Introduction

Heat stress (HS) is a non-specific physiological response of animals to ambient temperature when animals generate more heat than they lose (Yang, 2014). The decline in reproductive performance in dairy cows in subtropical and tropical climates under the influence of HS has been well studied. Considering global warming and intensive genetic progress for high productive performance, the decline in reproductive indicators is a limiting factor for dairy farming in the coming years (Roth, 2017). According to the author, heat stress in cows is no longer typical only to the hot regions of the planet. Studies on the negative effect of high temperatures on animals in regions of Europe with temperate and Mediterranean climates are also beginning to appear in the literature (Bernabucci et al., 2014). Holstein-Friesian is the most prevalent breed for milk production in the world. In summer, the ability of this breed to radiate body heat through the skin is limited by the relatively small body surface area relative to body weight, underdeveloped sweat glands, short and densely located hairs in coat (Yang et al., 2010). It is important from a scientific and practical point of view to study how different levels of HS in different climatic zones around the world affect the physiological response in Holstein-Friesian cows, considered as the main breed for milk in the world.

HS causes infertility in farm animals and is a major source of economic losses in the livestock sector. Animal fertility is reduced by high body temperature, which affects ovarian function, estrus manifestation, ovum quality and embryonic development. Protection against HS during the dry period is particularly important for highly productive cows, as it involves involution of the mammary gland and the subsequent rapid growth and development of the fetus and preparation for...
lactation (Aggarwal and Upadhyay, 2013). Dairy cows exposed to HS have lower plasma inhibin concentrations, which results in inhibition of folliculogenesis, as a considerable share of plasma inhibin comes from small and medium-sized follicles (Aggarwal and Upadhyay, 2013). Plasma follicle-stimulating hormone (FSH) concentrations are higher during the preovulatory period in summer and are associated with lower circulating inhibin concentrations (Ingraham et al., 1974). The study of Roth et al. (2000a) shows both immediate and delayed effects of heat stress on follicular dynamics, which were associated with high FSH and low inhibin concentrations in plasma, which could be related to low reproductive performance during the summer and autumn.

Neuroendocrine mechanisms controlling gonadotropin secretion are more sensitive to HS, especially in animals with low concentrations of plasma estradiol (Aggarwal and Upadhyay, 2013). In other words, HS causes a number of physiological and biochemical changes in endocrine functions and adversely affects milk production and reproductive efficiency in both male and female animals. High ambient temperatures in summer have been observed to adversely affect reproductive efficiency and drastically reduce conception rate (CR) and increase embryonic losses (Gwazdauskas et al., 1981). Roth (2008) points that reduced reproductive performance of lactating cows during the summer has been well-documented and is associated with decreased thermoregulatory capacity of the animal due to intensive genetic selection for high milk production.

Summer HS is a major factor contributing to low fertility among lactating dairy cows. This is a global problem that causes great economic losses and affects about 60% of the world’s cattle population. CR range from about 40-60% in the cooler months to 10-20% or lower in summer, depending on the severity of HS (Cavestany et al., 1985). Penev et al. (2020) found that with an increase in Temperature-humidity index above 78, the conception rate in Holstein-Friesian cows decreased significantly.

The most remarkable feature of summer infertility is its multifactorial nature, as hyperthermia directly alters and damages the cellular functions of various organs and tissues of the reproductive system. In addition, exposure of cattle to HS causes indirect reactions that can also affect reproductive processes. Such responses include redistribution of blood flow among the body’s organs, reduced food intake, respiratory alkalosis, and more (Wolfenson et al., 2000).

Although the effects of the various direct and indirect effects of HS on reproductive processes have never been quantified, the direct effect of hyperthermia on the deterioration of cellular functions is thought to be predominant. The significant increase in milk yield in recent years has exacerbated the low summer fertility syndrome due to the increase in metabolically produced heat (Wolfenson et al., 2000). The various cooling procedures used on farms fail to significantly improve the fertility and CR of lactating cows in summer, even on farms equipped with cooling systems, it is still significantly lower than that recorded in winter (Hansen, 1997b).

Low summer CR is usually associated mainly with the warm months of the year in the northern hemisphere, usually June, July, August and September. In a study by Penev et al. (2020), the lowest CR was registered in July and August, 19.4% and 16.7%, respectively. HS is a major contributing factor to the low CR of dairy cows inseminated in the late summer months (Ingraham et al., 1974; Ray et al., 1992; Thompson et al., 1996; Al-Katanani et al., 1999). The reduction in CRs during the hot months can vary between 20 and 30% compared to the winter months (Cavestany et al., 1985; Badinga et al., 1985; De Rensis et al., 2002). Lucy (2002) and Chebel et al. (2004) also reported for about a 20% to 27% drop in conception rates occurring in summer. There are clear seasonal differences in estrus detection, days to first insemination, and CR in dairy cows (Cavestany et al., 1985; Ryan et al., 1993; De Rensis et al., 2002; Almier et al., 2002) and lower levels of CR are constantly observed in the summer months compared to the winter. The effects of HS on fertility appear in the autumn (Badinga et al., 1985; Roth et al., 1997; Wolfenson et al., 1997; Drew, 1999). Low CR is usually clearly expressed during the warmer months of the year (June to September) and persists in the autumn (October and November), although cows are no longer exposed to HS (Hansen, 1997a). It is obvious that there is a delayed effect of summer HS on autumn fertility and it represents about one third or more of the total percentage of low summer fertility syndrome (Wolfenson et al., 2000). It has been suggested that this may be a lasting effect of HS during the hot months on the antral follicles, which will become large dominant follicles 40-50 days later (Roth et al., 2001a,b). The research of Penev et al. (2020) confirms the above thesis because the highest CR is registered in the winter months of November and December. Over the last decade, significant efforts have been made to clarify the HS-induced disorders in the processes in the reproductive system and the functioning of various parts of it. Ultrasonography, cell cultures, in vitro conceiving are among the ways to achieve a better understanding of the mechanisms by which HS adversely affects fertility in cattle (Wolfenson et al., 2000).

The aim of this review is to consider and discuss the scientific literature related to the effect of heat stress (HS) on reproductive performance in dairy cows and the opportunities to reduce/mitigate its effects.

**Influence of HS on the axis hypothalamus - pituitary - ovary**

Since the main factors regulating ovarian activity are gonadotropin-releasing hormone from the hypothalamus, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) from the anterior pituitary gland, some authors have studied the effect of HS on the secretion of these hormones. Some studies (Gwazdauskas et al., 1981; Gauthier, 1986) report unchanged concentrations of LH, while others report elevated concentrations (Roman-Ponce et al., 1981), and still others report decreased concentrations (Glad et al., 1993; Lee 1993) under HS conditions. These differences are probably
due to uncontrolled changes in other factors that affect progesterone levels in the blood.

Plasma inhibin concentrations in summer are lower in cows under HS (Wolfenson et al., 1995), probably due to reduced folliculogenesis. Low proportion of plasma inhibin comes from small and medium-sized follicles. Plasma FSH concentrations are higher during the preovulatory period in summer and are associated with lower circulating inhibin concentrations (Ingham et al., 1974).

Plasma estradiol concentrations are reduced by HS in dairy cows (Wolfenson et al., 1995, 1997; Wilson et al., 1998); an effect that is consistent with reduced LH concentrations and reduced dominance of a selected follicle. However, the effect of HS on plasma progesterone concentrations is controversial in the literature. Some studies (Wilson et al., 1998) found that HS had no effect on progesterone levels. Several other studies have reported increased (Trout et al., 1998), decreased (Howell et al., 1994; Ronchi et al., 2001) or unchanged (Roth et al., 2000; Guzeloglu et al., 2001) blood concentrations of this hormone during the summer HS in dairy cows. These differences are probably due to uncontrolled changes in other factors that affect progesterone levels in the blood. For example, the type of HS (i.e., acute or chronic) and differences in dry matter intake independently affect the concentration of progesterone in the blood and thus confuse the situation. Plasma progesterone concentrations are determined by differences between the speed of luteal production and the speed of hepatic metabolism, both of which are also affected by changes in dry matter intake.

If the concentration of plasma progesterone is reduced by HS, this would have implications for CR. Low plasma concentrations of progesterone during the luteal phase of the estrus cycle before fertilization can compromise follicular development, leading to abnormal egg maturation and early embryonic death (Ahmad et al., 1995). During the fertilization cycle, low progesterone concentrations can also lead to implantation failure (Mann et al., 1999; Lamming and Royal, 2001).

According to Wolfenson and Roth (2019), the reduced LH secretion is associated with reduced follicular estradiol secretion. Reduced dominance of the preovulatory follicle is reflected by reduced androstenedione and follicular estradiol concentrations and is associated with reduced estrous behavior. Increased number of medium-size follicles (6-9 mm in diameter), most likely due to reduced dominance, is associated with reduced inhibin and increased FSH concentrations. Reduced oocyte and embryo developmental competence is associated with disruption of nuclear and cytoplasmic maturation. Reduced plasma progesterone concentration is related to impaired function of the Corpus Luteum. Reduced fertility in heat-stressed cows is presumed to result from additive effects.

**Influence of HS on the development of follicles**

HS slows follicle selection and prolongs follicular wave and thus has potentially adverse effects on oocyte quality (Badinga et al., 1993; Roth et al., 2001a,b) and follicular steroidogenesis (Howell et al., 1994; Wolfenson et al., 1995; Roth et al., 2001a,b). Summer HS reduces the dominance of the dominant follicle and medium-sized subordinate follicles survive (Wolfenson et al., 1995; Wilson et al., 1998; Roth et al., 2000). When individual follicular dominance is reduced, more than one dominant follicle may develop, which may explain the increase in the percentage of twins that is often seen in cows inseminated and fertilised in the summer (Ryan and Boland, 1991). Thus, HS can simultaneously reduce the secretion of follicular steroids and increase the rate of twinning. In their review Negrón-Pérez et al. (2019) pointed the results of Schüller et al. (2017) in which the ovarian follicle size was 0.1 mm less per each additional unit on the temperature-humidity index on the day of estrus, which agrees with data obtained by Wilson et al. (1998), who found that the second-wave dominant follicle was initially larger in heat-stressed dairy cattle, but then those follicles grew more slowly and their final diameter was detrimentally affected. The follicle fluid also differs in composition during periods of heat stress (Alves et al., 2014), and many have reported differences in ovarian steroid concentrations between thermoneutral and heat-stressed animals (reviewed in Roth and Wolfenson, 2016).

**Influence of HS on the estrus manifestation in dairy cows**

In respect of estrus manifestation, the main clinical effect observed in animals under HS is that as many as 80% of estruses are not detected because of seasonal effect on estrous behavior. An extended period of high temperature shortens the duration and intensity of estrus signs. Furthermore, ovulations without estrous signs are more common during the warmer months of the year. Probably, the main cause of impaired heat detection is that HS reduces the steroidogenic capacity of theca and granulosa cells leading to diminished blood estradiol concentrations (De Rensis et al., 2015).

In cows under HS, the duration and intensity of estrus is reduced in some studies (Singh et al., 1989; IPCC, 2014), but remains unchanged in others (Das and Khan, 2010). The balance of these and other studies suggests that HS reduces the duration and intensity of estrus in dairy cows. For example, in summer, motor activity and other manifestations of estrus have been proven to decrease (Upadhyay et al., 2012; Dash, 2013) and the incidence of anestrus and delayed ovulation increased (Singh et al., 1989). Therefore, in hot climates there is a decrease in the number of inseminations and an increase in the number of inseminations needed for conception. Penev et al. (2020) found that the number of inseminations required for conception increases with increasing THI, the most significant being at THI over 90 - 4.24 inseminations per conception.
Influences of HS on the Days Open interval (DO) in dairy cows

In literature the Non-return rate (NR) is used as a measure of reproductive performance of cattle. Ravagnolo and Misztal (2002) reported that NR45 in Holstein cows showed a decrease of 0.005 per unit increase in THI on the day of insemination for THI>68 and also that NR45 in primiparous was significantly lower and more susceptible to an increase in THI compared to cows in later parities (decrease of 0.008 vs. 0.005).

There was a seasonal trend for change in DO in cattle. The DO in Holstein-Friesian cows is the longest (166 days) after calving in March / April and the shortest (130 days) after calving in September (Oseni et al., 2004). As a conclusion from the above study, the DO of cows is the longest for spring calving and the shortest for cows with autumn calving. The combined effect of high temperature with high humidity in spring and summer leads to physiological disorders affecting the digestive system, acid-alkaline balance of the blood, hormonal balance and finally leads to a longer DO of cows. In the study of Penev et al. (2020) the DO in cows increases with increasing THI values. In conditions of temperature comfort (THI<72) the authors establish a DO of 130.43 days, and under conditions of severe HS (THI>90) the DO reaches 206.7 days.

**Influence of HS on cow Conception rate (CR)**

HS has been shown to have a serious effect on CR in dairy cows (Ingraham et al., 1974). Statistical analysis of fertility in 20,606 cows from seven farms showed that the CR was 39.4% (THI<72.0), 38.5% (THI 72.0 to 73.9), 36.9% (THI 74.0 to 75.9), 32.5% (THI 76.0 to 77.9) and 31.6% (THI>78.0) (Lozano Domínguez et al., 2005). This shows that the CR of dairy cows decreases depending on THI. When the THI is greater than 72.0, there is a decrease in the CR by 1.03% per unit increase in THI (Lozano Domínguez et al., 2005). Penev et al. (2020) found that for THI over 72, the CR was higher in cows inseminated at THI up to or below 72. The effects of HS on CR were investigated in another study involving 1199 crossbred dairy cows for 30 days before and after insemination. In this study, according to whether the THI values were lower than 72 or not, the cows were divided into four groups: thermoneutral-thermoneutral (TN-TN), thermoneutral-HS (TN-HS), HS-thermoneutral (HS-TN) and HS-HS (HS-HS). The results show that the gestation rate in the HS-HS group (20.5%) is significantly lower than that of TN-TN (32.6%), TN-HS (30.3%) and HS-TN (31.8%) groups (p<0.05), while there are no significant differences between the last three groups (p>0.05) (Khan et al., 2013). Probably this is the reason for the results obtained by Penev et al. (2020). These studies show that the gestation rate of dairy cows is reduced in response to HS. Another study showed that the CR was significantly higher after embryo transfer than that obtained by artificial insemination (day 21, 47.6% vs. 18.0%; days 45 to 60, 29.2% vs. 13.5 %; p<0.001) (Putney et al., 1989b). Related studies have reported that HS adversely affects the development and condition of the bovine fallopian tube (Kobayashi et al., 2013) and egg quality (Al-Katanani et al., 2002). Therefore, the decrease in the CR after artificial insemination may be due to the reduced quality of the eggs and the function of the fallopian tubes in response to HS. Garcia-Ispierto et al. (2007) reported a negative effect of HS on this indicator in Holstein cows when THI>75, 3 days before artificial insemination. This decline was from 30.6% to 23% when THI was over 80 in northeastern Spain. The THI threshold for the effect of HS on the CR of lactating cows in Germany is 73 and the greatest negative impact of HS on this indicator is observed 21-1 days before insemination (Schuller et al., 2014). It has been found that the CR of Holstein cows is reduced during insemination in the hot months. Nabenishi et al. (2011) reported that the CR of dairy cows during the hot period (July to September) was 29.5% (p<0.01) significantly lower than the values established during the cold periods (October to June) - 38.2%.

The decrease in CR in hot periods is due to the combined effects of ambient heat, which leads to a change in the synthesis of reproductive hormones (Hahn et al., 2003). HS during the summer season is able to change the follicular microenvironment of highly productive dairy cows, and the harmful effect of HS is related to the physiological processes of the environment and maintenance of pregnancy after conception (Wolfenson et al., 2000).

**Influence of HS on embryos**

The intrauterine environment is also impaired in cows that are exposed to HS. It has been found that there is a decrease in blood flow to the uterus and an increase in uterine temperature (Gwazdauskas et al., 1975; Roman-Ponce et al., 1978). These changes inhibit embryonic development (Rivera and Hanssen, 2001), increase early embryonic losses, and reduce the proportion of successful inseminations. The high ambient temperature also affects the embryos in the attachment stage (Ray et al., 1992), but the degree of effect decreases with the development of the embryos (Ealy et al., 1993). Embryo production by superovulation is often reduced (Alfujairi et al., 1993) with compromised embryonic development observed (Putney et al., 1988) in hot seasons. HS can also affect the secretion of prostaglandin from the endometrium (Putney et al., 1989a), leading to premature luteolysis and embryo loss. In cows exposed to HS, most of the lost embryos were observed before day 42 (Vasconcelos et al., 1998).

**Influence of HS on the pregnancy rate**

In southeastern United States the highest pregnancy rate in Holstein-Friesian dairy cows was in September-November - 32%, while the lowest was in March-May - 24% (Oseni et al., 2005). The lowest pregnancy rate is due to the delay in insemination of cows during the summer months with high levels of HS. Moghaddam et al. (2009) established that cooling...
of dairy heifers for a short time before and after AI, especially with sprinkler and fan, can increase pregnancy rate during heat stress. Amundson et al. (2006) observed negative associations of THI with the pregnancy rate in crosses with Bos taurus for three intervals within the breeding season from 0 to 21 days, 0 to 42 days, and 0 to 60 days, respectively. However, the negative association was most pronounced during the first 21 days of the breeding season with a decrease of 2.06% of the pregnancy rate for each unit increase in THI.

Ways to improve reproduction performance in dairy cows under HS conditions

Estrus detection

One of the factors that increase the interval from calving to insemination of dairy cows during the hot season of the year is the untimely detection of the estrus. The use of paint on the sacrum, HeatWatch system, radiotelemetric pressure transducers and pedometers can improve the detection of estrus and thus CR. However, no studies have been published to evaluate the effects of these aids to detect estrus in summer infertility. Some dairy farmers in Italy are using natural mating in the summer in an attempt to overcome the poor detection of estrus and improve reproductive performance. However, the benefit of improved estrus detection is offset by deterioration in bull fertility caused by HS (Armstrong, 1994).

Microclimate control

Shelter (protection from solar radiation) is considered vital for cows to reduce losses of milk yield and those related to reproduction. It has been found that the total heat load can be reduced by 30 to 50% with a well-designed shelter. Cows in the shade, compared to those in the sun, have a lower body temperature (38.9 vs. 39.4°C), a lower respiratory rate (54 vs. 82 per min), and give 10% more milk (Collier et al., 2006). Researchers at the University of Arizona are considering the effect of shelter and cooling on cows, discussing the benefits and disadvantages of different types of shade shelter. According to them, the location and size of the shelter (shade) is important, and a different orientation is needed depending on whether the climate is dry or humid. Regardless of the climate, an adult dairy cow needs 3.5 to 4.5 m² of shade space, as well as a north-south orientation, so that sunlight can penetrate under the shade to dry the ground. The space under the shelter must be at least 4.3 m high, to reduce the reflected solar radiation from the roof of the shelter to the cow. The use of a porous material (e.g. fabric) is not as effective as a solid roof.

Cooling of cows

Although the shelter reduces the intensity of solar radiation on cows, it has no effect on temperature and relative humidity, so in areas with hot and humid climates, additional cooling of cows is needed (Brouk et al., 2001). Over the past 25 years, various foggers (steam condensed to fine particles of water or fine spray of water particles) and sprinklers spraying small droplets of water have been used to effectively cool the cows.

The need to use heat reduction strategies is a result of high metabolic heat production due to high milk yield, but also to the low level of sweating in cows - about 1/4 to 1/3 of that in horses and humans (Wolfenson and Roth, 2019). Until the 1980s, cooling was based on direct blocking of solar radiation and the use of ventilation, and did not involve spraying water on cows. However, these basic means failed to deal with hyperthermia in cows, which led researchers in Israel to use direct moisturizing of cows' skin to facilitate their cooling. This cooling approach is based on short-term spraying of water, followed by its evaporation from the skin by fan air (Flamenbaum et al., 1986; Berman and Wolfenson, 1992). A combination of spraying systems (wetting with water) and ventilation of dairy cows in hot climate countries is commonly used. Effective cooling requires several cooling cycles per day, consisting of cycles of continuous water spraying and ventilation of about 30-50 minutes each. An alternative approach to cooling cows is low-profile cross-ventilation in free stall barns. This approach requires closed barns and is based on evaporation and thus cooling of the microclimate in the barn. This cross-ventilation system is mainly used in the United States. Cooling efficiency in industrial and conventional farms can be compared by calculating milk productivity and CR in summer and winter. Calculations show that the effective management of cooling in highly productive dairy farms allows to maintain milk production in summer very close (98%) to values in winter (Wolfenson and Roth, 2019). The CR in summer reaches only 68% compared to winter values. Thus, it becomes clear that reproductive performance is much more susceptible to HS (Wolfenson and Roth, 2019).

Hormonal treatment

An alternative approach to improving reproductive performance in the summer is the use of reproductive hormones to stimulate estrus manifestation, synchronization and artificial insemination at a fixed time. Although it is not necessary to address the root causes of summer infertility, this approach offers an opportunity to overcome its effects on fertility. In stressed cows, treatment with GnRH induces follicular development of a preovulatory follicle (Guzeloglu et al., 2001). During the summer, the application of GnRH to lactating dairy cows in estrus increased the CR from 18 to 29% (Ullah et al., 1996). However, luteal support from a single application of hCG (3000 iu) on day 5 or 6 after insemination did not improve summer CR (Schmitt et al., 1996a). Similar results have been reported below from exogenous treatment with progesterone in the form of a CIDR device for intravaginal application (Wolfenson et al., 1994). In recent years, the effect of time-fixed artificial insemination (i.e., without estrus manifestation) on summer CR has been studied (Wolfenson et al., 1994; De la Sota et al., 1998). The results of these studies suggest that these techniques may help overcome the effects of HS and reduce summer infertility. The use of estrus synchronization protocols and breeding via time-fixed artificial insemination obviously has an advantage during the summer.
They are based on the treatment with GnRH or hCG to induce ovulation, followed by a luteolytic dose of prostaglandin F2α after 7 days and a second treatment with GnRH 48 hours after treatment with the luteolytic preparation to induce complete ovulation (Pursley et al., 1995; Schmitt et al., 1996 a,b). In the summer, these programs do not increase the number of cows that have conceived through application of schemes with fixed artificial insemination, but increase the proportion of cows that have conceived up to 120 days after calving and reduce the infertile days (Arechiga et al., 1998; De la Sota et al., 1998; Cartmill et al., 1999; Wolfenson et al., 2000). These approaches lead to an increase in the number of cows that have conceived by increasing the number of cows that were inseminated and although they limit the effect of summer HS on CR, they are not addressed to the main pathology, namely HS.

**Conclusion**

Numerous studies have been conducted over the last few decades on the effect of HS on reproduction in dairy cows. Many of the negative effects of HS are known, but this issue is still on the agenda in front of science and practice. The difficulties in increasing the reproductive performance of dairy cows stem from the multifactorial effect of heat on metabolism, and hence on their whole organism. In addition, we are witnessing drastic climate change expressed in global warming, even in regions of the world considered as optimal in terms of temperature for farming high-yielding dairy cows. Along with the above mentioned, the genetic progress and selection are advancing at a rapid pace with which the milk performance of dairy cows is increasing significantly. At the same time, however, the metabolically produced heat and the sensitivity of the cows to HS increase. All this shows that more work is needed to solve this problem. The differences in the climatic regions of the world, the feed base and the technological and managerial decisions place more questions to which answers must be found for increasing the economic efficiency in dairy cows farming. However, it is clear that more work is needed to find solutions of different nature to reduce the effects of HS.

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**References**


Cavestany D, El-Whishy AB, Foot RH, 1985. Effect of season


