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Effect of heat stress on reproduction and semen production: A review

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Abstract
In practically every section of the world, heat stress is one of the most important factors affecting bull reproductive capacity. Because hot, humid circumstances might decrease bull fertility, the current rise in global temperature poses a threat to future livestock output. When the body's natural physiological processes for regulating body temperature are overwhelmed by external factors, heat stress develops. The testes and scrotum each have their own intricate regulatory systems in place to safeguard growing sperm at their most vulnerable phases. Heat stress can affect the bull's endocrine system, causing sperm concentration and quality to drop. The negative effects on fertility may be caused by reactive oxygen species' effects on sperm DNA or by a disruption in the generation of antioxidants that normally preserve sperm from oxidative assault. The form of the sperm cell head and tailpiece can be changed by heat stress. Testicular degeneration and a decrease in the percentage of normal and viable spermatozoa in the ejaculate can also be caused by high ambient temperature. The purpose of this review is to see how heat stress affected bulls' sperm production.

Keywords: heat stress, spermatogenesis, scrotum, fertility, sperm quality, sperm morphology

1. Introduction
Climate change is one of the biggest concerns to the long-term viability of tropical cattle production systems. Climate change may have an impact on animal health and production due to changes in climatic factors such as air temperature, relative humidity, precipitation, and the frequency and amplitude of weather extremes (Gaughan *et al.*, 2009; Lacetera, 2019) [20, 30]. High temperature became one of the most significant variables influencing mammalian reproduction as global temperatures rise. Livestock animals have an endothermic temperature, which means they can maintain a stable body temperature by managing the amount of heat generated by their metabolism and the amount of heat lost to the environment. When the temperature of the environment changes, animals change their physiology and behavior to maintain a stable body temperature (DeShazer *et al*., 2009) [15]. Heat stress in livestock is caused by high ambient temperature and high relative humidity, the combined effect of the two having negative effects on livestock reproductive performance. Heat stress has a number of negative consequences for reproduction, ranging from gamete development to offspring growth. The degree and intensity of heat stress have an impact on animal reproductive success (Lacetera, 2019) [30].

2. Effect of Heat stress on Reproduction
Farm animal reproduction success is a significant driver of livestock-based industries and is required for species continuity and multiplication. Heat stress has been identified as a significant threat to animal welfare, reproduction, and output under the current global warming scenario. The negative effects of heat stress on reproductive capacity affect the fertility of both female and male animals (Hansen 2009; Ferro *et al* 2010) [23, 17]. Heat stress is a major contributor to livestock fertility problems. The first mechanism, which involves redistribution of blood flow from the body core to the periphery to increase sensible heat loss and reduced feed intake to reduce metabolic heat production, can lead to changes in energy balance and nutrient availability, which can have a negative impact on reproduction. The failure of homeokinetic mechanisms to control body temperature is another pathway for heat stress-induced disruption of reproduction (Hansen, 2009) [23]. The sensitivity of germinal cells to high temperatures varies. The male germ-line is distinguished by a germinal epithelium that undergoes continuous growth and is kept at a little lower temperature than the core body temperature by sophisticated thermoregulation mechanisms in most mammalian species.

The female germ-line, on the other hand, is segregated within the ovarian follicle for the rest of its life and is susceptible to environmental insults during its statics, growth, and maturation stages.

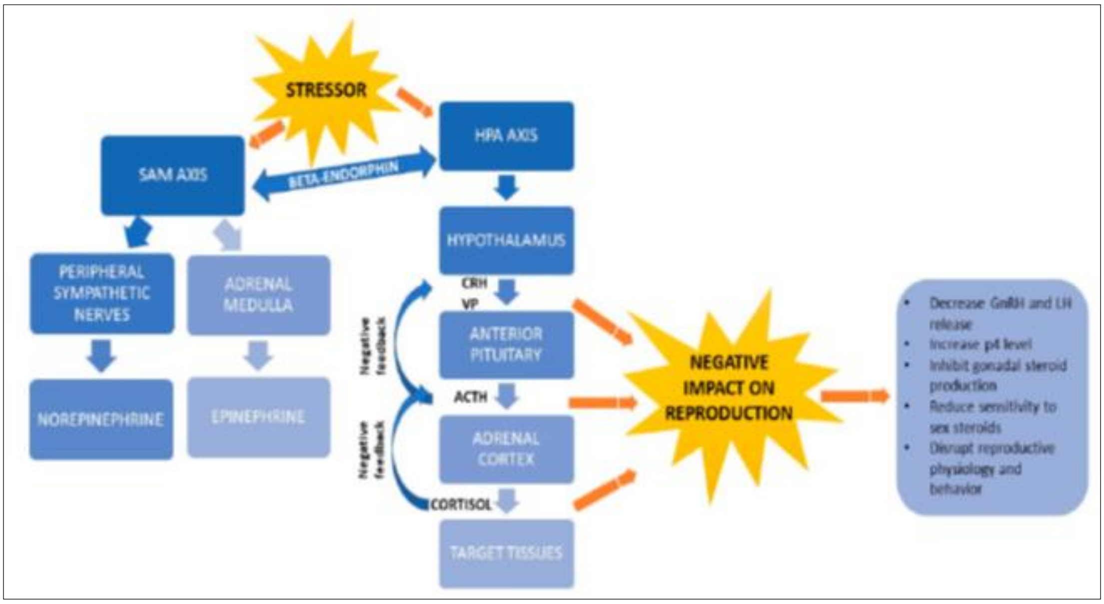

Fig 1: Mechanism of Heat Stress affecting Reproduction

2.1 Effects on Female reproduction

Heat stress may reduce the reproductive efficiency of both sexes of animals. Heat stress causes a decrease in the number of mounts in female animals, which makes it difficult to identify estrus (Pennington *et al*., 1985) [43]. Heat stress shortens the estrous cycle in cows and has a long-term negative impact on ovarian follicular dynamics and oocyte competence (de S Torres-Júnior *et al*., 2008) [14]. Heat stress reduces the estrogenic activity of developing follicles by inhibiting the expression of gonadotropin receptors in granulosa cells, as a result, decreased oestrogen production in animals may result in an irregular estrous cycle (Shimizu *et al*., 2005; Li *et al*., 2016) [60]. Heat shock during oocyte maturation can promote apoptotic response leading to disruption of the oocyte's capacity to support early embryonic development (Roth and Hansen, 2004) [55]. The pre-implantation developmental capacity of an oocyte is determined by cytoplasmic components rather than nuclear components, and the cytoplasmic maturation of oocyte is more susceptible to heat stress than nuclear maturation (Wang *et al*., 2009) [66].

The uterine blood flow is affected by thermal stress, which can compromise fetal development by altering the uterine environment. Heat stress does not impact the pace of cleavage, but it does slow the growth of blastocysts (de S Torres-Júnior *et al*., 2008) [14]. Heat stress impacts oocyte growth and quality in cows and pigs (Barati *et al*., 2008; Ronchi *et al*., 2001) [2, 54], as well as embryo development and pregnancy rate (Wolfenson *et al*., 2000; Hansen, 2007; Nardone *et al*., 2010) [67, 22]. Increased energy shortfalls and heat stress may compromise cow reproduction (De Rensis and Scaramuzzi, 2003; King *et al*., 2006) [13, 28].

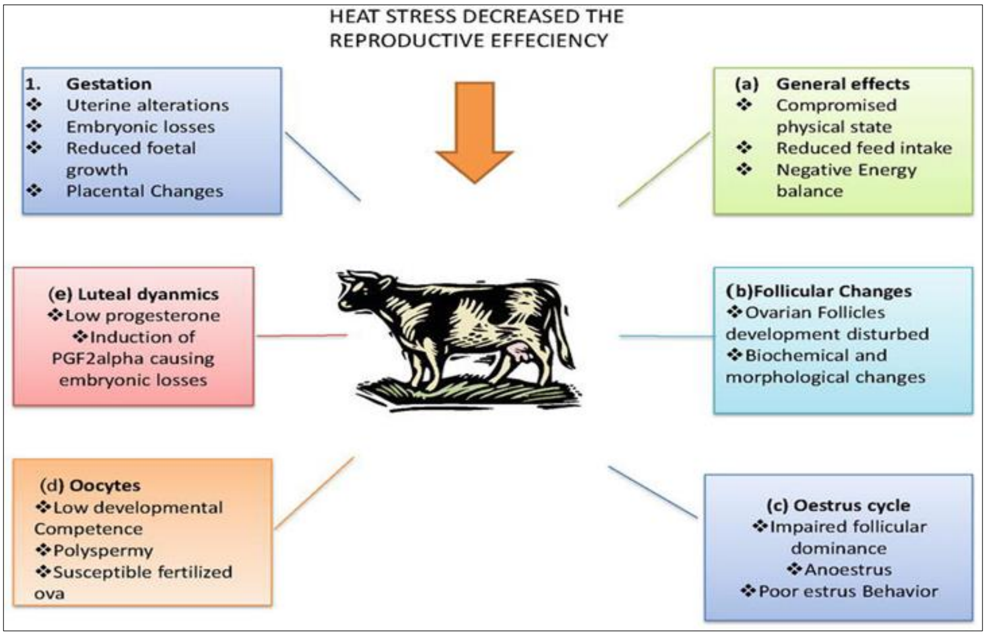

Fig 2: Effect of Heat Stress on Various Phase of Female Reproduction

2.2 Effects on Male reproduction

The key factors limiting male reproductive efficiency in a year are sexual behaviour, semen quality, and quantity. Because one bull can influence the pregnancy rate of 30 or 40 cows with natural mating, or thousands of cows with artificial insemination, the potential fertility of an individual bull is more relevant than that of an individual cow (Kastelic, 2013) [27]. Male animals' testicular endocrine function, metabolic condition, and gametogenesis functions are all affected by heat stress, but these functions are restored after a few weeks of acclimatisation to the hot environment. Heat stress lowers plasma luteinizing hormone (LH) levels in bulls (Rhynes and Ewing, 1973) [53] while raising plasma testosterone levels in boars (Murase *et al*., 2007) [39].

Increased testicular temperature causes hypoxia and increased reactive oxygen species (ROS) production as the ambient temperature rises. ROS are formed as a byproduct of cellular metabolism and are involved in sperm activity, sperm capacitation, and the acrosome reaction, as well as binding to the zona pellucida. As a result, no fertilisation occurs without ROS (de Lamirande *et al*., 1997) [12].

Males' reproductive performance and libido both suffer during periods of extreme heat (Gabaldi 2000) [18]. Changes in testicular diameter, texture, and mass were noticed on external examination and later changes in spermatogenesis and steroidogenesis functions, resulting in testicular degeneration and fibrosis, sterility, sperm mutation, germinal epithelium degeneration, and fertility loss (Gabaldi and Wolf, 2002; Santiago 2006; Ferro *et al* 2010) [19, 57, 17].

Heat stress makes sperm cells susceptible, causing them to die and lose their DNA integrity (Sinha *et al*., 2003; Banks *et al*., 2005) [61, 1]. The spermatozoa generated during the summer are either inherently less active at the moment of generation or have deteriorated during their travel along the reproductive tube prior to release in the ejaculate, even if they were normal at the time of genesis (Bhakat *et al*., 2014) [3]. Heat stress can also affect sperm production, motility, and the number of secondary abnormalities in sperm. The reaction time, total time taken for successful ejaculation, and dismount time were all found to be delayed in a study on heat stressed bulls, and this was linked by a low libido score (Mandal *et al*., 2000) [35].

Heat stress to the testis with acute scrotal heating has been shown to reduce not only semen quality, but also embryo quality following fertilization in female mice (Yaeram *et al*., 2006, Paul *et al*., 2008) [68, 41], as well as fetal growth (Jannes *et al*., 1998) [25]. Heat stress has been shown to lower male breeding effectiveness by reducing the quantity of testicular cells such as secondary spermatocytes and spermatids, as well as the ratio of sertoli cells to other cells and the diameter of the seminiferous tubules (Edwars and Hansen, 1997) [16].

3. Regulation of Scrotal and Testicular temperature

One of the most significant factors in the production of excellent spermatozoa is the environment. The production of viable spermatozoa requires a lower testicular temperature of roughly 32°C. The temperature of the testis is 2-6°celsius lower than that of the rest of the body. Increased testicular temperature can cause thermal stress, which can impact seminal and biochemical parameters, resulting in reproductive issues in bulls (Cardozo et.al 2006). High ambient temperatures, either alone or in combination with high humidity, impede evaporative heat escape from the scrotal surface, resulting in elevated testicular temperature. The testis is kept cool via a cooling system that includes the scrotum, muscles and pampiniform plexus (Senger, 2003) [58].

The bovine testes and epididymis are placed in the scrotum, which is below the abdomen, to keep them at a lower temperature for proper functioning (Brito *et al*., 2004) [8]. Scrotal skin in bulls is thin, hairless, and devoid of subcutaneous fat. It has a higher density of sweat glands than the skin on other regions of the body, allowing heat to be evacuated through sweating to facilitate heat transfer (Blazquez *et al*., 1988) [5]. To regulate temperature, smooth muscles in the cutaneous arterioles of the scrotum contract in cold weather and dilate in hot weather (Waites *et al*., 1970) [64].

In cold weather, the testes might be drawn towards the belly, whereas in hot weather, they can hang away from the body. The dartos muscle is a thin sheet of smooth muscle located just beneath the scrotal skin that lowers the volume of the scrotum during contraction. The vascular system also regulates temperature, the testicular artery provides blood to the testes and generates an extensive network of superficial capillaries. The testicular vascular cone functions as a classic counter-current heat exchanger. In the vascular cone, the artery is tortuous and interwoven with the venous complex (pampiniform complex), transferring heat from the artery to the vein, contributing to testicular cooling (Brito *et al*., 2004) [8].

4. Effect on Spermatogenesis

Spermatogenesis is a complicated biological process that occurs in the testis' seminiferous tubules. To ensure successful spermatogenesis in mammals, the testis temperature must be 2 to 8°C below body temperature (Senger, 2003; Banks *et al.*, 2005) [58, 1]. Germ cells are created in the testis, matured in the epididymis, and stored in the cauda epididymis in a dormant state in the male. Epididymal fluid and sperm are mixed with accessory sex gland secretions and deposited in the female reproductive tract after ejaculation.

Elevated temperature in the scrotum halt spermatogenesis and have a negative impact on testicular function, which causes sperm cells to degenerate, reducing spermatozoa's fertilizing capacity and ultimately causing infertility (Paul *et al.*, 2008; Rasooli *et al.*, 2010) [41, 49]. Because spermatogenesis in bulls takes around 60 days, changes in ejaculate characteristics caused by increase in testicular temperature, may not be seen immediately. However, 2 to 4 weeks following heat stress, potential spermatogenic cell damage can be found in the ejaculate (Vogler *et al*., 1993) [63] and it could take 6 to 12 weeks for sperm quality to return to normal (Kastelic *et al.*, 1997a; Hansen, 2009) [26, 23]. Heat stress has distinct effects on different types of cells in the testes, including apoptosis, DNA damage, disruption of the blood-testis barrier (BTB), and hormone secretion abnormalities.

Thermal damage to spermatogenic cells is caused by oxidative stress, which leads to apoptosis and DNA strand breakage (Perez-Crespo *et al*. 2008; Paul *et al*. 2008) [41]. All phases of spermatogenesis are affected, with the severity of the damage varying according to the magnitude and duration of the elevated temperature (Waites and Setchell, 1990) [65]. Because of the intense mitotic activity during the early stages of spermatogenesis, new germ cells (such as pachytene and diplotene spermatocytes) are vulnerable to high testicular temperatures (Pérez-Crespo *et al*., 2008; Houston *et al*., 2018) [44, 24]. High temperatures have been linked to the production of morphological and functional defects in sperm cells during the later stages of spermatogenesis i.e. epididymal maturation

(Rathore, 1969, 1970a, 1970b) [50, 51, 52].

Heat stress adversely affects testis function in numerous animal species, including bull (Roth *et al*., 2000) [56], boar (Malmgren and Larsson, 1984) [34], ram (Boland *et al.*, 1985) [6], and stallion (Love and Kenney, 1999). Heat stress causes the seminiferous tubules to lose spermatogonial germ cells, resulting in a reduction in sperm density (Rasooli *et al.*, 2010) [49]. Many animal species suffer from azoospermia or oligospermia as a result of harmful effects of exposure to heat. In hot weather, sperm quality deteriorates, and in many situations, sperm does not ejaculate (Hamilton *et al.*, 2016) [21]. According to Paul *et al*. (2009), transient mild testicular hyperthermia causes temperature-dependent germ cell death as well as a complicated stress response that includes oxidative stress and hypoxia.

5. Effect on Semen quality

Low sperm quality, which is linked to female infertility, may be caused by extremes in environmental temperature, as a result of low fertilization rates and increased embryonic death. Heat stress causes dramatic modifications in sperm quality and fertility (Collier *et al*., 2017) [10]. The capacity of spermatozoa to migrate from the location of semen deposition to the site of fertilization, to penetrate an egg and activate it to create an embryo is referred to as sperm quality. It can be classified based on motility, morphology, plasma membrane integrity, metabolic activity, and acrosome reaction capabilities (Moce and Graham, 2008; Morrell and Rodriguez-Martinez, 2009) [37, 38]. Extraneous factors such as environment, diet, and management practices can affect all of these sperm traits as well as intrinsic factors such as age (Snoj *et al*., 2013; Bhakat *et al*., 2014) [62, 3].

Heat stress affects certain bulls more than others, and a decrease in a bull's libido during heat stress drastically limits the semen harvest (Marshall, 1984) [65]. Changes in semen occur about two weeks after heat stress in the bull, whose spermatogenesis takes about 61 days, and do not return to normal until up to eight weeks after the end of heat stress. According to Rajoriya *et al*. (2013) [46], season had a substantial impact on ejaculate volume, sperm concentration, mass-motility, progressive motility, vigour, and morphological sperm abnormalities. Human fertility is influenced by genital temperature, which can be modified by the environment (Rao *et al*., 2015) [48]. As a result, the negative impact of heat stress on fertility was traditionally thought to be attributable to its effects on gametogenesis, but current research suggests that it may also influence sperm functioning by changing seminal plasma proteins (Rahman *et al*., 2018) [45].

Germ cell death, DNA damage, and disturbance of sperm development, including induction of morphological and functional abnormalities, are some of the impacts of higher testicular temperature on semen quality (Setchell, 1998; McDonald *et al*., 2007) [59, 36]. Heat stress reduces sperm mass motility significantly (Ram *et al*., 2017) [47]. During the summer, heat stressed bulls produced low-quality sperm with a large number of aberrant heads and cytoplasmic droplets (Koivisto *et al*., 2009) [29]. Simulated circadian thermal stress conditions in climatic chambers changed the percentage of rapidly motile sperm, as well as the linearity and average route velocity of spermatozoa in rams (from 38°C to 44°C after a daily routine for 8 weeks) (De *et al*., 2017) [11]. There were simultaneous changes in either spermatogenesis and semen characteristics or several sperm parameters affecting IVF and *in vitro* embryo development in bulls after a 72-hour scrotal insulation period (i.e., the scrotum was wrapped in cotton layers and covered with a plastic bag) or a high temperature exposure (35.5°C and 50% RH for 7 weeks) (Lucio *et al*., 2016) [33]. The acrosome integrity percent is lowest during summer as compared to winter season (Bhakat *et al*., 2014) [3]. Decrease in acrosomal function leads to reduction in sperm fertility (Birck *et al*., 2010) [4].

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