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Negative impact of heat stress on reproduction in cows: Animal husbandry and biotechnological viewpoints: A review

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Abstract: This review summarises current knowledge of the relationship between heat stress (HS) and reproduction in cattle. It focuses on research from the viewpoint of genetics (breed), from the viewpoint of reproduction physiology (*in vivo* and *in vitro*) and from the animal husbandry practice. From the viewpoint of animal husbandry, it was evidenced that heat stress influences reproduction before, during and after conception. Most publications suggest the negative impact of heat stress on the reproductive physiology of cows reflected in ovarian and follicular activity, in oocyte and embryo development, as well as in other processes studied under *in vivo* or *in vitro* conditions. There are also a number of products that the cell creates in response to heat stress, which is used as indicators of the stress (e.g. heat shock proteins). A number of publications also focus on how to prevent heat stress on the farm (e.g. shade, water shower) or during *in vitro* procedures, including the supplementation of the culture media with antioxidants like melatonin. Research of heat stress is very important in cattle breeding for preventing and reducing its effects on the farm and also in the context of climate changes and global atmospheric warming.

Keywords: dairy cows; physiology; embryo development

Introduction

In general, there is an effort to create the best possible conditions for the rearing of cattle. In this regard, there is a number of factors negatively affecting production, reproduction, health and other traits. One serious factor is heat stress, which has adverse effects on animal health, with a deterioration in production and reproduction leading to economic losses. The impact of heat stress on animals is an essential topic as it affects cattle in both tropical areas and areas with a moderate climate with fewer days of exposure to high temperatures (Schuller et al. 2014). Research in this area is very important, mainly in the context of climate changes and global atmospheric warming. Current climate analysis indicates an increase in human-induced warming by 0.2 °C per decade and estimates an increase in temperature by 1.5 °C between the years 2030–2052 (Allen et al. 2018). World climatic

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changes could lead to increased heat stress in cows and decreased production, reproduction and other traits (fitness, profitability).

Reproduction in cattle breeding is connected mainly with the turnover of the herd and is a key point in the livestock economy. Heat stress is one of the factors affecting reproduction. In general, heat stress is related to many problems, which can also be reflected in the significant economic losses in cattle breeding. Therefore, it is necessary to pay considerable attention to heat stress and to create the best possible environment for the animals.

It is important to distinguish between heat stress, temperature stress and thermal stress. Heat is energy (in Joule) transferred from one object to another (e.g. through conduction, radiation etc.), whereas temperature is a measure of energy (in Kelvin or in Celsius). From this point of view, heat stress is the negative effect of high external thermal energy on an individual (McGregor and Vanos 2018). On the other hand, thermal stress in animal physiology includes both heat and cold stress. In this context, a variety of stress indexes have been proposed, based mainly on maximal daily temperature and daily humidity (temperature-humidity index: THI).

The effect of heat stress has been confirmed for a number of physiological functions in both male and female animals. The present review focuses on the evaluation of heat stress consequences in cows from the viewpoints of genetics (breed), reproduction physiology and animal husbandry practice. Finally, we outline a possible preventive solution to heat stress or minimisation on the farm.

The role of genetics (breed) in animal tolerance to heat stress

It is evident that genetics plays a crucial role in the resistance of cows to heat stress. Genetic predisposition and thermo-tolerance of certain breeds can have an impact on the response of animals to heat stress, and therefore this factor (genetic and breed) must be considered. The characteristics of the hair coat (due to specific genes) play an important role in thermoregulation. Research in this area has provided evidence mainly due to the study of the "*slick*" gene described in Senepol and in Carora cattle (Olson et al. 2003). Animals carrying the dominant allele of the *slick* gene have a short, sleek hair coat. The *slick* locus has been localised on chromosome 20 (Mariasegaram et al. 2007), and cows that carry this gene are able to effectively regulate body temperature. This was confirmed in the Senepol breed and their crosses, including Holstein cows (Dikmen et al. 2008).

Thus, breed and genetics is a very important factor in the resistance of cows to heat stress. From this point of view, Silva et al. (2013) studied the effect of heat stress on the development and quality of embryos of *Bos taurus* (Jersey and Angus) and *Bos indicus* (Nellore), and they confirmed the hypothesis that the *Bos taurus* breeds are more affected by heat stress than the thermotolerant Nellore breed. Similarly, Rocha et al. (1998) tested the hypothesis that there would be a difference in the heat stress response between *Bos taurus* (Holstein) and *Bos indicus* (Brahman). The cows (Holstein, Brahman) in this study were superovulated; subsequently, the oocytes were aspirated and subjected to *in vitro* maturation and fertilisation. A significant relationship between heat stress and oocyte development was found only in Holstein and not Brahmans. In particular, Holstein cows showed a lower percentage of collected normal oocytes in the hot season versus cold season: 24.6% vs 80.0% (Brahman 77.0% vs 83.3%), fertilised oocytes developed to the 8-cell: 1.1% vs 44.4% (Brahman 69.9% vs 71.3%) and to the morula stage: 0.0% vs 34.2% (Brahman 58.1% vs 55.5%).

As mentioned above, an important role in the resistance of cows to heat stress is played by genetics (breed). Hence, efforts have been made to implement heat tolerance in cattle breeding programmes. In this regard, to overcome heat stress consequences in Australian dairy cattle, a new genomic breeding value for heat tolerance in Australia was established which enables the selection and improvement of this important trait (Nguyen et al. 2017).

Heat stress from the viewpoint of reproduction physiology (under *in vivo* or *in vitro* conditions)

Research under in vivo conditions

Reproduction, in relation to heat stress, can also be evaluated from physiological aspects, such as ovarian activity, oocyte quality, follicle size etc. This research under *in vivo* conditions plays a cru-

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cial role in understanding physiological aspects of reproduction.

The negative effect of heat stress on the physiology of both male (bulls) and female (cows; heifers) reproduction has been described in a number of studies. The impact of the hot summer period on the reproduction of bulls is manifested mainly by a deterioration in sperm quality (Dolezalova et al. 2016; Biniova et al. 2018; Zubor et al. 2020). Concerning female reproduction, an association between heat stress and ovarian activity, the number and quality of oocytes and transferable embryos can be probably reflected in other reproduction problems (Schuller et al. 2017; Bezdicek et al. 2019). Further, it will provide evidence on the influence of heat stress on ovarian activity and embryo development under *in vivo* conditions in different regions (tropical or moderate climate) and using other methods.

The relationship between stress factors and the spontaneous reduction of *corpora lutea* was studied in north-eastern Spain by Lopez-Gatius et al. (2010). These authors assumed that in the early fetal period, the embryos (including *corpora lutea*) are sensitive to stress factors such as the hot season. Wilson et al. (1998) also reported the negative effect of heat stress on ovarian activity in Holstein heifers. The authors performed this study at the University of Missouri-Columbia in heifers assigned to a thermoneutral or heat stress environment. They concluded that the heifers kept in the heat-stressed environment, had longer oestrus cycles (during inhibition of the function of the dominant follicle and often three follicular waves) in contrast to the heifers in a thermoneutral zone (22.9 ± 0.7 vs 20.5 ± 0.7 days). Heat stress was also studied in the moderate climate in central Europe, where high temperatures may last only several days. In particular, Bezdicek et al. (2019) studied the effect of mild heat stress on superovulated Holstein cows in the Czech Republic (moderate temperature climate) in a three season period: hot (mean THI = 75.7); optimal (mean THI = 65.9) and lower temperature season (mean THI = 45.6). The authors concluded that the hot season could negatively influence ovarian activity (number of *corpora lutea*) and the number of transferable embryos in superovulated cows.

Schuller et al. (2017) studied the influence of heat stress on follicle size in dairy cows in Germany (also a moderate temperature climate). The follicles, *corpora lutea* and cysts were measured and studied during oestrus by ultrasonography. The authors observed that follicular size was decreased in stressed cows by 0.1 mm with each increment in THI. The onset of heat stress was observed at a THI ≥ 68 (Schuller et al. 2017). The influence of heat stress on follicle size in Holstein cows assigned to a shady or non-shady environment was also studied by Badinga et al. (1993). In addition to the shade environment, a sprinkler-fan cooling system was used to cool the cows during the experiments. It was found that cows kept in the shade (cooler) management system had larger dominant follicles than those in the non-shade system (16.4 mm vs 14.5 mm). Moreover, the larger dominant follicles contained more follicular fluid (1.9 ml vs 1.1 ml). These studies thus confirm the negative effect of heat stress on the quality of the follicles in Holstein cows which are characterised by high milk production and intensive breeding programs. Whether this effect of heat stress is also true in other cattle breeds remains to be elucidated.

Research under in vitro conditions

The physiological aspects of reproduction have been studied in the last years also at the cellular level (e.g. oocytes) and in the early-stage of embryos (under *in vitro* conditions). The relationship between heat stress and oocyte/embryo development *in vitro* was studied by Al-Katanani et al. (2002), who collected ovaries from Holstein cows from a slaughterhouse in Florida in warm and cool seasons. After the *in vitro* maturation of oocytes and fertilisation, embryo development up to day 8 was monitored. The authors obtained the following results in relation to the hot or cold season (hot vs cold season): number of collected oocytes/per cow: 19.3 vs 25.1; total cell number: 68.7 vs 82.4; blastocyst rate on day 8 from total oocyte number: 11.4% vs 29.9%; blastocyst rate on day eight from cleaved embryos: 12.5% vs 34.3%. Al-Katanani et al. (2002) also reported a higher rectal temperature in cows during the hot period than during the cold period (39.8 °C vs 38.7 °C). Rocha et al. (1998) obtained a lower percentage of collected normal bovine oocytes (hot vs cool; 41.0% vs 75.9%) and lower embryo development rate to the 2-cell (45.0% vs 82.4%), 8-cell (21.2% vs 65.4%) and morula stage (6.0% vs 46.6%) following oocyte fertilisation.

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Sakatani et al. (2013) also found that bovine embryos at the morula stage are more resistant to heat shock (40 °C for 8 h) than early-stage embryos, and this resistance may arise as a result of the accumulation of specific proteins in the embryos in response to damage caused by free radicals. Similar conclusions were drawn earlier by Ortega et al. (2016), who also demonstrated that heat shock decreases the percentage of *in vitro* produced blastocysts developed from heat stress-exposed zygotes, and this negative effect is significantly dependent on the genotype, particularly in the *HSPA1L* gene (Ortega et al. 2016).

An important conclusion was also drawn by Ju et al. (1999), who found that only a higher temperature (43 °C; for 45 min or 60 min) reduced the formation of blastocysts, expanded blastocysts and hatched blastocysts. In particular, after 60 min of heat shock (43 °C) 9% of embryos developed to the blastocyst stage compared to 26% in the control group (39 °C; $P < 0.05$). In the case of mild heat shock (41.5 °C), the difference between the groups was insignificant (27% vs 32%). A similar tendency was also observed in the case of expanded and hatched blastocysts (Ju et al. 1999).

Generally, stress is associated with the whole internal environment of the cell. From this point of view, some authors have documented cellular changes in oocytes and embryos after an induced heat shock under *in vitro* conditions. These changes are not only related to some organelles and other cell structures (e.g. endoplasmatic reticulum, mitochondria, etc.) but also to the production of protective proteins against thermal stress – heat shock proteins (HSP). Several authors have studied cellular changes in bovine oocytes during stress (Tseng et al. 2004; Zhang et al. 2018; Takehara et al. 2020). In particular, Tseng et al. (2004) reported that prolonged heat stress (2–4 h; 41.5 °C) is associated with changes in the chromatin configuration, spindle and cytoplasmatic microtubules. Similar findings were also reported on porcine oocytes (Ju and Tseng 2004), where the damaging effect of thermal stress on the cytoskeleton of oocytes was irreversible.

Mitochondria play a significant role in thermal stress because these are associated primarily with energy production (ATP) and maternal genetics (own DNA). In the study by Gendelman and Roth (2012), an association between season and some mitochondrial features was found, especially concerning coenzyme Q10. The authors demonstrated that under moderate stress, coenzyme Q10 could improve some mitochondrial features (membrane polarisation, expression of mitochondrial genes, mitochondrial distribution) and can also be applied to improve embryo development *in vitro*.

Recent research has focused on the endoplasmic reticulum (ER). ER stress can result in the accumulation of misfolded and unfolded proteins, which leads to cell homeostasis disruption and, alternatively to apoptosis. One of the ER stress markers is *GRP78* (glucose-regulated protein 78), the *HSP70* family member. Takehara et al. (2020) demonstrated the relationship between the *GRP78* expression, oocyte quality and developmental characteristics, such as the age of oocytes or dead blastomeres rate in the blastocysts. Among other ER stress markers, there are: *ATF4* – activating transcription factor 4; *ATF6*; *XBP1* (x-box binding protein 1), etc.

Thus, the ER plays an important role in the homeostasis of eukaryotic cells. From this point of view, Khatun et al. (2020) reported that the deleterious effect of ER stress could be reduced in *in vitro* culture by the addition of tauroursodeoxycholic acid. Similarly, Zhang et al. (2018) reported that tauroursodeoxycholic acid is positively related to the inhibition of ER stress and apoptosis in bovine nucleus donor cells.

Concerning cellular stress, it is essential that the whole group of heat shock proteins ranged according to molecular size (from 10 kDa to more than 100 kDa) and located in different cellular locations, e.g. in the cytoplasm (HSP70), mitochondria (HSP10; HSP60), endoplasmic reticulum (HSP47), etc. According to Jee (2016), heat shock proteins prevent the denaturation of various cell molecules in a stressful situation and maintain cellular homeostasis. HSPs are synthesised not only in response to heat stress but also found in reaction to (in different animals) other stimuli, such as UV radiation, colds, infections and other factors (Kiriyama et al. 2001). The breed also plays an important role in the heat stress response and HSP expression, as it was shown in the comparison of Sahiwal (*Bos indicus*) and Frieswal (*Bos indicus* × *Bos taurus*) cattle (Deb et al. 2014). The study of HSP 90 protein revealed higher expression in Sahiwal cattle (Deb et al. 2014), which was associated with significantly greater viability of the peripheral blood mononuclear cells and a greater ability of Sahiwal cattle to regulate body temperature during heat stress.

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The published studies here demonstrate the negative effect of heat stress on ovarian and follicular activity, oocyte and embryo development and other processes under *in vivo* and *in vitro* conditions. The above-mentioned reports suggest the necessity to pay more attention to the action of heat stress also at the cellular level. Significant attention has been paid in the past years to cellular changes in oocytes in terms of cytoskeletal elements, mitochondrial activity, protection of cumulus cells, DNA changes, ER stress, as well as the role of heat shock proteins and other cellular changes during thermal stress. There are a number of mechanisms (products) by which the cell protects itself against external higher temperatures or other stress situations, which can be used as markers (indicators) of thermal stress.

Heat stress from the animal husbandry viewpoint

The effect of heat stress was evaluated from the animal husbandry viewpoint for days open (service period; Oseni et al. 2004), conception rate (Morton et al. 2007; Schuller et al. 2014), non-return rate (Al-Katanani et al. 1999; Ravagnolo and Misztal 2002) and others.

Service-period is a basic term in animal husbandry practice representing the number of days from calving to the next conception. It is comprehensible in research and practice and therefore used in studies including those on heat stress. In US Holstein cows, the relationship between days open and season has been studied by several authors (e.g. Oseni et al. 2004), who concluded unanimously that reproduction is worse (longer days open) in hot months. Specifically, Oseni et al. (2004) reported longer days open in the case of spring calving (March/April; 166 days) and the shortest for calving in the autumn (September/November; 130 days). The authors also found genetic correlations between days open and seasonal differences with a conclusion of close correlations (0.90) in both spring/summer and autumn/winter.

By evaluating reproduction traits in different animals, the genetic underpinning and individual genetic differences between animals were shown to play an important role. Therefore, it is advantageous to use cow-twins to eliminate the influence of genetic underpinning (influence of an ancestor – mother and father). From this viewpoint, Bezdicek et al. (2020) used the database of twins from the embryo transfer for evaluation of the effect of heat stress on the length of the service period. The first of the twins was calved during the summer months (affected by heat stress), and their full sister (fraternal twins) was calved in the cooler months. A significantly ($P < 0.05$) longer service period was recorded in cows calved in summer months in contrast to their sisters calved in cooler months (133.8 days vs 114.7 days). To compare the difference in the length of service period, the second group of twin pairs with reproduction only in the cold season (cold vs cold season; 58 twin pairs) was also created. No significant difference between twins in this period was revealed (113.6 days vs 119.4 days; Bezdicek et al. 2020).

The impact of seasonal heat stress on the conception rate in Holstein cows in Germany was studied by Schuller et al. (2014). For the evaluation of heat stress, the THI value (temperature-humidity index) was used, which included both the ambient temperature and relative humidity. Although the authors fixed the threshold for conception rate at the level of THI = 73, the influence of heat stress was also found at a lower level of THI. They concluded that the most sensitive period for heat stress in Holstein cows is in a moderate climate from 21 to one day before the service of cows.

A detailed analysis of the effect of heat stress on the conception rate was also performed by Morton et al. (2007) using the data from 16 878 services of Holstein-Friesan cows located in Australia (Atherton Tableland, North Queensland) were evaluated. The authors demonstrated that the conception rate was affected by heat-load not only on the days before but also after insemination. They also suggested that management steps to reduce the heat stress in cows should be implemented five weeks before and at least one week after insemination. From this point of view, Ravagnolo and Misztal (2002) studied the non-return rate in Holstein cows and concluded that THI is more informative about the heat stress of cows on the day of insemination. Al-Katanani et al. (1999) evaluated the effect of heat stress on Holstein cows in the area of north and south Florida. The authors recorded the following in the days –10; 0 and +10, the 90-day non-return rate (for cows exposed to temperatures > 20 °C vs ≤ 20 °C): 36.5% vs 60.1%; 41.4% vs 59.6% and 41.1% vs 56.9%. They concluded that the low 90-day non-return rate is

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associated with heat stress in cows before, after and on the day of breeding.

The influence of heat stress on reproduction traits was also studied by Chebel et al. (2004), who evaluated Holstein cows in the Central Valley of California, USA. In this study, temperature (heat stress) was defined as HS1 (at least one day of maximum temperature ≥ 29 °C; average daily maximum temperature lower than 29 °C), HS2 (average daily maximum temperature ≥ 29 °C), and NH (no heat stress). In particular, the conception rates in cows from groups HS1, HS2, and NH were 28.8%, 23.0%, and 31.3%, respectively. In the case of 20 days to insemination, the conception rates in the studied groups were 27.6%; 23.8% and 39.8%, resp. Therefore, Holstein cows were more sensitive to heat stress a few days before insemination. The conception rate was also affected by the parity, number of artificial inseminations, and post-parturient disease (Chebel et al. 2004).

The relationship between heat stress and conception rate was also studied in a population of Holstein cows in Mexico. Villa-Mancera et al. (2011) found lower conception rates ($P < 0.01$) in the warmer summer months (July to September; 32.1%) compared to the cooler months in winter (January to March; 36.9%).

These findings demonstrate the negative impact of heat stress on reproduction in cows from the animal husbandry viewpoint. This is reflected in poorer fertility resulting in a prolonged service period (days open), lower conception rate, lower non-return rate, etc. On the other hand, there is no agreement in time period crucial for the manifestation of heat stress (before, after, or on the day of insemination). In particular, Al-Katanani et al. (1999) concluded that for a non-return rate, the days −10, 0, and +10 are associated with heat stress. Morton et al. (2007) suggested that it is crucial to apply correct management, from the point of view of heat stress, five weeks before and at least one week after insemination. Schuller et al. (2014) reported the greatest negative effect of heat stress on the conception rate from −21 to −1 day before insemination (the most sensitive period). Some authors also emphasize the important factor of acclimatization to high temperatures in overcoming heat stress. Evaluation of fertility in relation to the service period, conception rate, and other animal husbandry traits is important in both research and practice.

Possible solutions to heat stress consequences in animal husbandry and oocyte/embryo development

The problem of heat stress in animals can be solved using different approaches. From the viewpoint of animal husbandry, the possibility of cooling using a water shower, providing shade, or a combination of both, is often discussed. Legrand et al. (2011) described a lower (by 0.2 °C) body temperature in the evening when cows were exposed to a shower during the day. Similarly, Kendal et al. (2007) found a significant influence of shade and shower on the body temperature (vaginal temperature), especially when the THI was ≥ 69. The authors showed the following results for vaginal temperature using different management strategies: with shower: 38.7 °C; shade and shower: 38.6 °C; only shade: 38.9 °C; control: 39.2 °C. Moreover, optimal ventilation in the stall (the movement of air) plays an important role in maintaining correct air parameters and welfare conditions (Herbut et al. 2015). Similar data on heat stress were also provided by other authors (Schutz et al. 2011; Broucek et al. 2020). For example, Schutz et al. (2011) recorded a decrease in the surface temperature of cows by 11.4% after the application of a shower (shade temperature decrease by 1%; control decrease – 1.4%). Using a water shower, ventilation and shade proved to be an appropriate solution for ensuring good environmental conditions for cows in the hot season.

Several authors have studied other options for resolving heat stress in the reproduction of cows, for example, by using the hormone melatonin either in *in vivo* (Garcia-Ispierto et al. 2013) or in *in vitro* (Garcia-Ispierto et al. 2013; Wang et al. 2017) experiments. Most research in this respect focuses on the role of melatonin as an important antioxidant in the development of embryos *in vitro*, particularly for its role in ROS reduction. Its positive effects on embryo development have been demonstrated in *in vitro* procedures not only in cattle but also in pigs, sheep, goats, and other animal species (Do et al. 2015; Li et al. 2015; Zhao et al. 2016; Soto-Heras et al. 2018). Some authors have studied the influence of retinoids as an oxidative-stress protector during *in vitro* oocyte maturation/culture. For example, Livingston et al. (2004) reported that the addition of 5 μM retinol to the *in vitro* maturation medium with bovine oocytes

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might have a positive effect on subsequent embryo development through an antioxidant mechanism. Retinol with an antioxidant activity was also studied by Duy et al. (2017), who found that all-*trans*-retinol acts as an effective antioxidant through donating a hydrogen atom.

Conclusion

A large number of studies have demonstrated a negative effect of heat stress on the reproduction of cows in *in vivo* (ovarian activity, number of transferable embryos, quality of *corpora lutea,* etc.) and *in vitro* experiments (*in vitro* oocyte maturation, *in vitro* embryo culture, etc.). In *in vivo* studies, heat stress influences reproduction physiology before, during, and after conception. The presented studies here demonstrate the damaging effect of heat stress on ovarian activity and embryo development under *in vivo* conditions, which is reflected in a significant reduction in the number of transferable embryos and *corpora lutea,* among others. Therefore, it is better to perform embryo transfers or *in vitro* procedures, etc. in the season when the animals are in their own thermoneutral (comfort) zone**.** In this context, an important role is also played by genetic factors, variability in thermotolerance between breeds, and thermotolerance-specific genes. The genetic predisposition and thermotolerance of certain breeds may also play an important role.

There are several suggested ways for preventing heat stress on the farm (shade, water shower) or during *in vitro* procedures (through the supplementation of the culture media with known antioxidants). Research in this area is extensive and ongoing in the context of climate change.

Conflict of interest

The authors declare no conflict of interest.

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