Impactos do estresse térmico na reprodução de fêmeas bovinas
Heat stress impact on bovine female reproduction

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Resumo
O estresse por calor (ET) ocorre quando a temperatura ambiente excede a zona de conforto térmico animal. Várias respostas corporais inespecíficas, capitaneadas pelos sistemas nervoso, neuroendócrino e imunológico são acionadas para manter a homeostase e resfriar o animal. O ET afeta o eixo hipotálamo-hipofisário-gonadal, comprometendo a liberação de gonadotrofinas, e promove o acúmulo de espécies reativas de oxigênio e proteínas anormais nas células ovarianas. Em resposta, as células ativam mecanismos antioxidantes e de reparação do DNA, que reduzem o metabolismo celular e aumentam as chances de sobrevivência; quando a reparação não é possível, acontece a apoptose. O ET impacta negativamente a produção de estradiol ovariano, o comportamento do estro, o desenvolvimento folicular, a competência dos oócitos e do embrião, as taxas de concepção, o estabelecimento e a manutenção da gravidez e até mesmo a eficiência reprodutiva da progênie. O combate ao ET inclui estratégias de combate ao aquecimento global progressivo e de manejo para resfriar os animais, e diminuir a produção de calor metabólico. O uso de biotecnologia reprodutiva e estratégias genéticas para gerar animais termotolerantes são também essenciais

Palavras-chave: Bovinos, Estresse térmico; Eficiência reprodutiva.

Abstract
Heat stress (HS), a harmful condition affecting animal production, reproduction, and welfare, occurs when an animal is exposed to temperatures that exceed its thermal comfort zone. Several nonspecific body responses involving neural, neuroendocrine, and immune systems are triggered to keep homeostasis in such conditions. These responses, primarily directed to cooling the body, also impact the hypothalamic-pituitary-gonadal axis, compromising the bovine female’s release of gonadotropins. Heat stress also promotes reactive oxygen species accumulation in ovarian cells, impairing protein folding and refolding, triggering antioxidant and DNA protection mechanisms. These mechanisms, directed to reduce cell metabolism and increase survival chances, are not always sufficient to protect the cell and result in apoptosis. Heat stress’s systemic and cellular consequences impact ovarian estradiol production, estrous behaviors, follicular development, oocytes and embryo competence, conception rates, pregnancy establishment and maintenance, and even the future reproductive efficiency of the progenies of cows exposed to HS during pregnancy. The combat of heat stress includes strategies to alleviate the effect of progressive global warming, management strategies to cool the animals, reduced metabolic heat, and methane production dietary approaches. The use of reproductive biotechs and genetic strategies to increase thermotolerant animals are also critical to overcoming the harmful effect of HS.

Keywords: Cattle; Heat stress; Reproductive efficiency.

Introduction
Heat stress (HS) occurs when an animal is exposed to temperatures above its thermal comfort zone (Ames, 1980). Additionally, ambient humidity, solar radiation, and wind exposure can also influence this response (Mader and Griffin, 2015).

HS has profound deleterious effects on animal production and reproduction (Roth, 2020) and based on predicted global warming, the harmful effects of HS on animals will be exacerbated (Masson-Delmotte et al., 2021). Therefore, a thorough understanding of the HS impacts on the reproductive
physiology of the animals is required. This review summarizes major impacts of HS on female bovine reproduction.

**Neuroendocrine responses to stress**

Stressful event activates the hypothalamic-hypophyseal axis, promoting hypothalamic secretion of the corticotropin-releasing hormone (CRH) and vasopressin (VP), triggering the adenohypophyseal secretion of the adrenocorticotropic hormone (ACTH) and β-endorphin. The ACTH acts on the adrenal cortex, stimulating synthesis of cortisol (Engler et al., 1989) and progesterone (P4) (Yoshida and Nakao, 2005; Maziero et al., 2011). Through a negative feedback mechanism, cortisol reduces the release of CRH and ACTH, whereas ACTH and β-endorphin lessen the secretion of CRH. However, these regulatory mechanisms also impact the hypothalamic-hypophyseal-gonadal axis, reducing secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus and luteinizing hormone (LH) by the adenohypophysis, lessening, therefore, the synthesis of 17β-estradiol (17β-E2) by ovarian follicles (Valsamakis et al., 2019).

**Heat stress systemic impacts**

The maintenance of a high production level, accompanied by high feed intake and, consequently, a high rate of metabolic heat production, increases HS risk (Kadzere et al., 2002). This phenomenon is particularly evident in Bos taurus dairy cows due to their higher production levels (Honig et al., 2012). Lactating Holstein cows need a temperature humidity index (THI)<72 to maintain thermoneutrality (Bohmanova et al., 2007), while non-lactating Holstein cows remain in thermal comfort, even with THI ~75.4 (Ferrazza et al., 2017).

Decreased dry matter intake (DMI) and increased respiratory rate are primary responses to HS (Ferrazza et al., 2017). The increased respiratory rate is an excellent indicator of HS (Ferrazza et al., 2017) and an essential thermoregulatory mechanism (McDowell et al., 1976). However, its excessive increase is considered a signal of low HS tolerance (Pereira et al., 2008). Additionally, HS increases water intake (Nardone et al., 1997), standing time (Allen et al., 2015), and sweating (Veissier et al., 2018).

Heat stress also depresses the immune system. The increased plasma cortisol concentrations impair phagocytic cells and lymphocytes (Sgorlon et al., 2012). Additionally, the increase in the Th1:Th2 ratio and plasma concentrations of transthyretin, tumor necrosis factor alpha (TNFα), and IL-6, well-known inflammatory biomarkers, characterize HS as a systemic inflammatory condition (Min et al., 2016).

**Cellular responses to heat stress**

HS increases the intracellular concentration of reactive oxygen species (ROS) (Yu et al., 2006; Gu et al., 2015) that promotes accumulation of unfolded/misfolded proteins (Read and Schröder, 2021) and DNA damage (Srinivas et al., 2019); ROS also activates the NRF2 transcription factor for antioxidant genes, e.g., catalase, and superoxide dismutase 1, which are fundamental for ROS excess neutralization (Amin et al., 2014).

To control the accumulation of unfolded/misfolded proteins, cells respond by increasing concentrations of chaperones, e.g., heat shock protein 60 (HSP60), HSP70, molecules with a primary function of supporting protein folding and refolding (Wang et al., 2020), mitigating deleterious effects of ROS and preventing apoptosis (Bindu et al., 2011). In response to DNA damage, the cell increases its P53 concentrations, which reduces metabolism and interrupts the cell cycle to focus on DNA repairs. However, if cell impairment is irreversible, P53 triggers apoptosis (Kruiswijk et al., 2015).

All cellular alterations induced by HS were recently described in bovine granulosa cells (BGC) cultured in vitro (Alelu et al., 2018; Wang et al., 2020; Khan et al., 2020). In these cells, HSP70 also reduces genes and protein expression of follicle stimulating hormone receptors and the aromatase, reducing 17β-E2 (Li et al., 2017).

**Heat stress reproductive effects**

_Estrus expression and sexual behavior_
HS severely impacts sexual behavior by decreasing physical activity, reducing mating and mating acceptance behaviors during estrus (Pennington et al., 1985). Furthermore, increased plasma concentrations of ACTH and cortisol are also related to reduced estrus expression, as cortisol decreases cerebral sensitivity to 17β-E₂. Furthermore, synthesis of 17β-E₂ is also impaired due to reduced LH pulsatility (Hein and Allrich, 1992) and reduced activity of ovarian steroidogenic enzymes (Wolfenson et al., 2000).

Reduced 17β-E₂ concentrations decrease estrus duration and intensity (Bolocan, 2009) and reduce vaginal mucosal hyperemia and mucus discharge (Schüller et al., 2017). HS drastically reduces the estrus detection rate (De Rensis and Scaramuzzi, 2003), and consequently, pregnancy rate (Black et al., 2018).

**Follicular development and ovulation**

Due to reduced LH pulsatility and direct effects of HS on theca and granulosa cells (Hein and Allrich, 1992), HS substantially impairs ovarian follicular development and oocyte and embryo competency (Wilson et al., 1998ab; Wolfenson et al., 2000). Compromised follicles produce less 17β-E₂ and inhibin, preventing control of plasma FSH and, consequently, delay follicle selection and deviation (Wolfenson et al., 1995; Roth et al., 2000). The delay reduces the probability of *B. taurus* cow having a follicle of ~12 mm, with full ovulatory capacity (Schüller et al., 2017; Sartori et al., 2001).

After a dairy cow is exposed to prolonged HS, conception rate (CR) will only return to normal in ~40 to 60 d (Morton et al., 2007). Similarly, in cattle more tolerant to heat, e.g., Gir cows (*B. indicus*), even at 100 d after the HS episode, still had lower follicular steroidogenesis and oocyte incompetence (Torres-Júnior et al., 2008).

**Luteogenesis, luteal function and luteolysis**

Cows kept permanently under HS initially have plasma P₄ concentrations similar to those in a thermoneutral (TN) environment, although there are lower P₄ concentrations between the 6 and 14 days after estrus (Howell et al., 1994; Wolfenson et al., 1988a; Alhussien et al., 2018), probably due to HS effects on luteal development. However, when HS occurs after ovulation, plasma P₄ concentrations are similar in HS and TN cows (Wilson et al., 1998b; Mogollón et al., 2020), implying that post-ovulation HS does not affect luteal steroidogenesis or P₄ metabolism.

In vitro, endometrial explants from pregnant or non-pregnant Angus and Brangus cows collected 17 days post-estrus produced more PGF2α when exposed to HS (42 ºC) than those kept under TN condition (39 ºC) (Putney et al., 1988, 1989). Additionally, oxytocin challenge increased PGF2α production of non-pregnant cow explants, independent of temperature, whereas in pregnant cows explants, an increase was only observed under HS (Putney et al., 1988). In another in vitro study, HS increased PGF2α production of endometrial explants from non-pregnant Brangus cows on the 17th day of the cycle but did not compromise the capacity of the INTr to halt this increase (Malayer et al., 1990). In a recent study, cells from the endometrial stroma of non-pregnant cows had increased in vitro synthesis of PGF2α under HS conditions; in addition, TNFα potentiated this increase, but IFNτ suppressed it (Sakai et al., 2021). However, in dairy cows, pregnant or not, on the 17th day post-estrus, 7.5 hours of HS did not alter plasma PGFM concentrations, indicating no increased PGF2α (Putney et al., 1989).

Contradictory results were also reported in vivo. In two studies, HS increased luteal phase ~9 and ~2 days in cows and heifers, respectively (Wilson et al., 1998a,b), implying delayed luteolysis. However, this was not confirmed in another study (Trout et al., 1998), and in yet another, there were indications of premature luteolysis (Wolfenson et al., 1988a). Lastly, in a study by our group, HS did not affect luteolytic responses in non-lactating Holstein cows given 5 or 12.5 mg of PGF2α, implying that HS did not alter PGF2α luteal sensitivity (Mogollón et al., 2020).

**Establishment and maintenance of pregnancy**

Cows under HS have lower rates of fertilization (Sartori et al., 2002) and conception (Schüller et al., 2017; Roth, 2020). Furthermore, as 17β-E2 are essential for expression of endometrial P4 receptors (Martin et al., 2008) and preparing the uterus for pregnancy (Binelli et al., 2014), the low plasma 17β-E2 observed during HS are likely insufficient to prepare the endometrium to support early embryo development.
and implantation. Also, HS reduces luteogenesis, plasma P4, and pregnancy establishment and maintenance (Alhussien et al., 2018). Besides being fundamental for maintenance of pregnancy, P4 also seems to be required for immune tolerance of the conceptus (Robinson and Klein, 2012). Importantly, in this review, the harmful impacts of HS upon oocytes and embryos will not be discussed. This subject was reviewed by Moura et al. (2021) and can be assessed in the Proceedings of this Congress.

Increased plasma concentrations of IL-2 and lower concentrations of IL-10 in cows under HS also seem to create unfavorable conditions for pregnancy maintenance (Alhussien et al., 2018). In women, the anti-inflammatory cytokines produced by Th-2 (e.g., IL-10) create favorable conditions for implantation, whereas pro-inflammatory cytokines from Th-1 cells (e.g., IL-2) are harmful to paternal antigens (Nickerson et al., 1994; Chatterjee et al., 2014).

As pregnancy advances, cows are more affected by deleterious effects of HS. In late pregnancy, HS impairs precalving mammary development (Tao and Dahl, 2013) and milk production in subsequent lactation (Do Amaral et al., 2009). Prepartum exposure to HS also delays the first breeding postpartum, reduces fertility, increases the calving to conception interval (Akbarinejad et al., 2017), and raises the risk of passive immunity transfer failure (Tao et al., 2012; Laporta et al., 2017).

Control of heat stress

Decreasing deleterious effects of HS depends on addressing global heating, including reductions in CO2 emissions by changing from fossil fuels to renewable energy sources (Tollefson, 2018). Also, as dairy cattle are responsible for 20% of global methane production, another greenhouse gas, an increased efficiency is also necessary, enabling sustained or increased production with fewer cows (Pryce and Bell, 2017).

The urgency and need for HS mitigating measures depend on the stage of the production cycle. In dairy cows, critical stages are the dry, transition, and lactation periods (Collier et al., 1982; Wolfenson et al. 1988b). Holstein cows prefer shade areas (Schütz et al., 2011), and even if such shade does not provide a THI < 72, it reduces respiratory rate and increases DMI (Abreu et al., 2020).

As ambient temperature increases, animal cooling mechanisms change from evaporative to non-evaporative. Novel ambient cooling systems, integrating fans and water sprinklers, are highly efficient in supporting evaporative heat loss (Negrón-Pérez et al., 2019). Increased evaporative heat loss, even when implemented for short intervals, before and after the artificial insemination, increased pregnancy rates in Holstein cows exposed to HS (Moghaddam et al., 2009).

Feeding management can also be used to mitigate increased ambient temperature. Feeding animals in the coolest periods of the day can increase dry matter intake (Hicks et al.,1989), whereas diets with less forage (Mader et al. 1999) and richer in fats and carbohydrates seem to reduce metabolic heat (West, 1999). Another nutritional approach to reduce impacts of HS and improve fertility is diets containing melatonin, chromium, immunomodulatory supplements, and antioxidants (Hall et al., 2014; Negrón-Pérez et al., 2019).

In addition, fixed-time artificial insemination protocols can also mitigate HS harmful effects on estrus behavior display, improve oocyte quality and increase service and pregnancy rates (De La Sota et al., 1998). Furthermore, in vitro embryo production under thermoneutral conditions attenuates adverse effects of HS during initial development, enabling transfer of a morula or blastocyst that is more resistant to HS, increasing fertility (Hansen and Areéchiga, 1999).

Intense genetic selection for milk production reduced cattle HS tolerance due to a strong negative correlation (Pryce and Haile-Mariam, 2020). Therefore, global warming challenges the scientific community to pursue genetic improvement strategies that restore thermotolerance without impairing production (Scheper et al., 2016).

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