



## Review article

## Heat stress on cattle embryo: gene regulation and adaptation

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## ABSTRACT

Global warming has been affecting animal husbandry and farming production worldwide via changes in organisms and their habitats. In the tropics, these conditions are adverse for agriculture and animal production in some areas, due to high temperatures and relative humidity, affecting competitiveness related to economic activities. These environments have deteriorated livestock production, due to periods of drought, reduction in forage quality and heat stress, eliciting negative effects on reproduction, weight gain, and reduced meat and milk production. However, the use of animals adapted to tropics such as breeds derived from subspecies *Bos primigenius indicus* and native breeds from tropical countries or their crossings, is an alternative to improve production under high-temperature conditions. Therefore, physiological adaptation including gene expression induced by heat stress have been studied to understand the response of animals and to improve cross-breeding between cattle breeds to maintain high productivity in adverse weather conditions. Heat stress has been associated with lower reproductive performance in cows, due to the impact on blastocyst production, decreased implantation and increased embryonic death. Thus, for decades, *in vitro* fertilization and embryo transfer techniques have focused on studying the optimal conditions for production of high-quality embryos to transfer. The aim of this review is to discuss the effects of heat stress in bovine embryos, and their physiological and genetic modulation, focusing on the genes that are related with major adaptability to heat stress conditions and their relationship with different embryonic stages.

## 1. Introduction

The increase of the world population has demanded a greater production of protein and generates pressure on animal production (Hill and Wall, 2017; Van Zanten et al., 2018), including livestock in tropical and sub-tropical developing countries, which supply a high percentage of the world's meat consumption (Renaudeau et al., 2012). However, climate change and environmental conditions are critical issues on cattle production worldwide (Rust, 2019; Ealy et al., 1993; El-Sayed and Kamel, 2020). The combination of high temperature with humidity may induce physiological changes in animals, leading to low reproductive performance during the hot seasons (Ju, 2005); which can be aggravated by low nutritional value of fodders, infectious and parasitic diseases (Hernández-Castellano et al., 2019).

Stress describes a neuroendocrine response in front of internal or external stimuli (*i.e.* stressor) that causes an imbalance of homeostasis and alters physiological conditions (Fraisse and Cockrem, 2006; Koolhaas and Van Reenen, 2016; Chrousos and Gold, 1992; Smith and Vale, 2006). The stress response is divided into acute and chronic phases (Koolhaas and Van Reenen, 2016), and its physiological consequences are influenced by the individual's perception of their ability to cope with the stressor (Fink, 2016; Koolhaas and Van Reenen, 2016; Lucy, 2019). The acute stress responses can last from minutes to days after the beginning of the exposure to the stimuli, and it is driven by the autonomic nervous system, promoting the release of catecholamines and glucocorticoids (Koolhaas and Van Reenen, 2016; Collier et al., 2017). In the chronic response, stress is driven mainly by the endocrine system and it is associated with alterations in the homeostatic signals (Collier et al., 2017). The stress response has similar phases to the general adaptation

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syndrome described by Selye in the '30s and '70s, suggesting three consecutive stages, the first is the alarm reaction, referred to as “shock”; the second one is the stage of resistance; and the third is the stage of exhaustion (Selye, 1946; Koolhaas and Van Reenen, 2016).

## 2. Neuroendocrine stress regulation

Two different neuroendocrine signaling axis have been identified in mammals producing an integrated physiological stress response: first, hypothalamic–pituitary–adrenocortical axis (HPA), which elicits an endocrine response mediated by the release of glucocorticoids from the adrenal cortex (Herman and Cullinan, 1997); second, neuroendocrine signaling, which is mediated by sympathetic-adrenal-medullary (SAM) axis, and involves the release of catecholamines from the adrenal medulla, and initiates the “fight or flight” responses, altering the metabolic and immune cell function (Smith and Vale, 2006; Aich et al., 2007; Kolli et al., 2014; Chen et al., 2015; Fink, 2016).

In the stress responses, the HPA axis is activated (Collier et al., 2017). The hypothalamus secretes corticotropin-releasing hormone (CRH). This CRH travels through the hypothalamic-pituitary portal system and causes the release of adrenocorticotropic hormone (ACTH) from the pituitary gland, which leads the adrenal gland to synthesize and secrete glucocorticoid (Lucy, 2019). There are indirect inhibitory effects of glucocorticoids on GnRH (Gonadotropin-releasing hormone) and LH (luteinizing hormone). These effects are mediated by the KNDy cells (Kisspeptin, neurokinin B, and dynorphin neurons) which possess glucocorticoid receptor, and transmit the glucocorticoid signal to the GnRH neurons in the hypothalamus (Ralph et al., 2016; Lucy, 2019; Scott et al., 2018).

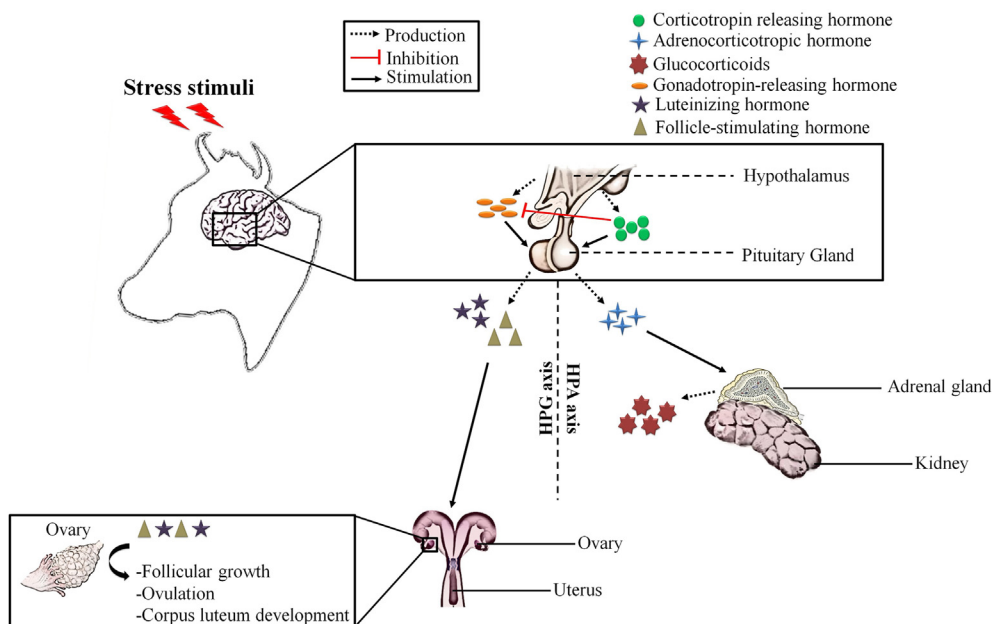
Glucocorticoids improve energy mobilization through hepatic gluconeogenesis to maintain the energy balance (Ayoob et al., 2018), however, an increased level of cortisol would affect the expression of genes related to metabolism, including fructose and pentose phosphate pathways in response to stress (Kolli et al., 2014). Besides, cortisol affects negatively the synthesis of GnRH, which is responsible for the regulation of LH and follicle-stimulating hormone (FSH) in the pituitary gland, impacting the reproductive performance in animal production, due to the role of these hormones in mammalian sexual maturation and reproductive function, including gametogenesis, steroidogenesis, and ovulation

(Roth et al., 2000; Whitfield, 2016; Wolfenson and Roth, 2019) (Figure 1).

The gonadotropins play an important role in ovarian function, including the regulation of follicular growth, ovulation, and corpus luteum development (Wolfenson and Roth, 2019). However, stimuli as heat stress alter steroid and hormone levels, with detrimental effects on ovarian function, resulting in higher levels of progesterone with inhibition of LH secretion, leading to a disturbance of the growth of dominant follicles and oocyte fertilization, carrying finally to rejection of the embryo (Al-Katanani et al., 2002; Cotter, 2017; Khan et al., 2020; Rocha et al., 1998; Roth et al., 2000; Whitfield, 2016). Additionally, the immediate effect of heat stress was evidenced in the depression of follicular dominance, assessed by a decrease in the plasma inhibin levels, and consequently higher FSH concentrations (Roth et al., 2000).

## 3. Heat stress

Heat stress is defined as a misbalance between the proportion of heat acquired by different sources as the body metabolism and the environmental conditions, against the heat dissipation system by the body that triggers an increase in body temperature of the animal (Brown-Brandl, 2018; Thatcher, Flamenbaum, Block and Bilby, 2010; Bernabucci et al., 2014; Collier et al., 2017; Lees et al., 2019). In tropical climates, cattle production systems are mostly grazing, and animals are constantly exposed to solar radiation, high ambient temperature, high humidity, and wind speed, increasing the effective temperature of the environment above the thermoneutral zone of livestock which generates heat stress (Bernabucci et al., 2010; Barragán-Hernández et al., 2015; Belhadj Slimen et al., 2016). Mammalian thermoregulatory mechanisms such as conduction, convection, and radiation are effective ways to lose heat, however, when these routes of heat exchange are lost, the remaining route of heat loss is through evaporative routes such as sweating and panting (Collier et al., 2019). Higher respiratory frequency, sweat rate, and peripheral vasodilation are several effector responses in front of heat stress, determining the internal body temperature (Scharf et al., 2010). Likewise, these responses may be insufficient at high temperature and high ambient humidity conditions, decreasing the rate of sweat evaporation and consequently, the body's capacity to dissipate heat (Silanikove, 2000; Cordeiro et al., 2020; Collier et al., 2019).



**Figure 1.** Inhibition of the hypothalamic-pituitary-gonadal (HPG) axis by the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The activation of HPA axis is a conserved response to stress in mammals, which begins with the production of CRH induced by a stress stimulus, leading to the production of ACTH from the pituitary gland and stimulating the adrenal gland to produce glucocorticoids. High levels of CRH reduce the production of the GnRH that stimulate the liberation of LH and FSH from the pituitary gland. These hormones allow the follicular growth in the ovary, ovulation and corpus luteum development, thereby the reduction in their level compromise the ovary function.

The cellular response to heat stress is one component of the acute systemic phase, involving genes modulation in different cells and tissues, activation of intra and extracellular signaling pathways that modify the body metabolism (Collier et al., 2008). Transcriptomic analyses have shown that environmental conditions can alter the expression of stress-related genes (Deb et al., 2014; Kumar et al., 2015), performance (Finney, 2018), metabolism (Bernabucci et al., 2014), fertility (Li et al., 2016), semen quality (Llamas Luceño et al., 2020; Rajoriya et al., 2014), and embryo's quality and viability (Silva et al., 2013). In cattle, a short-term increase in temperature during oocyte maturation has a deep impact on embryo yield and quality, induces alterations in oxidative balance within the oocyte and the surrounding cumulus cells, and produce significant alterations in the gene expression in the oocyte, cumulus cells and blastocysts (Stamperna et al., 2020).

Heat stress has been shown short and long term negative effects on reproduction in cattle (Wolfenson et al., 1997), including decreased fertility (Khan et al., 2020; Wolfenson and Roth, 2019; Mellado et al., 2013; Hansen, 2019), reduction in the follicular growth (de et al., 2008), postpartum anoestrus (Benyei et al., 2001), oocyte quality (Abdelatty et al., 2018; Paula-Lopes and Hansen, 2002; Rispoli et al., 2011), implantation and viability of blastocyst (Burkuš et al., 2015; Vasconcelos et al., 2006) and fetal development (Koch et al., 2016).

Currently, the adaptation to the tropic weather is a concern in the use of some cattle breeds, due to an inverse relationship between production level and heat tolerance, related to environmental temperature (Renau-deau et al., 2012). The use of breeds adapted to specific tropical regions together with the improvement of quality and digestibility of diverse crops, tolerant to droughts, and high temperatures, will be essential in the coming years for dairy and meat production in the tropics (Hernández-Castellano et al., 2019). Crossbreeding is one of the alternatives to enhance the productivity in hot climates; based on the crossing of heat-tolerant breeds (e.g. zebu and creole) with high production European breeds (Hansen et al., 2004). Thus, the identification of specific genes conferring thermotolerance in cattle breeds and single animals may be an additional strategy to improve the genetic background in production breeds (Hassan et al., 2019). This review aims to discuss the impact of heat stress on gene expression and modulation in cows and cattle embryos.

#### 4. Heat stress response in cattle

Cattle ethology is considered an important adaptive aspect inherently related to breed, leading to a higher thermal tolerance as observed in the Brahman breed and other *Bos indicus* cattle, compared to *Bos taurus* breeds (Brown-Brandl et al., 2006; Kadzere et al., 2002). To ameliorate the negative effects of direct heat, some cattle breeds spend more time in the shade (Adamczyk et al., 2015; Curtis et al., 2017; Shilja et al., 2016), compared to highly adapted tropical native breeds (Sejian et al., 2018). In desert regions, adapted breeds spend more time in standing positions which allows them to reorient themselves in different directions, avoiding direct solar and ground radiation. It also facilitates the dissipation of body heat to the surroundings by increasing the amount of skin exposed to airflow or wind (Chedid et al., 2014).

In addition to behavioral adaptation, the physiological response to dissipate body heat includes an increase in respiration rate, panting, higher drinking frequency, sweating, and reducing food intake (Rensis and Scaramuzzi, 2003; Schütz et al., 2009). However, physiological adaptations to heat stress can compromise other systems, e.g. in the cardiovascular system, the redistribution of blood flow from the viscera to the periphery during heat stress allows the dissipation of body heat, but it also leads to reduced perfusion of the placental vasculature and a delay in fetal growth (Hansen and Areéchiga, 1999). In Holstein heifers, the effect of heat stress during the final 46 days of gestation affects growth and milk production (Monteiro et al., 2016; Brown et al., 2016a, 2016b).

Heat stress can also impair the production of the hormones that regulate ovarian function such as GnRH, LH, and FSH, leading to poor

follicle maturation (Abdelatty et al., 2018; Khodaei et al., 2011). Impaired ovarian function determines a reduction in the number of ovulations and the number of viable embryos measured in the embryo recovery rate, pregnancy, and conception rate (Benyei et al., 2001). Despite beef cattle are less affected by heat stress due to their lower metabolic rate and lower body heat production compared to dairy cattle; its pregnancy rate can be negatively affected (Amundson et al., 2006). Both, dairy and beef cattle exposed to heat stress show a decrease in welfare and productivity performance (Summer et al., 2019), leading to a reduction in the conception rate between 20% and 30% (Rensis and Scaramuzzi, 2003). Similar alterations have been observed in buffaloes under heat stress (Dash et al., 2016).

#### 5. Heat stress in cattle embryo

##### 5.1. Embryo environment

Initially, the embryo's environment refers to the conditions in the oviduct, since there the fertilization takes place through the modulation of the composition of the medium to allow sperm capacitation, transport of mature sperm in the ampulla until reaching the oocyte, and early embryonic development in the isthmus (Leese et al., 2007; Leese et al., 2001). The bovine oviduct is a small-elongated organ, which connects the ovaries to the tip of the uterine horns; when gametes enter the oviduct and meet in the ampulla, where fertilization occurs (Maillo et al., 2016). Bovine embryos remain in the oviduct from 3,5 to 4 days and migrate to the distal end of the isthmus before they enter the uterus (Besenfelder et al., 2020).

The oviductal fluid contains simple and complex carbohydrates, ions, lipids, phospholipids, and proteins (Avilés et al., 2010; Mouguelar et al., 2015; Yániz et al., 2000; Kölle et al., 2009; Yániz et al., 2000; Mouguelar et al., 2015), which serve as substrates to produce lactate, pyruvate, and glucose, as well as amino acids, to maintain the embryo (Hugentobler et al., 2007). Some proteins as glucodelins and lactoferrin present in the oviductal fluid are involved in the interaction with gametes (Ghersevich et al., 2015), and others such as oviductin (OVPG1), osteopontin, and complement protein C3 contribute to early embryonic development (Tse et al., 2008). OVPG1 promotes sperm capacitation while maintaining mobility and viability (Coy et al., 2012), and it has been suggested as a protein with a protective activity of the early embryo (Ghersevich et al., 2015). Oviductin is found in the perivitelline space and membrane of embryos of different species before implantation, which could act as a protective 'shield' around the early embryo (Ghersevich et al., 2015). Thus, changes in the composition of the oviductal fluid and gene expression reflect the ability of the oviduct to adapt to the environment in different events from fertilization to early embryonic development (Maillo et al., 2015).

In the same way, the fertilization protocols and maturation embryo techniques *in vitro* are designed to mimic at laboratory conditions the environment provided by the oviduct through supplements in the culture media (van der Weijden et al., 2017). Despite this, gene expression analysis between *in vitro* and *in vivo* conditions has shown a down-regulation of genes related to RNA processing in the *in vitro* model (Smith et al., 2009), exhibiting the differences only in the embryo production process. Besides the changes inherently to embryo culture conditions, it has been demonstrated that an increase in the environment temperature of the donor females caused by the season change, directly decreases the proportion of the viable oocytes (Al-Katanani et al., 2002).

Regarding the impairment of oocyte function in heat-stressed cattle, this may result from alteration of oocyte membrane characteristics such as fatty acid composition, physical properties, or changes in follicular fluids contents such as insulin-like growth factor binding proteins or steroids (Lopes et al., 2012; Roth, 2017; De Rensis et al., 2017).

## 5.2. Effects of heat stress in cattle embryo

The effects of heat stress in the embryo quality can come from environmental exposure; it has been shown that heat stress can disturb the oocyte RNA, preformed related proteins as heat shock proteins and other components including antioxidants, compromising the later phases of its development (Ealy et al., 1993; Pavani et al., 2015; Verdoljak et al., 2018; Sakatani, 2017). Rispoli et al. (2011) showed a decrease in the cleavage rate, embryo maturation, and the percentage of blastocyst coming from oocytes exposed to heat stress (12 h of heat stress at 41 °C).

In the same way, changes in sperm conditions can alter the outcome of embryo development (Rahman et al., 2018). An increase of the body temperature has been shown to reduce the total sperm count and motility, including changes in the morphology of the spermatozoa and an increase in the ROS (reactive oxygen species) level (Skinner and Louw, 1966; Rahman et al., 2018). In Nelore bulls, sperm exposed to hydrogen peroxide (an oxidant agent) was related to lower cleavage and blastocyst rates, supporting the idea of the effect of sperm conditions before the fertilization on the embryo development (de Castro et al., 2016).

Embryonic death has been related to heat stress due to the increase of the cow internal temperature (over 39 °C) which occurs within the first six days of embryo development, because of the lack of production of heat-tolerant proteins that protect the embryo in the uterus (Bailey et al., 2016). Besides, it probably reduces the ability of the embryo to become transcriptionally competent (Hansen, 2007). In the case of the 2-cell embryo stage, exposure to high temperatures causes changes in microfilament and microtubule network (Rivera et al., 2004); depolarization of the cell by the increase in the number of swollen mitochondria (Rivera et al., 2003), and production of ROS (Sakatani et al., 2004). Later embryos, such as blastocyst, develop thermotolerance by the accumulation of antioxidants such as reduced glutathione (GSH), in response to heat-inducible production of ROS and the ability to synthesize heat shock protein 70, HSP70 (Boni, 2019). In blastomeres after exposure to 41 °C for 9 h, it was found a high percentage (15,8%) of embryos with DNA fragmentation (Paula-Lopes and Hansen, 2002). Similarly, *in vitro* embryo fertilization and development are susceptible to the heat stress before the EGA (embryonic genome activation) at the 8-cell stage, *i.e.* between the oocyte and cleavage stage (Graf et al., 2014a,b). Exposure of the zygote and 2-cell embryo to the high temperatures causes a large reduction in the percentage of embryos that reach the blastocyst stage (Hansen, 2019). This susceptibility has been associated with a reduction in the antioxidant level and the addition of antioxidants in culture media reduces the effects of heat stress in embryos produced *in vitro* (Sakatani, 2017; Torres-Osorio et al., 2019).

The bovine embryos are susceptible to oxidative damage, especially the IVP (*in vitro* production) embryos (Rocha-Frigoni et al., 2014). However, it has been reported that the Glutathione (GSH) and other antioxidants maintain the intracellular redox balance and increase the developmental rate of the stages morula (13%) and blastocyst (20%) (Guo et al., 2020; Takahashi et al., 1993). The embryo resides in the oviductal microenvironment for the first 3–4 days until a 16-cells stage before to enter to the uterus; here the embryo interacts with oviductal

fluid proteins as the insulin-like growth factors, glycoproteins, and binding proteins that allow the embryo development; these proteins have been selected as targets to improve *in vitro* embryo production methods (Pillai et al., 2017). Jousan and Hansen (2007) showed that the addition of insulin-like growth factor I (IGF-I) to *in vitro* cultures inhibited heat-induced apoptosis, leading to embryonic survival. This apoptosis is mediated by activation of group II caspases that are responsible for the destruction of structural and regulatory proteins that lead to DNA damage and cell demise (Hansen, 2007; Wolfenson and Roth, 2019). Changes in the blastocyst rate, cleavage rate, and other effects of heat stress can be seen in Table 1.

On the other hand, heat stress can alter DNA replication, protein assembly, and membrane fluidity (Higashikubo et al., 1993; Horvath et al., 1998; Kapila et al., 2016). Heat shock proteins (HSPs), also known as chaperones, have a pivotal role in the response to thermal stress (Li and Srivastava, 2004) and their level of expression depends on the magnitude of the stressor stimuli, *e.g.* HSP70 usually increases its expression at severe heat exposures more than 3 °C to normal temperature in short periods. HSPs act as a molecular chaperone protecting the protein machinery and avoiding caspase-mediated cell death or necrosis induced by oxidative stress (Creagh et al., 2000; Sonna et al., 2002). Also, several genes are activated in front of a variety of stress stimuli including cyclooxygenase 1 and 2 (COX1, COX2), X-linked inhibitor of apoptosis (XIAP), B-cell lymphoma 2 (BCL-2), among others (Creagh et al., 2000; Hiramatsu et al., 2014; Pihan et al., 2017; Rossi et al., 2012).

These changes in the expression of genes associated with heat shock response have boosted the development of new studies to characterize bovine breeds and improve the *in vivo* and *in vitro* fertilization protocols (Silva et al., 2013; Urrego et al., 2014), to select crossbreeds that can tolerate adverse temperatures and sustain high productivity. Thus, previous reports have demonstrated the effects of heat stress on the nuclear and cytoplasmic maturation, in addition to alterations in mRNA transcripts level, protein synthesis, and membrane phospholipid composition, in cattle oocytes and embryos (Al-Katanani et al., 2002; Edwards et al., 2005; Memili and First, 2000).

## 5.3. Transcriptomic response to heat stress in cattle embryo

Activation of gene machinery (demethylation or acetylation of histones, activation of transcription factors, and enhancers) to increase the transcription of HSPs genes (Cagnone and Sirard, 2016; Cassar-Malek et al., 2008) is an embryonic response to heat stress. However, early embryonic development is initially carried out by maternal transcripts and different proteins produced by the oocyte during oogenesis (Graf et al., 2014). This occurs from the fertilization to 2-cell stage in mouse (Schultz, 1993), 4–8 cells stage in humans (Braude et al., 1988) and 8–16 cells stage in bovines (Jiang et al., 2014).

The transcription of heat stress-related genes during embryonic development not only depends on transcription factors and EGA but also on epigenetic regulation (de Barros and Paula-Lopes, 2018). This includes the methylation patterns made by the DNA methyltransferases (DNMT) in the process called embryo epigenetic reprogramming, which

**Table 1.** Effects of heat stress in bovine embryos.

	Decrease in the blastocyst developmental rate	Decrease in pregnancy rates	Decrease in cleavage rate	Alteration in the cell structural morphology	Increase in the ROS	Increase in the number of apoptotic cells
Breed	NS <sup>a,d,g</sup> , Jersey <sup>h</sup> , Angus <sup>h</sup> , Japanese black <sup>b</sup> , Holstein <sup>c</sup>	Holstein <sup>l,k</sup> , Holstein Friesian <sup>i</sup>	NS <sup>e</sup>	Angus <sup>l</sup>	NS <sup>a</sup>	NS <sup>g,m</sup> , Holstein <sup>c</sup>
Type of study	IVF <sup>a-h</sup> , IVM <sup>d-h</sup>	IVS <sup>i-k</sup>	IVF <sup>e</sup> , IVM <sup>e</sup>	IVF <sup>l</sup> , IVM <sup>l</sup>	IVF <sup>a</sup>	IVF <sup>c,g,m</sup> , IVM <sup>g,m</sup>

IVM: *In vitro* maturation; IVF: *In vitro* fertilization; IVS: *In vivo* study; NS: Does not specify.

<sup>a</sup>(Sakatani et al., 2004); <sup>b</sup>(Sakatani et al., 2012); <sup>c</sup>(Ferreira et al., 2011); <sup>d</sup>(Sugiyama et al., 2007); <sup>e</sup>(Sakatani et al., 2015); <sup>f</sup>(Bonilla et al., 2011); <sup>g</sup>(Nabenishi et al., 2012); <sup>h</sup>(Silva et al., 2013); <sup>i</sup>(Nabenishi et al., 2011); <sup>j</sup>(Ealy et al., 1993); <sup>k</sup>(Moghaddam et al., 2009); <sup>l</sup>(Rivera et al., 2003); <sup>m</sup>(Paula-Lopes and Hansen, 2002).



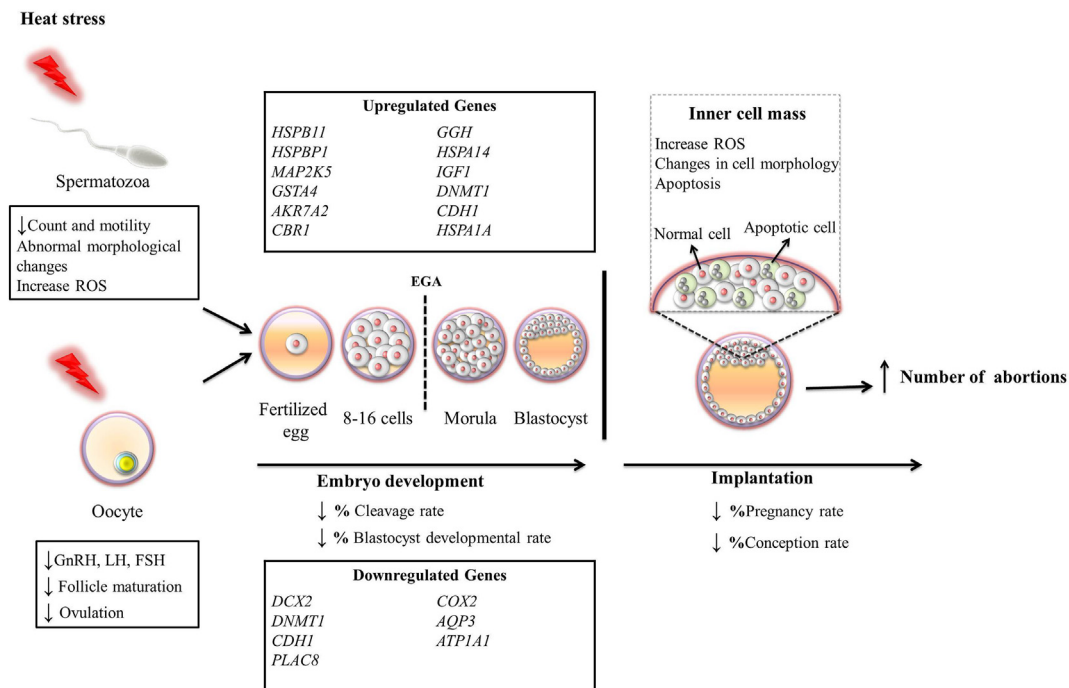
involve both paternal and maternal genome demethylation with de novo embryo methylations in 8- to 16-cell stage (Dobbs et al., 2013; Dean et al., 2001). This process can be affected by heat stress leading to markedly differences in gene expression (de Barros and Paula-Lopes, 2018). A recent study has been demonstrated that heat stress can lead to an increase of acetylation levels on the promoter for the *HSP70* gene in harsh-temperature-conditioned individuals (Kisliouk et al., 2017). Additionally, Camargo et al. (2019b) showed by immunofluorescence analysis an aggregation of the histone H3 lysine 9 trimethylation (H3K9me3) together with heterochromatin protein 1 (HP1) in the nuclei of embryos derived from heat-shocked bovine oocytes, suggesting abnormal chromatin compaction that can reduce the transcription of genes associated with embryo development leading to an increase of the proportion of apoptotic cells seen in these blastocysts compared to embryos derived from no heat-shocked oocytes (Camargo et al., 2019b). Despite this, epigenetic regulation under heat stress conditions and its effects on embryo development in livestock production is poorly understood. At the 8–16 cells stage coordinating with embryo epigenetic reprogramming, maternally derived RNA and proteins are degraded, and the EGA is initiated. This process has been named maternal-to-embryonic transition and includes the replacement of transcripts from the oocyte to the brand-new embryonic transcripts, like ribosomal RNAs (Graf et al., 2014). It has been reported that embryos in a preimplantation stage (before late blastocyst state) are more sensitive to stress conditions despite the EGA, and the heat sensitivity depends on the stage of the embryo (Sakatani et al., 2004).

One of the first produced proteins to respond to heat stress is the HSP70, which inhibits the activation of the caspase pathway, preventing the release of cytochrome C from the mitochondria and participates in the stabilization and refolding of the proteins damaged by heating (Mosser et al., 2000). In 2 and 4-cells embryos exposed to 42 °C, mRNA levels of HSP70 were increased (Chandolia et al., 1999). Sakatani et al. (2012), showing an increase in the expression of the *HSPA14* gene (a member of the HSP70 family) in the morula stage embryo exposed to thermal stress (40 °C, for 24 h). Additionally, the *HSPA14* gene has been

reported as the major inducible gene associated with heat shock response, protecting the embryo from death (Hassan et al., 2019).

Pavani et al. (2016) showed the relative gene expression of *HSPA14*, *DNMT1*, *Cx43*, and *CDH1* in different embryo stages (2-cells, 4-cells, morula, and blastocyst) under heat stress conditions. It was found that *in vitro* bovine embryos extracted from warm months have lower expression levels of *HSPA14* and *DNMT1* compared to embryos from cold months in 2-cells, 4-cells, and morula stage. The *Cx43* gene was downregulated in 2- and 4-cell stages from oocytes obtained in warm months. However, embryos from oocytes matured at 38,5 °C (control group), 39,5, and 40,5 °C (heat shock groups) for 24 h, showed upregulation of the *DNMT1* gene in 2-cells, 4-cell, and blastocyst stage in the heat shock groups. For the *HSPA14* gene was observed a downregulation in 2-cells, 4-cells, morula, and blastocyst stage in the heat shock groups compared to control. This gene has been associated as one of the first responder proteins to heat stress (Archana et al., 2017; Camargo et al., 2007). Interestingly, the gene to the cadherin-1 *CDH1* showed a bimodal response, initially showing a downregulation in the 2-cells, 4-cells, and morula stage in the heat shock groups, and then an upregulation in blastocyst stage in heat shock groups. The protein encoded by this gene is associated with intercellular interactions and possibly is related to late embryonic stages where the inner cell mass and trophectoderm begin the differentiation process. In the case of the Gap Junction Protein Alpha 1 or connexin-43 (*Cx43*), an upregulation in the expression of the gene was observed in the 4-cell stage in heat shock groups. The function of this protein in embryo response to heat shock has been associated with cell survival; it is even used as a marker for embryo quality and survival rate (Pavani et al., 2016).

Sakatani et al. (2013) analyzed the cattle embryo transcriptome using a 3'-tag digital gene expression, showing differences in the gene expression in morula-stage embryos cultured at 40 °C for 8 h compared to control (38 °C), increasing mRNA levels of *HSPB11* and *HSPBP1* related to heat shock proteins and in the *AKR7A2*, *CBR1*, *GGH*, *GSTA4*, and *MAP2K5* associated with oxidative stress. Interestingly, 38 out of 173 genes altered by heat shock were related to the ubiquitin signaling



**Figure 2.** Effects of heat stress in the different stages on embryo development. The figure shows the effects of heat stress either in the sperm, oocyte and early embryonic development, including the upregulated and downregulated genes in each embryo development stage (8–16 cells, morula, and blastocyst), lead to compromise the outcome the final stages of the embryo, reducing the pregnancy and conception rate by spontaneous abortion (Camargo et al., 2019a,b; Chandolia et al., 1999; Mosser et al., 2000; Pavani et al., 2016; Sakatani et al., 2012; Sakatani et al., 2013; Satrapa et al., 2013).

pathway, possibly as a mechanism to reduce the accumulation of denatured proteins, leading to degradation of those by the proteasome (Sakatani et al., 2013). These and other genes related to a heat stress response are summarized in Figure 2 (Figure 2).

Heat shock can downregulate the expression of specific genes, as shown by Camargo et al. (2019a), which made a comparison between *in vitro* fertilized (IVF) or parthenogenetic (Part) blastocysts derived from oocytes matured *in vitro* (IVM) under 41°C/12 h (heat shock treatment) followed by 38,5°C/12 h. This study showed a downregulation of the aquaporin 3 gene *AQP3* and upregulation of HSP70.1 (*HSPA1*) in blastocysts, and downregulation of the *ATP1A1* gene in IVF blastocysts. The aquaporin *AQP3* protein plays an important role in the cavitation of the blastocyst allowing the transport of water through the membrane and the *ATP1A1* protein is related to ion transport across the trophoctoderm. Both allow the embryo development and their downregulation may be associated with a higher apoptotic index observed in the heat stress embryos, which may serve as an indicator of quality and viability in embryo development (Camargo et al., 2019a).

Khan et al. (2017) showed the effects of coagulysin-A (coa-A) (a steroidal lactone that functions as an antioxidant and apoptosis regulator) in bovine embryos matured and fertilized *in vitro*. They found that embryos supplemented with coa-A showed an increase in HSP70 and PI3K (phosphatidylinositol 3-kinase) expression at a protein and genetic level. Additionally, the *BCL-2* gene was upregulated and *COX-2*, *iNOS*, *BAX*, *CASP3*, *p53*, *p21*, and *NF-κB* showed downregulation. The relevance of these findings is related to the high free radicals as ROS that is produced during a heat stress state leading to cell death; thus, the coa-A supplementation could be a way to reduce the effects of the heat stress in bovine embryos developed *in vitro* (Khan et al., 2017).

## 6. Concluding remarks

The heat stress response in bovine embryos are associated with an increase of the expression of chaperon proteins related genes of the HSPs family in early stages, genes related to oxidative stress *COX 1*, *GGH* and *DNMT*; protein signaling *MAP2K5*; cell to cell interaction *CDH1* and cell survival *BCL2*, *XIAP*. This response can affect the embryo development decreasing the cleavage rate by altering cell morphology, increasing ROS production, and the number of apoptotic cells, leading finally to a decreasing in the pregnancy rate. These effects are markedly higher in heat susceptible bovines as *Bos taurus* breeds in comparison with *Bos indicus* breeds. Thus, further studies are necessary to understand the genetic and protein modulation elicited by a heat stress response in cattle embryos as the HSP proteins, including their polymorphisms and expression levels in different breed crosses taking into account bovines susceptible and tolerant to heating; all of this considering several factors that influence the gene expression, as the time of exposure to heat stress, temperature level, embryo stage and type of fertilization technique, among others.

## Declarations

### Author contribution statement

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The authors declare no conflict of interest.

## Additional information

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## References

- Adamczyk, K., Górecka-Bruzda, A., Nowicki, J., Gumulka, M., Edyta, M., Schwarz, T., Czesław, K., 2015. Perception of environment in farm animals – a review. *Ann. Anim. Sci.* 15, 565–589.
- Abdelatty, A.M., Iwaniuk, M.E., Potts, S.B., Gad, A., 2018. Influence of maternal nutrition and heat stress on bovine oocyte and embryo development. *Int. J. Vet. Sci. Med.* 6 (Suppl), S1–S5.
- Aich, P., Jalal, S., Czuba, C., Schatte, G., Herzog, K., Olson, D.J., Griebel, P., 2007. Comparative approaches to the investigation of responses to stress and viral infection in cattle. *OMICS* 11 (4), 413–434.
- Al-Katanani, Y.M., Paula-Lopes, F.F., Hansen, P.J., 2002. Effect of season and exposure to heat stress on oocyte competence in Holstein cows. *J. Dairy Sci.* 85 (2), 390–396.
- Amundson, J.L., Mader, T.L., Rasby, R.J., Hu, Q.S., 2006. Environmental effects on pregnancy rate in beef cattle. *J. Anim. Sci.* 84 (12), 3415–3420.
- Archana, P., Joy, A., Prathap, P., Vidya, M., Niyas, P.A., Madijagan, B., Bhatta, R., 2017. Role of heat shock proteins in livestock adaptation to heat stress. *J. Dairy Vet. Anim. Res.* 5, 00127.
- Avilés, M., Gutiérrez-Adán, A., Coy, P., 2010. Oviductal secretions: will they be key factors for the future ARTs? *Mol. Hum. Reprod.* 16 (12), 896–906.
- Ayoub, A., Sejian, V., Madijagan, B., Krishnan, G., 2018. Heat stress and livestock adaptation: Neuro-endocrine regulation. *Int. J. Vet. Anim. Med.* 1 (2), 1–8.
- Bailey, T., Sheets, J., McClary, D., Smith, S., Bridges, A., 2016. Heat Abatement. Retrieved from. <https://assets.ctfassets.net>.
- Barragán-Hernández, Wilson, Andrés, Mahecha-Ledesma, Liliana, Cajas-Girón, Socorro, Yasmin, 2015. Variables Fisiológicas-Metabólicas de Estrés Calórico en Vacas Bajo Silvopastoreo y Pradera sin Árboles. *Agron. Mesoam.* 26 (2), 211–223.
- Belhadj Slimen, I., Najar, T., Ghram, A., Abdrabba, M., 2016. Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review. *J. Anim. Physiol. Anim. Nutr. (Berl)* 100 (3), 401–412.
- Benyei, B., Gaspard, A., Barros, C.W., 2001. Changes in embryo production results and ovarian recrudescence during the acclimatisation to the semiarid tropics of embryo donor Holstein-Friesian cows raised in a temperate climate. *Anim. Reprod. Sci.* 68 (1–2), 57–68.
- Bernabucci, U., Lacetera, N., Baumgard, L.H., Rhoads, R.P., Ronchi, B., Nardone, A., 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal* 4 (7), 1167–1183.
- Bernabucci, U., Biffani, S., Buggiotti, L., Vitali, A., Lacetera, N., Nardone, A., 2014. The effects of heat stress in Italian Holstein dairy cattle. *J. Dairy Sci.* 97 (1), 471–486.
- Besenfelder, U., Brem, G., Havlicek, V., 2020. Environmental impact on early embryonic development in the bovine species. *Animal* 14 (S1), s103–s122.
- Boni, R., 2019. Heat stress, a serious threat to reproductive function in animals and humans. *Mol. Reprod. Dev.* 86 (10), 1307–1323.
- Bonilla, A.Q., Oliveira, L.J., Ozawa, M., Newsom, E.M., Lucy, M.C., Hansen, P.J., 2011. Developmental changes in thermoprotective actions of insulin-like growth factor-1 on the preimplantation bovine embryo. *Mol. Cell. Endocrinol.* 332 (1–2), 170–179.
- Braude, P., Bolton, V., Moore, S., 1988. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature* 332 (6163), 459–461.
- Brown-Brandt, T., Nienaber, J., Eigenberg, R., Mader, T., Morrow, J.L., Dailey, J.W., 2006. Comparison of heat tolerance of feedlot heifers of different breeds. *Livest. Sci.* 105.
- Brown, B.M., Stallings, J.W., Clay, J.S., Rhoads, M.L., 2016a. Periconceptual heat stress of holstein dams is associated with differences in daughter milk production during their first lactation. *PLoS One* 11 (2), e0148234.
- Brown, B.M., Stallings, J.W., Clay, J.S., Rhoads, M.L., 2016b. Correction: periconceptual heat stress of holstein dams is associated with differences in daughter milk production and composition during multiple lactations. *PLoS One* 11 (2), e0150049.
- Brown-Brandt, Tami M., 2018. Understanding heat stress in beef cattle. *Rev. Bras. Zootec.* 47, e20160414. Epub November 29, 2018.
- Burkus, J., Kšiňanová, M., Babelová, J., Kokošová, N., Fabianová, K., Fabian, D., Čikoš, Š., 2015. Stress exposure during the preimplantation period affects blastocyst lineages and offspring development. *J. Reprod. Dev.* 61.
- Cagnone, G., Sirard, M.A., 2016. The embryonic stress response to *in vitro* culture: insight from genomic analysis. *Reproduction* 152 (6), R247–R261.
- Camargo, L.S., Viana, J.H., Ramos, A.A., Serapiao, R.V., de Sa, W.F., Ferreira, A.M., do Vale Filho, V.R., 2007. Developmental competence and expression of the Hsp 70.1 gene in oocytes obtained from *Bos indicus* and *Bos taurus* dairy cows in a tropical environment. *Theriogenology* 68 (4), 626–632.
- Camargo, L.S.A., Costa, F.Q., Munk, M., Wohlrés-Viana, S., Serapiao, R.V., Carvalho, B.C., Viana, J.H.M., 2019a. Contrasting effects of heat shock during *in vitro* maturation on development of *in vitro*-fertilized and parthenogenetic bovine embryos. *Reprod. Domest. Anim.* 54 (10), 1357–1365.
- Camargo, L.S.A., Aguirre-Lavin, T., Adenot, P., Araujo, T.D., Mendes, V.R.A., Louro, I.D., Souza, E.D., 2019b. Heat shock during *in vitro* maturation induces chromatin modifications in the bovine embryo. *Reproduction* 158 (4), 313–322.

- Cassar-Malek, I., Picard, B., Bernard, C., Hocquette, J.-F., 2008. Application of gene expression studies in livestock production systems: a European perspective. *Aust. J. Exp. Agric.* 48 (7), 701–710.
- Chandolia, R.K., Peltier, M.R., Tian, W., Hansen, P.J., 1999. Transcriptional control of development, protein synthesis, and heat-induced heat shock protein 70 synthesis in 2-cell bovine embryos. *Biol. Reprod.* 61 (6), 1644–1648.
- Chedid, M., Jaber, L., Giger-Reverdin, S., Duvaux-Ponter, C., Hamadeh, S., 2014. Review: water stress in sheep raised under arid conditions. *Can. J. Anim. Sci.* 94, 243–257.
- Chen, Y., Arsenaault, R., Napper, S., Griebel, P., 2015. Models and methods to investigate acute stress responses in cattle. *Animals (Basel)* 5 (4), 1268–1295.
- Chrousos, G.P., Gold, P.W., 1992. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 267 (9), 1244–1252.
- Collier, R.J., Collier, J.L., Rhoads, R.P., Baumgard, L.H., 2008. Invited review: genes involved in the bovine heat stress response. *J. Dairy Sci.* 91 (2), 445–454.
- Collier, R.J., Renquist, B.J., Xiao, Y., 2017. A 100-Year Review: stress physiology including heat stress. *J. Dairy Sci.* 100 (12), 10367–10380.
- Collier, R.J., Baumgard, L.H., Zimelman, R.B., Xiao, Y., 2019. Heat stress: physiology of acclimation and adaptation. *Anim. Front.* 9 (1), 12–19.
- Cordeiro, A.L.L., Satrapa, R.A., Gregianini, H.A.G., Gregianini, J.T.F., Maia, G.F.N., Landim-Alvarenga, F.C., 2020. Influence of temperature-humidity index on conception rate of Nelore embryos produced in vitro in northern Brazil. *Trop. Anim. Health Prod.* 52 (3), 1527–1532.
- Cotter, P., 2017. Basophilia and basophilosis in caged hens at 18 and 77 Weeks. *Int. J. Poultry Sci.* 16, 23–30.
- Coy, P., Garcia-Vázquez, F.A., Visconti, P.E., Avilés, M., 2012. Roles of the oviduct in mammalian fertilization. *Reproduction (Cambridge, England)* 144 (6), 649.
- Creagh, E.M., Sheehan, D., Cotter, T.G., 2000. Heat shock proteins—modulators of apoptosis in tumour cells. *Leukemia* 14 (7), 1161–1173.
- Curtis, A.K., Scharf, B., Eichen, P.A., Spiers, D.E., 2017. Relationships between ambient conditions, thermal status, and feed intake of cattle during summer heat stress with access to shade. *J. Therm. Biol.* 63, 104–111.
- Dash, S., Chakravarty, A.K., Singh, A., Upadhyay, A., Singh, M., Yousuf, S., 2016. Effect of heat stress on reproductive performances of dairy cattle and buffaloes: a review. *Vet. World* 9 (3), 235–244.
- Dean, W., Santos, F., Stojkovic, M., Zakhartchenko, V., Walter, J., Wolf, E., Reik, W., 2001. Conservation of methylation reprogramming in mammalian development: aberrant reprogramming in cloned embryos. *Proc. Natl. Acad. Sci. U. S. A.* 98 (24), 13734–13738.
- de Barros, F.R.O., Paula-Lopes, F.F., 2018. Cellular and epigenetic changes induced by heat stress in bovine preimplantation embryos. *Mol. Reprod. Dev.* 85 (11), 810–820.
- Deb, R., Sajjanar, B., Singh, U., Kumar, S., Singh, R., Sengar, G., Sharma, A., 2014. Effect of heat stress on the expression profile of Hsp90 among Sahiwal (*Bos indicus*) and Frieswal (*Bos indicus* x *Bos taurus*) breed of cattle: a comparative study. *Genus* 536 (2), 435–440.
- de Castro, L.S., de Assis, P.M., Siqueira, A.F., Hamilton, T.R., Mendes, C.M., Losano, J.D., Nichi, M., Visintin, J.A., Assumpção, M.E., 2016. Sperm oxidative stress is detrimental to embryo development: a dose-dependent study model and a new and more sensitive oxidative status evaluation. *Oxidat. Med. Cell. Longev.* 2016, 8213071.
- De Renzis, F., Lopez-Gatius, F., García-Ispuerto, I., Morini, G., Scaramuzzi, R.J., 2017. Causes of declining fertility in dairy cows during the warm season. *Theriogenology* 91, 145–153.
- de, S.T.-J.J.R., de, F.A.P.M., de, S.A., de, W.F., de, M.F.A., Viana, J.H., Camargo, L.S., Baruselli, P.S., 2008. Effect of maternal heat-stress on follicular growth and oocyte competence in *Bos indicus* cattle. *Theriogenology* 69 (2), 155–166.
- Dobbs, K.B., Rodriguez, M., Sudano, M.J., Ortega, M.S., Hansen, P.J., 2013. Dynamics of DNA methylation during early development of the preimplantation bovine embryo. *PLoS One* 8 (6), e66230.
- Ealy, A.D., Drost, M., Hansen, P.J., 1993. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. *J. Dairy Sci.* 76 (10), 2899–2905.
- Edwards, J.L., Saxton, A.M., Lawrence, J.L., Payton, R.R., Dunlap, J.R., 2005. Exposure to a physiologically relevant elevated temperature hastens in vitro maturation in bovine oocytes. *J. Dairy Sci.* 88 (12), 4326–4333.
- El-Sayed, A., Kamel, M., 2020. Climatic changes and their role in emergence and re-emergence of diseases. *Environ. Sci. Pollut. Res. Int.* 27 (18), 22336–22352.
- Ferreira, R.M., Ayres, H., Chiaratti, M.R., Ferraz, M.L., Araujo, A.B., Rodrigues, C.A., Baruselli, P.S., 2011. The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *J. Dairy Sci.* 94 (5), 2383–2392.
- Finney, A., 2018. Relationships Among Beef Cow Productivity Traits and Single Nucleotide Polymorphisms in the Bovine Heat Shock Protein 70 Gene. *Animal Science Undergraduate Honors Theses*. Retrieved from: <https://scholarworks.uark.edu/ansucht/19>.
- Fink, G., 2016. Chapter 1 - stress, definitions, mechanisms, and effects outlined: lessons from anxiety. In: Fink, G. (Ed.), *Stress: Concepts, Cognition, Emotion, and Behavior*. Academic Press, San Diego, pp. 3–11.
- Fraisse, F., Cockrem, J.F., 2006. Corticosterone and fear behaviour in white and brown caged laying hens. *Br. Poultry Sci.* 47 (2), 110–119.
- Ghersevich, S., Massa, E., Zumoffen, C., 2015. Oviductal secretion and gamete interaction. *Reproduction* 149 (1), R1–R14.
- Graf, A., Krebs, S., Heininen-Brown, M., Zakhartchenko, V., Blum, H., Wolf, E., 2014a. Genome activation in bovine embryos: review of the literature and new insights from RNA sequencing experiments. *Anim. Reprod. Sci.* 149 (1–2), 46–58.
- Graf, A., Krebs, S., Zakhartchenko, V., Schwalb, B., Blum, H., Wolf, E., 2014b. Fine mapping of genome activation in bovine embryos by RNA sequencing. *Proc. Natl. Acad. Sci. U. S. A.* 111 (11), 4139–4144.
- Guo, Q., Cui, L., Sun, W., Li, F., Hao, H., Zhao, X., Du, W., 2020. Long non-coding RNA and mRNA profiling in early-stage bovine embryos treated with glutathione. *Antioxidants (Basel)* 9 (5).
- Hansen, P.J., Arechiga, C.F., 1999. Strategies for managing reproduction in the heat-stressed dairy cow. *J. Anim. Sci.* 77 (Suppl 2), 36–50.
- Hansen, M., Lund, M.S., Pedersen, J., Christensen, L.G., 2004. Gestation length in Danish Holsteins has weak genetic associations with stillbirth, calving difficulty, and calf size. *Livest. Prod. Sci.* 91 (1), 23–33.
- Hansen, P.J., 2007. To be or not to be—determinants of embryonic survival following heat shock. *Theriogenology* 68 (Suppl 1), S40–48.
- Hansen, P.J., 2019. Reproductive physiology of the heat-stressed dairy cow: implications for fertility and assisted reproduction. *Anim. Reprod.* 16 (3), 497–507.
- Hassan, F.U., Nawaz, A., Rehman, M.S., Ali, M.A., Dilshad, S.M.R., Yang, C., 2019. Prospects of HSP70 as a genetic marker for thermo-tolerance and immunomodulation in animals under climate change scenario. *Anim. Nutr.* 5 (4), 340–350.
- Herman, J.P., Cullinan, W.E., 1997. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci.* 20 (2), 78–84.
- Hernandez-Castellano, L.E., Nally, J.E., Lindahl, J., Wanapat, M., Alhidary, I.A., Fanguero, D., de Almeida, A.M., 2019. Dairy science and health in the tropics: challenges and opportunities for the next decades. *Trop. Anim. Health Prod.* 51 (5), 1009–1017.
- Higashikubo, R., White, R.A., Roti Roti, J.L., 1993. Flow cytometric BrdUrd-pulse-chase study of heat-induced cell-cycle progression delays. *Cell Prolif.* 26 (4), 337–348.
- Hill, D.L., Wall, E., 2017. Weather influences feed intake and feed efficiency in a temperate climate. *J. Dairy Sci.* 100 (3), 2240–2257.
- Hiramatsu, N., Messah, C., Han, J., LaVail, M.M., Kaufman, R.J., Lin, J.H., 2014. Translational and posttranslational regulation of XIAP by eIF2alpha and ATF4 promotes ER stress-induced cell death during the unfolded protein response. *Mol. Biol. Cell* 25 (9), 1411–1420.
- Horvath, I., Glatz, A., Varvasovszki, V., Torok, Z., Pali, T., Balogh, G., Vigh, L., 1998. Membrane physical state controls the signaling mechanism of the heat shock response in *Synechocystis* PCC 6803: identification of hsp17 as a “fluidity gene”. *Proc. Natl. Acad. Sci. U. S. A.* 95 (7), 3513–3518.
- Hugentobler, S.A., Diskin, M.G., Leese, H.J., Humpherson, P.G., Watson, T., Sreenan, J.M., Morris, D.G., 2007. Amino acids in oviduct and uterine fluid and blood plasma during the estrous cycle in the bovine. *Mol. Reprod. Dev.: Incomp. Gamete Res.* 74 (4), 445–454.
- Jiang, Z., Sun, J., Dong, H., Luo, O., Zheng, X., Obergfell, C., Tian, X.C., 2014. Transcriptional profiles of bovine in vivo pre-implantation development. *BMC Genom.* 15, 756.
- Jousan, F.D., Hansen, P.J., 2007. Insulin-like growth factor-I promotes resistance of bovine preimplantation embryos to heat shock through actions independent of its anti-apoptotic actions requiring PI3K signaling. *Mol. Reprod. Dev.* 74 (2), 189–196.
- Ju, J.C., 2005. Cellular responses of oocytes and embryos under thermal stress: hints to molecular signaling. *Anim. Reprod.* 2 (2), 79–90.
- Kadzere, C.T., Murphy, M.R., Silanikove, N., Maltz, E., 2002. Heat stress in lactating dairy cows: a review. *Livest. Prod. Sci.* 77 (1), 59–91.
- Kapila, N., Sharma, A., Kishore, A., Sodhi, M., Tripathi, P.K., Mohanty, A.K., Mukesh, M., 2016. Impact of heat stress on cellular and transcriptional adaptation of mammary epithelial cells in Riverine buffalo (*Bubalus bubalis*). *PLoS One* 11 (9), e0157237.
- Khan, I., Lee, K.L., Xu, L., Mesalam, A., Chowdhury, M.M., Joo, M.D., Kong, I.K., 2017. Improvement of in vitro-produced bovine embryo treated with coagulansin-A under heat-stressed condition. *Reproduction* 153 (4), 421–431.
- Khan, A., Dou, J., Wang, Y., Jiang, X., Khan, M.Z., Luo, H., Zhu, H., 2020. Evaluation of heat stress effects on cellular and transcriptional adaptation of bovine granulosa cells. *J. Anim. Sci. Biotechnol.* 11, 25.
- Khodaei, M., Zare Shahneh, A., Masoumi, R., Derensis, F., 2011. Alteration in reproductive hormones during heat stress in dairy cattle. *Afr. J. Biotechnol.* 10 (29), 5552–5558.
- Kisliouk, T., Cramer, T., Meiri, N., 2017. Methyl CpG level at distal part of heat-shock protein promoter HSP70 exhibits epigenetic memory for heat stress by modulating recruitment of POU2F1-associated nucleosome-remodeling deacetylase (NuRD) complex. *J. Neurochem.* 141 (3), 358–372.
- Koch, F., Lamp, O., Eslamizad, M., Weitzel, J., Kuhla, B., 2016. Metabolic response to heat stress in late-pregnant and early lactation dairy cows: implications to liver-muscle crosstalk. *PLoS One* 11 (8), e0160912.
- Kölle, S., Dubielzig, S., Reese, S., Wehrend, A., König, P., Kummer, W., 2009. Ciliary transport, gamete interaction, and effects of the early embryo in the oviduct: ex vivo analyses using a new digital videomicroscopic system in the cow. *Biol. Reprod.* 81 (2), 267–274.
- Kolli, V., Upadhyay, R.C., Singh, D., 2014. Peripheral blood leukocytes transcriptomic signature highlights the altered metabolic pathways by heat stress in zebu cattle. *Res. Vet. Sci.* 96 (1), 102–110.
- Koolhaas, J.M., Van Reenen, C.G., 2016. Animal behavior and well-being symposium: interaction between coping style/personality, stress, and welfare: relevance for domestic farm animals. *J. Anim. Sci.* 94 (6), 2284–2296.
- Kumar, A., Ashraf, S., Goud, T.S., Grewal, A., Singh, S.V., Yadav, B.R., Upadhyay, R.C., 2015. Expression profiling of major heat shock protein genes during different seasons in cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) under tropical climatic condition. *J. Therm. Biol.* 51, 55–64.



- Lees, A.M., Sejian, V., Wallage, A.L., Steel, C.C., Mader, T.L., Lees, J.C., Gaughan, J.B., 2019. The impact of heat load on cattle. *Animals (Basel)* 9 (6).
- Leese, H.J., Hugentobler, S.A., Gray, S.M., Morris, D.G., Sturme, R.G., Whitear, S.-L., Sreenan, J.M., 2007. Female reproductive tract fluids: composition, mechanism of formation and potential role in the developmental origins of health and disease. *Reprod. Fertil. Dev.* 20 (1), 1–8.
- Leese, H.J., Tay, J.L., Reischl, J., Downing, S.J., 2001. Formation of Fallopian tubal fluid: role of a neglected epithelium. *Reproduction (Cambridge, England)* 121 (3), 339–346.
- Li, L., Wu, J., Luo, M., Sun, Y., Wang, G., 2016. The effect of heat stress on gene expression, synthesis of steroids, and apoptosis in bovine granulosa cells. *Cell Stress Chaperones* 21 (3), 467–475.
- Li, Z., Srivastava, P., 2004. Heat-shock proteins. *Curr. Protoc. Immunol.*, Appendix 1. Appendix 1T.
- Llamas Luceno, N., de Souza Ramos Angrimani, D., de Cassia Bicudo, L., Szymanska, K.J., Van Poucke, M., Demeyere, K., Van Soom, A., 2020. Exposing dairy bulls to high temperature-humidity index during spermatogenesis compromises subsequent embryo development in vitro. *Theriogenology* 141, 16–25.
- Lopes, F.F.D.P., Lima, R.S., Risolia, P.H.B., Ispada, J., Assumpção, M.E.O., Visintin, J.A., 2012. Heat stress induced alteration in bovine oocytes: functional and cellular aspects. *Anim. Reprod.* 395–403.
- Lucy, M.C., 2019. Stress, strain, and pregnancy outcome in postpartum cows. *Anim. Reprod.* 16 (3), 455–464.
- Maillo, V., Gaora, P.Ó., Forde, N., Besenfelder, U., Havlicek, V., Burns, G.W., Rizos, D., 2015. Oviduct-embryo interactions in cattle: two-way traffic or a one-way street? *Biol. Reprod.* 92 (6), 144, 141–148.
- Maillo, V., Sánchez-Calabuig, M.J., Lopera-Vasquez, R., Hamdi, M., Gutierrez-Adan, A., Lonergan, P., Rizos, D., 2016. Oviductal response to gametes and early embryos in mammals. *Reproduction* 152 (4), R127–R141.
- Mellado, M., Sepulveda, E., Meza-Herrera, C., Veliz, F.G., Arevalo, J.R., Mellado, J., De Santiago, A., 2013. Effects of heat stress on reproductive efficiency of high yielding Holstein cows in a hot-arid environment. *Rev. Colombiana Ciencias Pecuarias* 26 (3), 193–200.
- Memili, E., First, N.L., 2000. Zygotic and embryonic gene expression in cow: a review of timing and mechanisms of early gene expression as compared with other species. *Zygote* 8 (1), 87–96.
- Moghaddam, A., Karimi, I., Pooyanmehr, M., 2009. Effects of short-term cooling on pregnancy rate of dairy heifers under summer heat stress. *Vet. Res. Commun.* 33 (6), 567–575.
- Monteiro, A.P.A., Tao, S., Thompson, I.M.T., Dahl, G.E., 2016. In utero heat stress decreases calf survival and performance through the first lactation. *J. Dairy Sci.* 99 (10), 8443–8450.
- Mosser, D.D., Caron, A.W., Bourget, L., Meriin, A.B., Sherman, M.Y., Morimoto, R.I., Massie, B., 2000. The chaperone function of hsp70 is required for protection against stress-induced apoptosis. *Mol. Cell Biol.* 20 (19), 7146–7159.
- Mouguelar, H., Díaz, T., Borghi, D., Quinteros, R., Bonino, F., Apichela, S.A., Aguilar, J.J., 2015. Morphometric study of the mare oviductal mucosa at different reproductive stages. *Anat. Rec.* 298 (11), 1950–1959.
- Nabenishi, H., Ohta, H., Nishimoto, T., Morita, T., Ashizawa, K., Tsuzuki, Y., 2011. Effect of the temperature-humidity index on body temperature and conception rate of lactating dairy cows in southwestern Japan. *J. Reprod. Dev.* 57 (4), 450–456.
- Nabenishi, H., Ohta, H., Nishimoto, T., Morita, T., Ashizawa, K., Tsuzuki, Y., 2012. The effects of cysteine addition during in vitro maturation on the developmental competence, ROS, GSH and apoptosis level of bovine oocytes exposed to heat stress. *Zygote* 20 (3), 249–259.
- Paula-Lopes, F.F., Hansen, P.J., 2002. Heat shock-induced apoptosis in preimplantation bovine embryos is a developmentally regulated phenomenon. *Biol. Reprod.* 66 (4), 1169–1177.
- Pavani, K., Carvalhais, I., Faheem, M., Chaveiro, A., Reis, F.V., da Silva, F.M., 2015. Reproductive performance of holstein dairy cows grazing in dry-summer subtropical climatic conditions: effect of heat stress and heat shock on meiotic competence and in vitro fertilization. *Asian-Australas. J. Anim. Sci.* 28 (3), 334–342.
- Pavani, K.C., Baron, E., Correia, P., Lourenco, J., Bettencourt, B.F., Sousa, M., da Silva, F.M., 2016. Gene expression, oocyte nuclear maturation and developmental competence of bovine oocytes and embryos produced after in vivo and in vitro heat shock. *Zygote* 24 (5), 748–759.
- Pihan, P., Carreras-Sureda, A., Hetz, C., 2017. BCL-2 family: integrating stress responses at the ER to control cell demise. *Cell Death Differ.* 24 (9), 1478–1487.
- Pillai, V.V., Weber, D.M., Phinney, B.S., Selvaraj, V., 2017. Profiling of proteins secreted in the bovine oviduct reveals diverse functions of this luminal microenvironment. *PLoS One* 12 (11), e0188105.
- Rahman, M.B., Schellander, K., Luceno, N.L., Van Soom, A., 2018. Heat stress responses in spermatozoa: mechanisms and consequences for cattle fertility. *Theriogenology* 113, 102–112.
- Rajoriya, d. j., Prasad, J., Ghosh, S., Perumal, P., Kumar, A., Kaushal, S., Ramteke, S., 2014. Studies on effect of different seasons on expression of HSP70 and HSP90 gene in sperm of Tharparkar bull semen. *Asian Pac. J. Reprod.* 3, 192–199.
- Ralph, C.R., Tilbrook, A.J., 2016. The usefulness of measuring glucocorticoids for assessing animal welfare. *J. Anim. Sci.* 94 (2), 457–470.
- Renaudeau, D., Collin, A., Yahav, S., de Basilio, V., Gourdiere, J.L., Collier, R.J., 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* 6 (5), 707–728.
- Rensis, F.D., Scaramuzzi, R.J., 2003. Heat stress and seasonal effects on reproduction in the dairy cow—a review. *Theriogenology* 60 (6), 1139–1151.
- Rispoli, L.A., Lawrence, J.L., Payton, R.R., Saxton, A.M., Schrock, G.E., Schrick, F.N., Edwards, J.L., 2011. Disparate consequences of heat stress exposure during meiotic maturation: embryo development after chemical activation vs fertilization of bovine oocytes. *Reproduction* 142 (6), 831–843.
- Rivera, R.M., Kelley, K.L., Erdos, G.W., Hansen, P.J., 2003. Alterations in ultrastructural morphology of two-cell bovine embryos produced in vitro and in vivo following a physiologically relevant heat shock. *Biol. Reprod.* 69 (6), 2068–2077.
- Rivera, R.M., Kelley, K.L., Erdos, G.W., Hansen, P.J., 2004. Reorganization of microfilaments and microtubules by thermal stress in two-cell bovine embryos. *Biol. Reprod.* 70 (6), 1852–1862.
- Rocha, A., Randel, R.D., Broussard, J.R., Lim, J.M., Blair, R.M., Rousel, J.D., et al., 1998. High environmental temperature and humidity decrease oocyte quality in *Bos taurus* but not in *Bos indicus* cows. *Theriogenology* 49 (3), 657–665.
- Rocha-Frigoni, N.A., Leao, B.C., Nogueira, E., Accorsi, M.F., Mingoti, G.Z., 2014. Reduced levels of intracellular reactive oxygen species and apoptotic status are not correlated with increases in cryotolerance of bovine embryos produced in vitro in the presence of antioxidants. *Reprod. Fertil. Dev.* 26 (6), 797–805.
- Rossi, A., Coccia, M., Trotta, E., Angelini, M., Santoro, M.G., 2012. Regulation of cyclooxygenase-2 expression by heat: a novel aspect of heat shock factor 1 function in human cells. *PLoS One* 7 (2), e31304.
- Roth, Z., Meidan, R., Braw-Tal, R., Wolfenson, D., 2000. Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows. *J. Reprod. Fertil.* 120 (1), 83–90.
- Roth, Z., 2017. Effect of heat stress on reproduction in dairy cows: insights into the cellular and molecular responses of the oocyte. *Ann. Rev. Anim. Biosci.* 5, 151–170.
- Rust, J.M., 2019. The impact of climate change on extensive and intensive livestock production systems. *Anim. Front.* 9 (1), 20–25.
- Sakatani, M., Kobayashi, S., Takahashi, M., 2004. Effects of heat shock on in vitro development and intracellular oxidative state of bovine preimplantation embryos. *Mol. Reprod. Dev.* 67 (1), 77–82.
- Sakatani, M., Alvarez, N.V., Takahashi, M., Hansen, P.J., 2012. Consequences of physiological heat shock beginning at the zygote stage on embryonic development and expression of stress response genes in cattle. *J. Dairy Sci.* 95 (6), 3080–3091.
- Sakatani, M., Bonilla, L., Dobbs, K.B., Block, J., Ozawa, M., Shanker, S., Hansen, P.J., 2013. Changes in the transcriptome of morula-stage bovine embryos caused by heat shock: relationship to developmental acquisition of thermotolerance. *Reprod. Biol. Endocrinol.* 11, 3.
- Sakatani, M., Yamanaka, K., Balboula, A.Z., Takenouchi, N., Takahashi, M., 2015. Heat stress during in vitro fertilization decreases fertilization success by disrupting anti-polyspermy systems of the oocytes. *Mol. Reprod. Dev.* 82 (1), 36–47.
- Sakatani, M., 2017. Effects of heat stress on bovine preimplantation embryos produced in vitro. *J. Reprod. Dev.* 63 (4), 347–352.
- Satrapa, R.A., Razza, E.M., Castilho, A.C., Simoes, R.A., Silva, C.F., Nabhan, T., Barros, C.M., 2013. Differential expression of IGF family members in heat-stressed embryos produced in vitro from OPU-derived oocytes of Nelore (*Bos indicus*) and Holstein (*Bos taurus*) cows. *Reprod. Domest. Anim.* 48 (6), 1043–1048.
- Scharf, B., Carroll, J.A., Riley, D.G., Chase Jr., C.C., Coleman, S.W., Keisler, D.H., Spiers, D.E., 2010. Evaluation of physiological and blood serum differences in heat-tolerant (Romosinuano) and heat-susceptible (Angus) *Bos taurus* cattle during controlled heat challenge. *J. Anim. Sci.* 88 (7), 2321–2336.
- Schultz, R.M., 1993. Regulation of zygotic gene activation in the mouse. *Bioessays* 15 (8), 531–538.
- Schütz, K.E., Rogers, A.R., Cox, N.R., Tucker, C.B., 2009. Dairy cows prefer shade that offers greater protection against solar radiation in summer: shade use, behaviour, and body temperature. *Appl. Anim. Behav. Sci.* 116 (1), 28–34.
- Scott, C.J., Rose, J.L., Gunn, A.J., McGrath, B.M., 2018. Kisspeptin and the regulation of the reproductive axis in domestic animals. *J. Endocrinol. JOE-18-0485*, Advance online publication.
- Selye, H., 1946. The general adaptation syndrome and the diseases of adaptation. *J. Clin. Endocrinol. Metab.* 6, 117–230.
- Sejian, V., Bhatta, R., Gaughan, J.B., Dunshea, F.R., Lacetera, N., 2018. Review: adaptation of animals to heat stress. *Animal* 12 (s2), s431–s444.
- Shilja, S., Sejian, V., Bagath, M., Mech, A., David, C.G., Kurien, E.K., Bhatta, R., 2016. Adaptive capability as indicated by behavioral and physiological responses, plasma HSP70 level, and PBMC HSP70 mRNA expression in Osmanabadi goats subjected to combined (heat and nutritional) stressors. *Int. J. Biometeorol.* 60 (9), 1311–1323.
- Silanikove, N., 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest. Sci.* 67 (1–2), 1–18.
- Silva, C.F., Sartorelli, E.S., Castilho, A.C., Satrapa, R.A., Puelker, R.Z., Razza, E.M., Barros, C.M., 2013. Effects of heat stress on development, quality and survival of *Bos indicus* and *Bos taurus* embryos produced in vitro. *Theriogenology* 79 (2), 351–357.
- Skinner, J.D., Louw, G.N., 1966. Heat stress and spermatogenesis in *Bos indicus* and *Bos taurus* cattle. *J. Appl. Physiol.* 21 (6), 1784–1790.
- Smith, S.M., Vale, W.W., 2006. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin. Neurosci.* 8 (4), 383–395.
- Smith, S.L., Everts, R.E., Sung, L.Y., Du, F., Page, R.L., Henderson, B., Tian, X.C., 2009. Gene expression profiling of single bovine embryos uncovers significant effects of in vitro maturation, fertilization and culture. *Mol. Reprod. Dev.* 76 (1), 38–47.
- Sonna, L.A., Fujita, J., Gaffin, S.L., Lilly, C.M., 2002. Invited review: effects of heat and cold stress on mammalian gene expression. *J. Appl. Physiol.* (1985) 92 (4), 1725–1742.
- Stamperna, K., Giannoulis, T., Nanas, I., Kalemkeridou, M., Dadouli, K., Moutou, K., Dovoliou, E., 2020. Short term temperature elevation during IVM affects embryo yield and alters gene expression pattern in oocytes, cumulus cells and blastocysts in cattle. *Theriogenology* 156, 36–45.



- Sugiyama, S., McGowan, M., Phillips, N., Kafi, M., Young, M., 2007. Effects of increased ambient temperature during IVM and/or IVF on the in vitro development of bovine zygotes. *Reprod. Domest. Anim.* 42 (3), 271–274.
- Summer, A., Lora, I., Formaggioni, P., Gottardo, F., 2019. Impact of heat stress on milk and meat production. *Anim. Front.* 9 (1), 39–46.
- Takahashi, M., Nagai, T., Hamano, S., Kuwayama, M., Okamura, N., Okano, A., 1993. Effect of thiol compounds on in vitro development and intracellular glutathione content of bovine embryos. *Biol. Reprod.* 49 (2), 228–232.
- Torres-Osorio, V., Urrego, R., Echeverri-Zuluaga, J.J., López-Herrera, A., 2019. Estrés oxidativo y el uso de antioxidantes en la producción in vitro de embriones mamíferos. Revisión. *Revista mexicana de ciencias pecuarias* 10 (2), 433–459.
- Tse, P.-K., Lee, Y.-L., Chow, W.-N., Luk, J.M., Lee, K.-F., Yeung, W.S., 2008. Preimplantation embryos cooperate with oviductal cells to produce embryotrophic inactivated complement-3b. *Endocrinology* 149 (3), 1268–1276.
- Urrego, R., Rodríguez-Osorio, N., Niemann, H., 2014. Epigenetic disorders and altered gene expression after use of Assisted Reproductive Technologies in domestic cattle. *Epigenetics* 9 (6), 803–815.
- van der Weijden, V.A., Chen, S., Bauersachs, S., Ulbrich, S.E., Schoen, J., 2017. Gene expression of bovine embryos developing at the air-liquid interface on oviductal epithelial cells (ALI-BOEC). *Reprod. Biol. Endocrinol.* 15 (1), 91.
- Van Zanten, H.H.E., Herrero, M., Van Hal, O., Roos, E., Muller, A., Garnett, T., De Boer, I.J.M., 2018. Defining a land boundary for sustainable livestock consumption. *Global Change Biol.* 24 (9), 4185–4194.
- Vasconcelos, J.L., Demetrio, D.G., Santos, R.M., Chiari, J.R., Rodrigues, C.A., Sa Filho, O.G., 2006. Factors potentially affecting fertility of lactating dairy cow recipients. *Theriogenology* 65 (1), 192–200.
- Verdoljak, J., Pereira, M., Gándara, L., Acosta, F., Fernández-López, C., Martínez-González, J., 2018. Reproducción y mortalidad de razas bovinas en clima subtropical de Argentina. *Abanico Vet.* 8 (1), 28–35.
- Whitfield, L.K., 2016. Heat stress and its impact on fertility in dairy cattle. *Livestock* 21, 218–221.
- Wolfenson, D., Lew, B.J., Thatcher, W.W., Graber, Y., Meidan, R., 1997. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. *Anim. Reprod. Sci.* 47 (1-2), 9–19.
- Wolfenson, D., Roth, Z., 2019. Impact of heat stress on cow reproduction and fertility. *Anim. Front.* 9 (1), 32–38.
- Yániz, J.L., Lopez Gatiús, F., Santolaria, P., Mullins, K.J., 2000. Study of the functional anatomy of bovine oviductal mucosa. *Anat. Rec.* 260 (3), 268–278.