

Effect of stress on dairy animal reproduction

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Abstract

Stress is a strong barrier to dairy animal reproduction and, hence, poses an alarming threat to combat it. The economic sustainability of a dairy herd mainly depends on getting utmost reproductive efficiency. In contrast to extensive research on infectious diseases, nutrition, infertility etc. less is known about animal welfare and its interaction on reproductive efficiency. Animal welfare means paying attention to animal's social relation, environment, climate and availability of adlib food and water. Welfare is a condition in which animals do not feel a mental or physical discomfort and do not feel stress or pain. A loss of welfare brings the animals into stress, which affects their production and reproduction. The natural, physical and environmental factors affecting animals are air temperature, relative humidity, solar radiation, atmospheric pressure and wind speed. Summer stress reduces productive and reproductive efficiency of dairy animal. The neuroendocrine mechanism of heat stress has been extensively reviewed. Heat stress compromises hypothalamic GnRH secretion, and circulating concentration of LH and FSH reduces. In addition, there is also evidence that HS affects follicular dynamics and ovulation.

Key words: anestrous; dairy animal; reproduction; stress; thermal

Introduction

Stress can be defined as any non-specific response of the body to any stimuli/demand. It is further defined as a biological response exhibited when an individual perceives a threat to its homeostasis (Fernandez-Novo *et al.*, 2020). It is also defined as a result of an external event or condition that places a strain on a biological system (Collier *et al.*, 2017). Stress is a series of physiological reactions which occurs in order to adapt to different conditions. In general, stressors elicit metabolic, behavioral and physiological changes in animals (Colier and Gebremedhin, 2015). A stress can be acute (persists for minutes up to few days and elicits “fight-or-flight” response); chronic (long term stress leads to health disorder) (Brown and Vosloo, 2017). Due to stress, animal exhibits reduced feed intake, reduced energy level, weight loss, reduced conception rate, reduced milk production, early embryonic death, small or premature calves, weak immune system and progression of diseases. Dairy cattle can respond to stress through acclimatization (adapts to stressors within its natural environment), acclimation (animal’s phenotypic response to stressors) and temperament.

Classification of stressors

There can be several types of stressors. It can be internal or external. It can also be classified as biotic and abiotic. Diseases causing pathogens are the commonest causes of biotic stress (Jaya *et al.*, 2016). Biotic stresses are caused by living organisms and diseases affect animal’s production and reproduction. These include bacteria, virus, fungus, mycoplasma, insects, parasites etc. The abiotic stressors mainly include cold stress, heat stress, poor housing, social stress, loud noise and transportation particularly during noon hours cause stress, also reduction in feed quality and quantity lead stressful conditions (Gadzhiev *et al.*, 2021). Psychological stressors include separation of young calves from dam, isolation of animals during contagious diseases and mixing of animals during low land availability or low space for housing. Physiological stressors include parturition and lactation during which animals need high energy demand. Stressors can also be classified as endogenous, if they have a genetic or physical origin and exogenous, if they arise from the social and physical environment.

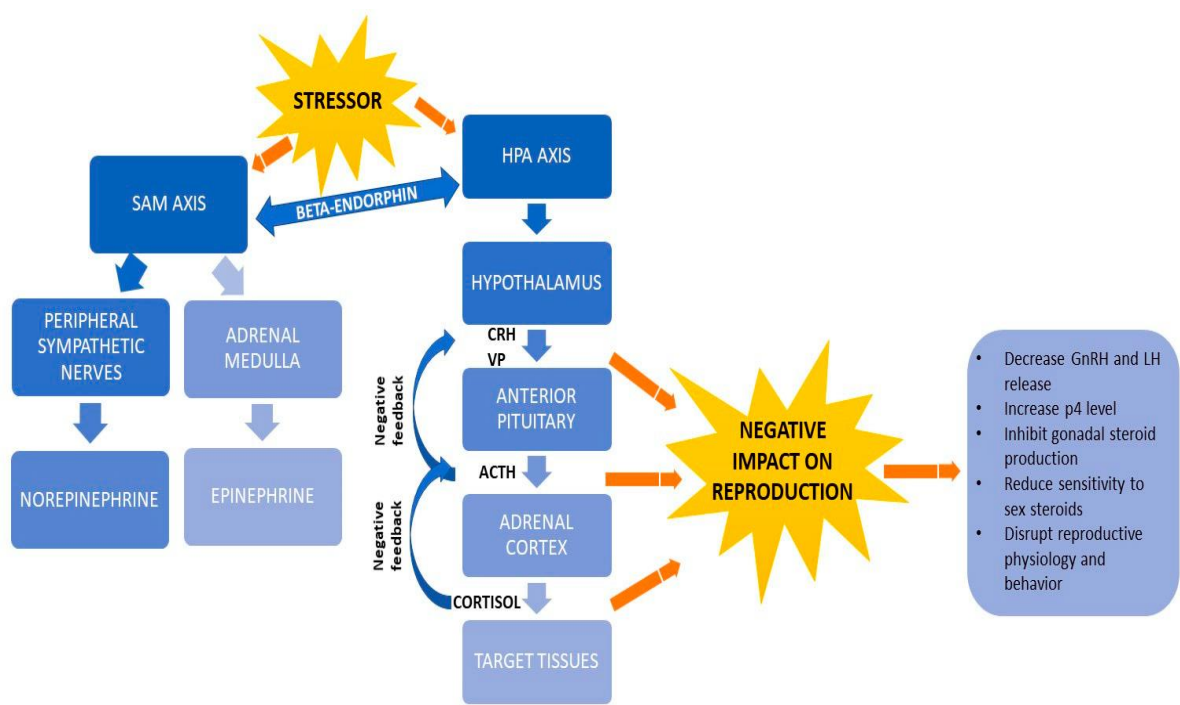


Fig 1. Physiology of stress (Fernandez-Novo *et al.*, 2020)

The afferent pathways transmit stress signal into various part of central nervous system, including thalamus, hypothalamus and adrenal cortex. The efferent pathways are activated to give rise to a stress response. The sympathetic-adrenal-medullary (SAM) axis mediates acute stress responses (Chen *et al.*, 2015) while the hypothalamus-pituitary-adrenocortical (HPA) axis mediates both acute and chronic responses (Kumar *et al.*, 2012). In the acute stress response, changes in environment activates receptors in the body, which triggers the SAM axis to turn on production of two catecholamines, epinephrine in the adrenal medulla and norepinephrine in the peripheral sympathetic nerves. These hormones trigger a fight-or-flight response in which heart and respiration rate increases and blood pressure rises. The HPA axis induces the hypothalamus to secrete corticotropin-releasing hormone and vasopressin (Mormede *et al.*, 2007), which in turn triggers the pituitary

gland to release adrenocorticotrophic hormone (ACTH). ACTH causes adrenal cortex to secrete glucocorticoids, mainly cortisol. Glucocorticoids can influence a broad range of innate and acquired immune responses. They can induce a pro-inflammatory response and acute-phase protein production. In 2011, Cooke and others had first described the CRH-induced release of pro-inflammatory cytokines and acute-phase proteins in dairy cattle. These cytokines are carried to the liver, where they trigger the synthesis of haptoglobin and acute-phase proteins in hepatocytes, like serum amyloid-A (SAA). Homeostasis cannot be restored if stressor is present and HPA axis is unable to control its effect resulting in allostatic overload. If such overload is chronic then it harms immune system, reproductive system and consequently animal welfare. In reproductive system, reduction in GnRH and LH secretion, increase progesterone level, prevent gonadal steroid production, decreased sensitivity to sex steroids and disruption of reproductive physiology.

Quantification of stress

The negative influence of heat stress on reproduction traits can be quantified through formulating Temperature Humidity Index (THI). Heat load index (HLI) is another to measure the level of heat stress in cattle (Gaughan *et al.*, 2008). Several indicators have been described to evaluate the level of stress in dairy cattle.

Behavioral Indicators

It includes temperament, handling, exit velocity, pen score, feed intake, and time standing. Additional behavioral indicators include movement-measuring devices, a docility test, strain gauges, race score, qualitative behavioral assessment and a four-platform standing scale, amount and time of feed intake, time standing and lying down. Most of these behavioral scales have been shown to be objective, repeatable and correlated with different biomarkers, including cortisol, haptoglobin, substance P and prolactin.

Animal Based Indicators

They are more reliable & more indicative eg, temperature, heart rate (HR), respiration rate (RR) & lesions or injuries. Stressors initiate 'fight-or-flight' response that increase heart & respiration rate. A feed intake index can measure stress around feeding, which may due to fear of human or hierarchy or chronic stress. Study on such index shown that hierarchical dams consume more feed and that under heat stress. Skin injuries or inadequate housing conditions cause aggression within animals. Stressors compromise the immune system and predispose to diseases, thereby increasing morbidity and mortality (Chen *et al.*, 2015).

Biomarkers

These are objective indicators of medical state that can be observed from outside the individual and can be measured accurately and reproducibly (Strimbu and Tavel, 2010). Biomarkers of stress are cortisol, corticosteron & its metabolites, haptoglobin, prolactin, serum amyloid-A & substance P measured in blood, saliva, urine, milk & faeces (De Vane *et al.*, 2001). Prolactin is not routinely used in this way because its levels are strongly influenced by dairy husbandry practices. C-reactive protein (CRP) is usually used as a stress biomarker in dogs and human but occasionally in dairy cattle. Cattle have negligible concentrations of haptoglobin but levels can increase 100-folds in presence of stress. Serum amyloid-A proteins, which are apolipoprotein associated with high-density lipoprotein, are synthesized by the liver during the acute-phase stress response. Substance P can be used as a stress biomarker and its levels were higher in aggressive dairy heifers than in calm heifers and its levels increased in heifers undergoing AI.

Types of stress in dairy animals

Management Stress

This stress include handling stress, isolation of animals during disease lead to social stress, separation of young from his mother lead to weaning stress, long transport of animals leads to transport stress. When animals are managed in groups, they face social stress and hierarchies. Facilities should be designed to reduce individual stress and the amount of handling by humans. Higher cortisol concentrations in excitable dams reduce GnRH and LH, harming the reproductive performance and longer postpartum anestrus. Excitable dams are more sensitive to environmental threats and show signs of chronic distress, leading to lower feed intake and lower Body Condition Score (BCS). This hierarchy influences feed intake, social behavior, relationships between dams and group creation. Weaning stress leads to an increase in movements and vocalizations, which leads an increase in cortisol and weight loss. Calves should not be allowed to suckle too long, since this interferes with GnRH secretion by the hypothalamus and even the mother's seeing and smelling the calf can prevent the GnRH secretion and the LH release, prolonging of anestrus in dairy animals (Orihuela and Galina, 2019).

Chronic Stress

It includes locomotory diseases in dairy animals, such as lameness, have a negative influence on the estrus behavior, fertilization rate, conception rate and early embryo death (Blackie and Maclaurin, 2019).

Nutritional Stress

When animal faces malnutrition there be a negative energy balance cause secretion of ghrelin from gastro intestinal track and negative impact on ovarian functionality, leptin in adipose tissue and activation of hypothalamic neurons by kisspeptin and effect on GnRH producing neurons and positive effect on ovarian

functionality. Another IGF1 (insulin like growth factors-1) release from liver that positively affect to achieve puberty and shorten estrus postpartum and also negative effect lead to delay puberty and longer estrus postpartum (Fernandez-Novo *et al.*, 2020). The infertility period may be extended during lactation when energy demand is more (El-Tarabany, 2016). In high-yielding cows, fertility is often reduced or even absent (Braganca and Zangirolamo, 2018). Mycotoxins in food can cause abortion and endometritis and pesticides cause hormonal dysfunction in the H-P-G axis (Wrzecinska *et al.*, 2021).

Thermal Stress

It includes abiotic stress caused by excessive heat, cold, wind and rain. Environmentally induced hyperthermia is called heat stress. Fundamental factors in environmental conditions are air movement, relative humidity, as well as sunlight. Heat stress is related to the external / internal environment. Heat stress can compromise reproduction by decreasing the expression of estrous behavior, altering ovarian follicular development, compromising oocyte competence and inhibiting embryonic development. Heat stress is responsible for lowering corpus luteum weight and diameter leading to less production of progesterone. It has also been associated with lower conception rate; lower concentrations of progesterone, prolactin and estradiol and higher rate of pregnancy loss.

A temperature-humidity index (THI) is a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress. This index has been developed as a weather safety index to monitor and reduce heat-stress-related losses. The THI is computed from measures of atmospheric moisture other than wet-bulb temperature. For example, $THI = 0.55T + 0.2T_d + 17.5$ involves air temperature T (°F) and dew-point T_d (°F). The ideal THI value for dairy cattle is 72 in Indian subcontinent, above which they experience thermal stress generally during October and mid-March to mid-July. Higher THI has been linked to changes in estrus behavior of dairy cattle, which is heavily influenced by herd hierarchy. Heat stress also alters the endometrial environment, such as by up-regulating glycoprotein 2 and neurotensin, which may contribute to infertility in the summer (Sakumoto *et al.*, 2015). All these changes lower the fertilization rate and reduce the quality of embryos, increasing the risk of pregnancy loss and decreasing reproductive performance (Fernandez-Novo *et al.*, 2020).

Effect of Heat Stress on Estrus

Cattle exhibit estrus signs during cool hours, mostly during night hours. The intensity and length of estrus are inversely associated with environmental temperatures. Higher the temperature, increase the anestrus and silent heat in farm animals (Singh *et al.*, 2021). Dairy animals exposed to high temperature may become physically lethargic. The decreased concentration of oestradiol-17 beta in summer reduces the intensity of estrus manifestation, results in silent heat in buffaloes (Dash *et al.*, 2015). Mean plasma prolactin concentration was significantly higher in summer than winter cause acyclicity/infertility in buffaloes (Roy and Prakash, 2007). Heat stress can cause an increase in cortisol secretion, which block estradiol and subside estrus behavior by increasing progesterone concentrations in blood resulting in negative feedback on the hypothalamus, which decreases GnRH, and in turn, reduces LH and estradiol concentrations in blood.

Effect of Stress on Ovarian Follicle

There is no direct evidence of effect of HS on primary follicles. The fertility is restored only in 40–60 days after heat stress period (De Rensis, 2021). In a recent in vitro study, growing pre-antral follicles, including primary and secondary follicles, were found susceptible HS (Aguilar *et al.*, 2020). Exposure of heat stress to lactating dairy cows has been associated with a reduction of granulosa cell-specific gene expression (Vanselow *et al.*, 2016). The size of the pre-ovulatory follicle reduced with a high THI (Jitumrong *et al.*, 2020). In fact, the diameter of the pre-ovulatory follicle is reduced by an estimated 0.1 mm for each additional point on the THI value on the day of estrus (Schuller *et al.*, 2017). Roth *et al.* (2001) conducted a study and found detrimental effect of thermal stress on follicular characteristics in cows as follow.

Effect of Heat Stress on Oocyte and Embryo

There is reduction in the synthesis of LH and estradiol during higher THI and heat stress period. Oocyte competence is lower in summer than winter (Rutledge *et al.*, 1989). Due to increased THI and heat stress, decrease in blood flow to the uterus and elevated temperature suppresses embryo development and early embryonic death occurs. Direct exposure of bovine oocytes to temperature 41.8°C for 12 hours reduced their ability to complete nuclear maturation and development after fertilization (Payton *et al.*, 2004). Moreover, some studies demonstrated DNA fragmentation and disruption of oocytes after direct exposure to high temperature before or during maturation (Roth and Hansen, 2004). When animals are exposed to high THI and long-term chronic thermal stress, luteal insufficiency occurs which leads to decrease progesterone, impaired oocyte maturation, implantation failure, early embryonic death and pregnancy loss. (Wolfeson and Roth, 2019).

Effect of Heat Stress on Conception Rate

If THI on the day of service is more than 72, it decreases the conception rate in dairy cattle. The high heat load 3-5 weeks pre-service and 1 week post-service is associated with reduced conception rate and early embryonic death in cattle. The reduction in conception rate in hot days are due to environmental heat which

Table-1: Reproductive problems in dairy cows due to heat stress

1	Lower estrus detection rates	Collier <i>et al.</i> , 1982
2	Reduction in size of ovarian follicle	Schuller <i>et al.</i> , 2017
3	Altered follicular fluid composition & abnormal concentrations of ovarian steroids	Roth and Wolfenson, 2016
4	Relative location, morphology, and function of ooplasmic organelles (especially mitochondria) are altered by heat stress	Silva <i>et al.</i> , 2013
5	Transient effects on reproduction up to 50 days after exposure to heat stress	Roth <i>et al.</i> , 2001
6	Decreased sperm concentration, motility & increased morphological abnormalities	Rahman <i>et al.</i> , 2018
7	Abnormal condensation of chromatin in sperm	Rahman <i>et al.</i> , 2018
8	Early embryonic death under heat stress	Edwards and Hansen, 1997

Table-2: Effect of thermal stress on follicular characteristics in cows

S.N.	Characteristic	Control cows	Heat stressed cows
1	No. of Cows	6	5
2	Total no. of follicles/cow	2.6	2.6
3	No. of healthy follicles/cow	1.5	1.0
4	Diameter (mm)	7.7 ± 0.4	6.7 ± 0.7
5	Follicular fluid volume (ml)	0.4 ± 0.1	0.3 ± 0.2
6	No. of granulosa cells 106/follicle	2.0 ± 0.5	1.5 ± 1.0
7	Viability of granulosa cells (%)	60.2 ± 4.8	54.0 ± 5.8
8	Viability of thecal cells (%)	>90	>90

Table-3: Effect of Heat Stress on service period (SP)

S.N.	Species	Higher SP			Lower SP			Reference
		SP (days)	Month	THI	SP (days)	Month	THI	
1	Thailand cattle	299±11	Feb.	80	133±7	Dece	72	Kaewlamun <i>et al.</i> , 2011
2	HF	166	Mar-Apr	-	130	Sept	-	Oseni <i>et al.</i> , 2005
3	HF	154	Mar	-	128	Octo	-	Boonkum <i>et al.</i> , 2011
4	Cows	159±3	Summer	-	148±4	Autumn	-	Kumar <i>et al.</i> , 2012
5	Murrah buffalo	180	May	80.27	119	March	67	Dash, 2013

Table-4: Effect of heat stress on conception rate

S.N.	Species	Higher CR			Lower CR			Reference
		CR (%)	Month	THI	CR (%)	Month	THI	
1	Holstein cows	30.6	winter	75	23	summer	80	Garcia-Ispuerto <i>et al.</i> , 2007
2	Holstein cows	-	-	-	Lower	summer	-	Dash <i>et al.</i> , 2016
3	Dairy cows	38.2	Oct-Jan	-	29.5	Jul-Sep	-	Nabenishi <i>et al.</i> , 2011
4	Murrah buffalo	78	Oct	75	59	August	81	Dash, 2013

Table-5: Effect of Heat Stress on Pregnancy Rate

S.N.	Species	THI	PR (%)	Month	Reference
1	Dairy cattle	74	34.1	July	El-Wishy, 2013
2	Dairy cattle	69	15.7	May	El-Wishy, 2013
3	HF	<70	35.8	-	El-Tarabany and El-Bayoumi, 2015
4	HF	>70	29.4	-	El-Tarabany and El-Bayoumi, 2015
5	HF	-	32	Sept-Nov	Oseni <i>et al.</i> , 2005
6	HF	-	24	March - May	Oseni <i>et al.</i> , 2005
7	Murrah Buffalo	66.09	58	November	Dash <i>et al.</i> , 2015
8	Murrah Buffalo	80.88	25	July	Dash <i>et al.</i> , 2015

Table-6: Anomalies in Bull Sperm after Heat Stress

S.N.	Anomalies	References
1	Insufficient increase in glutathione peroxidase (GPx) activity to minimize damage caused by ROS	Nichi <i>et al.</i> , 2006
2	Impaired motility, genetic modifications	Al-Kanaan <i>et al.</i> , 2015
3	Significant DNA fragmentation	Valeanu <i>et al.</i> , 2015
4	Decreased live and total sperm count	Sharma <i>et al.</i> , 2017
5	Decreased seminal pH	Sharma <i>et al.</i> , 2018
6	Affects seminal quality during spermatogenesis	Sabes-Alcina <i>et al.</i> , 2019
7	Sperm morphological defects, DNA fragmentation	Garcia-Oliveros <i>et al.</i> , 2020
8	Reduced viability of sperm	Luceno <i>et al.</i> , 2020

alters the synthesis of reproductive hormones and changes follicular microenvironment in high producing dairy cows.

Effect of Heat Stress on Pregnancy Rate

When dairy cattle are in thermo neutral zone, the pregnancy rate estimated as 32.6%, but significantly decreased to 20.5% when the animals came into heat stress and high THI zone (Khan et al., 2013). The ideal TNZ in Murrah buffalo is 72 THI. (Dash et al, 2015). Animals exposed to heat stress showed increased uterine temperature which lead to reduced fetal viability, early embryonic death and finally cause pregnancy loss.

Effect of stress on male

Normal spermatogenesis takes 61 days in healthy bulls. Additionally, it takes 14 days to travel from testis to tail of epididymis, where it waits for an insemination/ ejaculation. In a normal healthy bull, pampiniform plexus is a network of arteries and veins called the testicular vascular cone (Mete *et al.*, 2012) responsible for the thermoregulation of the testis. After thermal stress, sperm production normally takes 6 – 12 weeks. So, in such cases, it is crucial to test semen quality before breeding/ AI (Wrzecinska *et al.*, 2021). Several studies have reported negative effects heat stress on male reproduction (Shahat *et al.*, 2020; Capela *et al.*, 2022). It is observed that bulls exposed to high environmental temperatures (40°C or more) it lowers sperm quality. In addition, there is a significant decrease in testicular blood supply during warm months, causing deleterious effects on enzymatic activity and various aspects of semen and seminal plasma quality. Higher percentage of sperm defects in summer were recorded compared to winter in Simmental (*Bos taurus*) bulls due to high ambient temperatures (Koivisto *et al.*, 2009).

The thermal stress induces an increase in testicular temperature, leads to increased sperm abnormalities. The proportion, appearance and severity of sperm abnormalities vary according to the intensity and duration of heat stress and the developmental stages of the affected germ cells. It is noteworthy that abnormalities are predominantly located in the sperm head, micro and macrocephalic heads, piriform heads and presence of acrosomal defects (Sharma *et al.*, 2017). Sperm motility can also be compromised during 14–21 days by short-term heat stress, results in aggravations of developmental stages of spermatid and spermatocyte.

Mitigation strategies for stress

Mitigation of stress is most important because farmers have to bear great economic losses by reduced production and various reproductive problems due to stress. The stress can be managed by physical modification of environment, nutritional management, genetic selection of heat tolerant breeds, hormonal treatments and assisted reproductive technologies (ARTs).

Provision of shade and cooling techniques include fogging and misting systems effectively decreases heat stress on dairy animals (Dash *et al.*, 2016). One can protect his animal from direct and indirect solar radiation by providing cost effective house. Tree shades have been proved to be more efficient. If enough natural shade is unavailable, artificial structures may be constructed. Shades with straw roofs are best, as they have a high insulation value and a reflective surface. Increasing the air circulation in the sheds will help in dissipating heat. The air circulation inside shed can be increased by keeping half side wall, i.e., open housing system, use of exhaust fan and increasing height of the shed.

Water is arguably the most important nutrient requirement for the dairy cows. Water intake increased by 1.2 kg/°C increase in ambient temperature, but regardless, it is obvious that abundant water must be available all times under shed during hot conditions.

Dietary fat at the level of 2-6% or high-quality bypass fat will provide extra energy during negative energy balance. Animals undergo mild to severe heat stress needs 7–25% extra maintenance requirements (Singh *et al.*, 2021). Heat stress induced problems like poor immunity, decrease feed intake, decrease weight gain, oxidative stress, body temperature, fertility and semen quality can be ameliorated by inclusion of vitamin A, C, E, zinc and other electrolytes in feed (Abidin and Khatoon, 2013).

Cattle with shorter hair, hair of greater diameter and lighter coat color are more adapted to hot environments than those with longer hair coats and darker colors (Bernabucci *et al.*, 2010). So, such breeds should be improved in hot areas. Administration of GnRH in the early stage of estrus with endogenous LH improves conception rate during summer season (Singh *et al.*, 2021). The TAI program also improves summer fertility using GnRH followed by PGF2 α 7 days later to regress the CL which permits the final maturation of ovulatory follicles. Further, a second dose of GnRH 48 h after PGF2 α may induce ovulation and insemination of cows at 16 h (Richard *et al.*, 1998). Uses of ARTs can be helpful in mitigating summer sterility include a Multiple Ovulation and Embryo Transfer (MOET).

Conclusions

Managerial practices, dietary interventions, various cooling methods and supplementations that promote acceptable temperament, like low-stress stockman-ship, and acclimation of animals to handling are low-cost ways to significantly impact reproduction and reduce stress for both cattle and producers. Genetic parameters

and use of ART will help to improve fertility; TAI programs eliminate failure to detect estrus as a reason for reproductive inefficiency under stress. Research is needed to evaluate molecular and cellular mechanism of heat induced cell damage, especially deleterious effect of heat on puberty, oocyte or embryonic viability are poorly understood and potential of crossbreeding dairy breeds to enhance reproductive capability in stress.

Futuristic perspectives

Use of genome-wide association studies to identify the relationship of DNA markers linked production and reproduction traits would be a strategic approach on the productivity and selection of animals with greater adaptive capability. A wide range of available options, including proper housing, new approaches to shading, advanced cooling systems, advanced biotechnological strategies, are required to reduce environmental stresses. One of the most critical research issues is to elucidate the mechanisms of heat shock proteins (HSP) on livestock fertility (Vaidya et al 2023). Additional research will be needed for the incorporation of new technologies, such as transgenesis, which could enable the genetic improvement of livestock in the future through the knock-out of undesirable genes or the knock-in of desirable genes to reduce the impact of stress on animal productivity. Exact and complete mechanism by which HS affects reproduction is yet unclear and need further exploration.

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