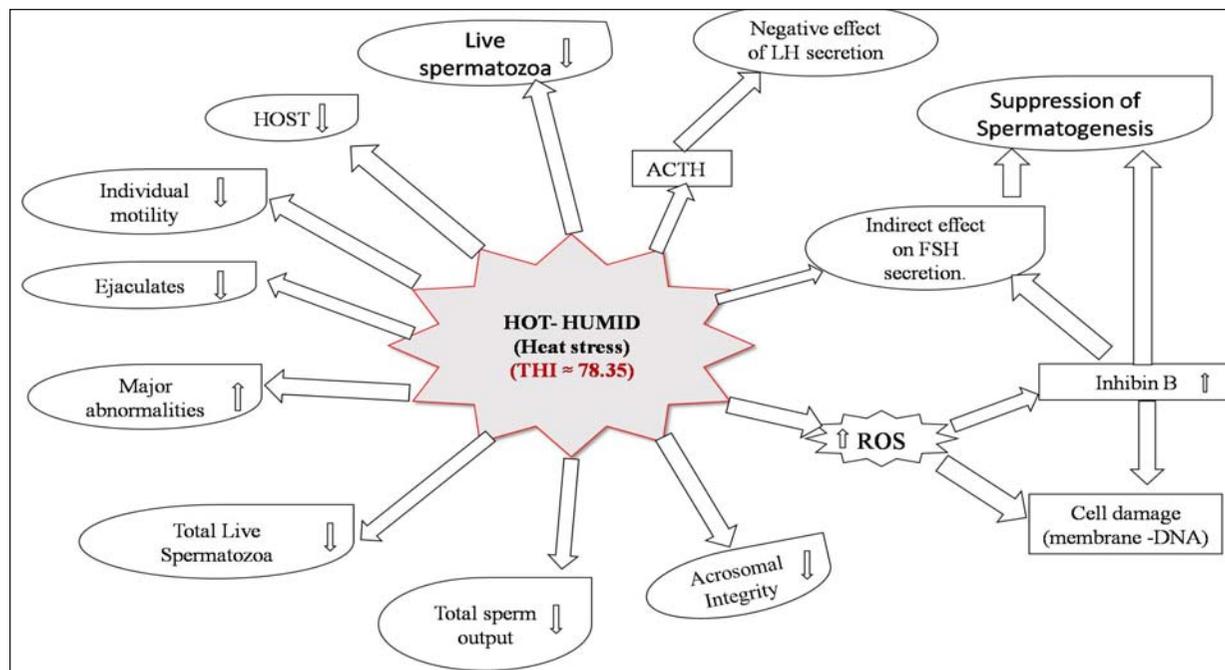
**Heat stress on physical parameters of semen**

The maintenance of testicular temperature is necessary for optimum production of morphologically normal and

fertile spermatozoa. The embryonic death during summer may also be contributed from the quality of semen affected by the heat stress (Das *et al.*, 2016). A little emphasis has also been given on bull management and its improvement under heat stress. The higher sperm abnormalities and reduced sperm output were observed during summer season in crossbred bulls (Soren *et al.*, 2017a). The elevated testicular temperature decreased the viability and acrosome integrity of spermatozoa. The percentage of live spermatozoa with intact acrosome decreased during hot season (Mandal *et al.*, 2000). The optimum ambient temperature for spermatogenesis is around 5 to 15°C (Fuerst-Waltl *et al.*, 2006). The higher percentage of sperm abnormalities is one of the important indicators of disturbed spermatogenesis. Kumar *et al.* (2016) found less number of hypo-osmotically swelled spermatozoa in ejaculates of crossbred Karan Fries bulls as compared to ejaculates of Sahiwal bulls of similar mass activities, and proposed that the semen parameters of crossbred bulls are more vulnerable to heat stress as compared to zebu bulls. Bhakat *et al.* (2014) observed the significant effects



**Fig. 1:** The possible events, which can compromise the semen quality of crossbred bulls under environmental factors (heat stress). Some diagrams were adapted from internet sources (Soren, 2015).



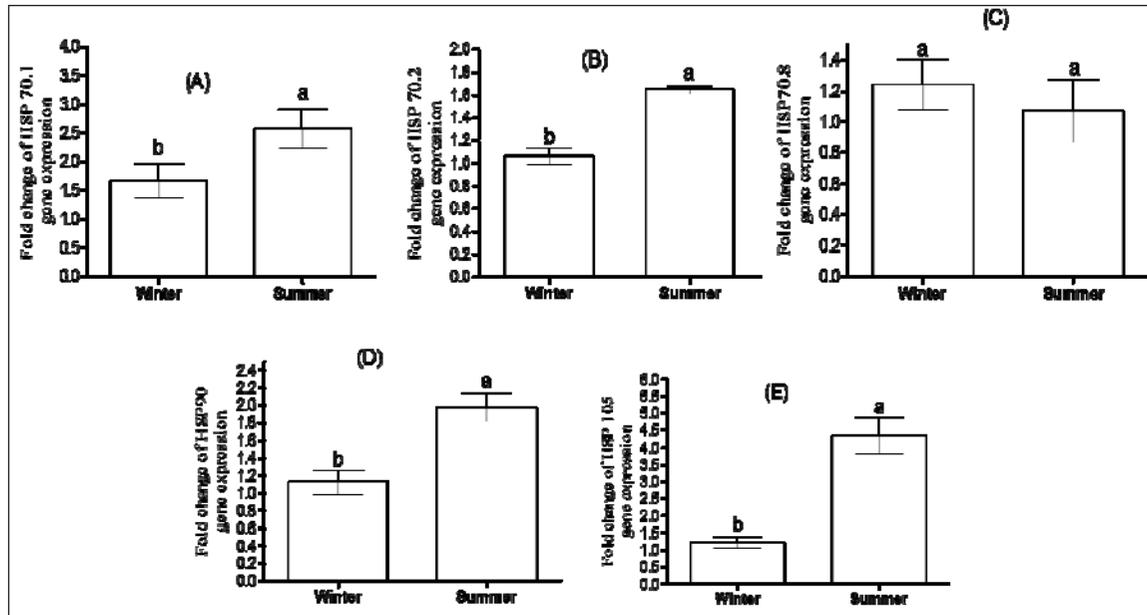
**Fig. 2.** The functional parameters of semen affected during hot humid season under tropical climatic condition and other associated factors considerable affect the spermatogenesis process (Soren, 2015)

of heat stress on initial motility, non-eosinophilic count, acrosome integrity, HOST, sperm concentration, sperm abnormalities and osmolality of semen in crossbred Karan Fries bulls. The functional parameters of semen affected significantly at THI 78 in crossbred bulls represented in Fig. 2 (Soren, 2015).

### Lipid peroxidation and semen quality

The elevation of ambient temperature influences the production of reactive oxygen species (ROS) or free radicals i.e. atom or molecules with one or more unpaired electrons. When the concentration of free radicals becomes higher than physiological level can detrimental to cell metabolism. It damages the biological structure of DNA, lipids, carbohydrates and proteins (Agarwal *et al.*, 2014). The hydroxyl radical ( $\text{OH}^\cdot$ ), the superoxide anion ( $\text{O}_2^\cdot$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), are produced endogenously through cellular pathways of the mitochondria. It is necessary to maintain the balance between antioxidant enzymes and ROS production for normal metabolism of sperm cells. The mammalian sperm membrane is rich in phospholipids, sterols and polyunsaturated fatty acids. The higher contents of phospholipids and polyunsaturated

fatty acids make them more susceptible to free radical attack. Mild amount of lipid peroxidation is said to be necessary for sperm functions. For neutralization of higher concentration of free radicals, the sperm membrane has to be dependent on antioxidant levels in the seminal plasma (Agarwal *et al.*, 2014). The cellular defence system (enzymatic and non-enzymatic antioxidant) in seminal plasma counteracts the ROS produced from the damage of sperm membrane. Lipid peroxidation resulted in the loss of fluidity of sperm membrane and compromise fertilizing ability of sperm. A balance has to be maintained between ROS production and the antioxidant activity. When the ROS concentrations increase continuously, lead to impairment of spermatozoa functions (Agarwal *et al.*, 2014). Spermatogenesis is the highly active replicative process producing approximately 1000 sperm per second (Aitken and Roman, 2008). It demands high rate of mitochondrial oxygen consumption by germinal epithelium. Therefore, spermatozoa are always vulnerable to ROS attack. The higher concentration of antioxidant enzymes were estimated during summer season in the semen of Karan Fires bulls, indicating higher ROS production (Soren *et al.*, 2016a; Soren *et al.*, 2018). The higher concentration of



**Fig. 3:** Variation in the fold change of HSP70.1 (A), HSP70.2 (B), HSP70.8 (C), HSP90 (D) and HSP105 (E) during winter and summer in spermatozoa of Karan Fries bulls under tropical climate (Soren *et al.*, 2018)

ROS correlated with low fertility (Agarwal *et al.*, 2014). The neutralization of ROS is necessary to maintain good quality semen. The routine analysis of semen doesn't incorporate ROS estimation or antioxidant enzymes assay. The reduction of antioxidant activity in semen plasma was found to have strong significance. The activities of glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase were higher in semen of poor quality during non-breeding season, which might be an indicative of protective mechanism to maintain the sperm quality under adverse conditions (Cardozo *et al.*, 2006). The higher lipid peroxidation was estimated during the summer season compared to the winter in Karan Fries bulls under tropical climatic conditions (Soren *et al.*, 2016b). The higher level of glutathione activity in seminal plasma of *Bos taurus* versus *Bos indicus* bulls during summer season is an indicative of oxidative stress (Nichi *et al.*, 2006). Bulls with lower lipid peroxidation had higher siring calves (Kasimanickam *et al.*, 2007). The antioxidant enzymes assay in seminal plasma is one of the important semen quality parameters for assisted reproductive biotechnology.

### Heat shock proteins (HSPs)

Induction of HSPs occurs in cells exposed to thermal,

chemical or physical stress, viral infection, drugs and transforming agents. These HSPs refolds the denatured and misfolded proteins, which mainly occurs due to different stresses, thereby avoiding the cell death due to protein toxicity. HSPs are also induced by physiological events such as cell growth, differentiation and ageing (Calderwood *et al.*, 2006). HSPs are highly conserved proteins and its expression is influenced by heat stress with important role to help and cope with heat stress (Van Oosten-Hawle *et al.*, 2013). The HSPs play crucial roles in thermal adaptation (Sorensen *et al.*, 2003).

Several studies indicated that the constitutive elevation of inducible HSPs levels provides cytoprotection against thermal stress (Oksala *et al.*, 2014). Aberrant expression of HSPs and heat shock factors (HSFs) seemed to cause male infertility (Ji *et al.*, 2012). Normally 1-2% of HSPs are present in the cells out of total proteins; which may increase up to 4 to 6% under stressful condition (Crevel *et al.*, 2001). Cells respond to heat stress stimuli by increasing the synthesis of HSPs (Oksala *et al.*, 2014). Soren *et al.* (2018) observed the higher expression of HSP70.1, HSP70.2, HSP70.8, HSP90 and HSP105 in spermatozoa of Karan Fries bulls during summer season than winter season (Fig. 3).

### Heat shock protein 70 (HSP70)

The heat shock protein 70 (HSP70) assumed to regulate the activity of some antioxidant enzymes (superoxide dismutase) in the sperm cells (Zhang *et al.*, 2015). The increasing level of HSP70 in cells restrained the activity of protein kinase including p38 and Jun N-terminal kinase (JNK), prevented cell apoptosis and strengthened the resistance of cell (Kennedy *et al.*, 2014). The reduced expression of HSP70.2 was observed in maturation arrest testes compared to normal testes (Feng *et al.*, 2001) and suggested crucial role of HSP70.2 for spermatogenesis. Its expression correlated with maturity, function and fertility (Huszar *et al.*, 2000). The higher expression of HSP70 observed in ejaculated spermatozoa during summer (Soren *et al.*, 2018) indicated their important role in maintaining the quality of semen.

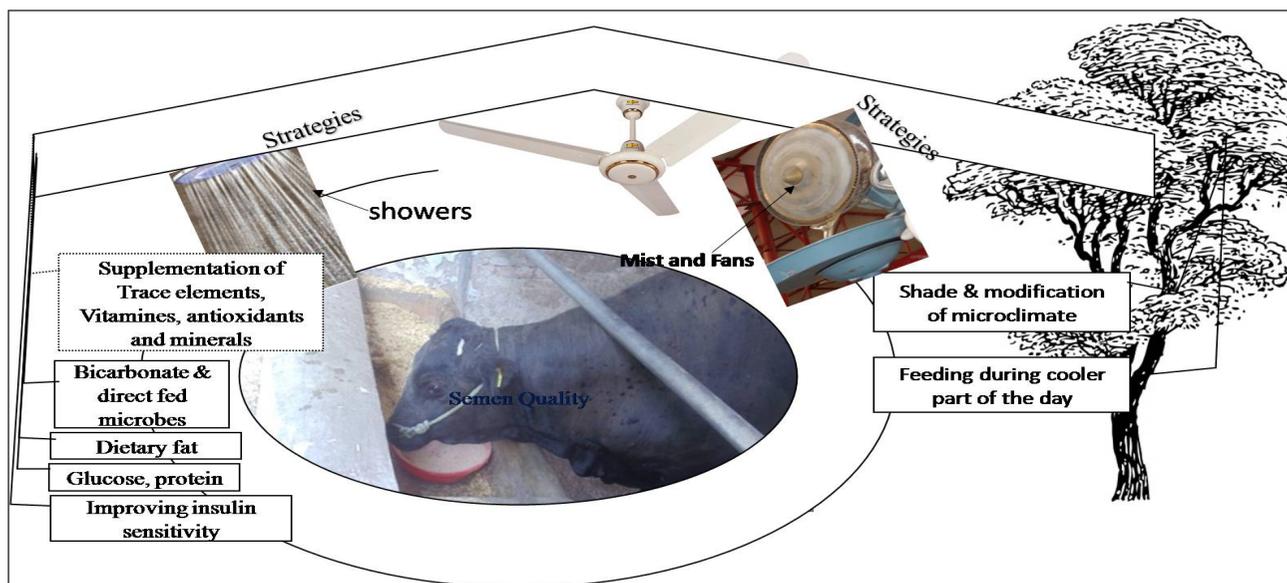
### Heat shock protein 90 (HSP90)

The Heat shock protein 90 (HSP90) expressed considerably under heat stress and displayed its role in protein folding (Oksala *et al.*, 2014). It is localised in the sperm tail in all species and associated with sperm motility and fertility (Wang *et al.*, 2012). Many proteins like tyrosin kinases, serine-threonine kinases and other enzymes

(Pratt, 1998) are associated with HSP90, which may have direct or indirect influence on sperm motility (Huang *et al.*, 2000). Zhang *et al.* (2015) observed the expression level of HSP90 decreasing gradually in bull spermatozoa during the process of freezing-thawing, therefore, it was suggested that the HSP90 might be associated with sperm motility, plasma membrane and acrosome integrity. In respect to the external stresses, increasing HSPs is to maintain the metabolic and structural integrity of the cells and try to counteract the negative effect of stress condition (Kwon *et al.*, 2002). The decreased level of HSP90 was also observed by Cao *et al.* (2003), which were associated with decline in semen characteristics (Wang *et al.*, 2005). The HSP90 may be involved in protection of cells from ROS (Oksala *et al.*, 2014). The protective role of HSP90 against oxidative stress during cryopreservation is yet to be clarified. It was demonstrated that HSP90 was involved in ATP metabolism (Prodromou *et al.*, 1997).

### Heat shock protein 105 (HSP105)

Heat shock protein105 (HSP105) forms a complex with p53 at the scrotal temperature and dissociates from it at suprascrotal temperatures (Kumagai *et al.*, 2000). HSP105 may contribute to the stabilization of p53 proteins in the cytoplasm of the germ cells and thus preventing



**Fig. 4:** Possible strategies for improvement of semen quality during summer season under tropical climatic conditions (Soren *et al.*, 2017b)

the potential induction of apoptosis by p53 at scrotal temperature (Kumagai *et al.*, 2000). Zhang *et al.* (2005) observed that the transient increase of scrotal temperature at 43 °C for 30 minutes once daily for two consecutive days induced germ cells apoptosis in non-human primates. The sperm concentration was decreased by 8.4 % on 28<sup>th</sup> day after heat shock compared to pretreatment levels and recovered back to normal on day 144. Increased numbers of apoptotic spermatocytes and round spermatids were detected by TUNEL assay on days 3, 8 and 30 after heat treatment. HSP105 decreased dramatically with the loss of spermatids on days 3, 8 and 30 after heat treatment and the expression of HSP60 was high on days 3, 8 and 30, detected in sertoli and spermatogonial cells. The decreased expression of HSP105 with germ cell death suggested their involvement in regulation of germ cell apoptosis. The increase numbers of dead spermatozoa during summer than winter consequently increase expression of HSP105 might afford to prevent sperm cell death (Soren *et al.*, 2018).

### Nutritional management

Supplementation of antioxidants minimizes the load of oxidative stress which protects the sperm cells from cellular oxidants and prevents the accumulation of oxidative damaged molecules. Antioxidants, both enzymatic and non-enzymatic are necessary to supply as an amelioration measure against heat stress. Some of the strategies were presented in a graphical presentation adopted from Soren *et al.* (2017b) with minor modification.

### CONCLUSION

Sustainable dairy growth has to be achieved in the present scenario to fulfil the demand of increasing population. Global warming is a threat to animal's productivity under tropical climatic condition. The failure in artificial insemination and conception during hot climatic condition is not only due to female factor but also contributed by male factor. The physical, biochemical and molecular properties of semen are affected significantly under heat stress. Some defects of sperm cannot be detected through routine evaluation tests. Therefore, more studies are necessary for evaluating the quality of semen at the molecular level and formulating ameliorative measures during summer season under tropical climatic conditions.

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