Acta Scientiae Veterinariae, 2022. 50: 1870.

RESEARCH ARTICLE
Pub. 1870

ISSN 1679-9216

Effects of Heat-Stress on Oocyte Number and Quality and *In Vitro* Embryo Production in Holstein Heifers

Sakine Ulkum Cizmeci[1], Dursun Ali Dinc[1], Omer Faruk Yesilkaya[1], Muhammed Furkan Ciftci[1], Abdurrahman Takci[2] & Mustafa Numan Bucak[3]

ABSTRACT

***Background*:** This study aimed to determine the effects of environmental temperature on the number and quality of oocytes and embryo production rates obtained by performing ovum pick up (OPU). Heat stress leads to long-term, short-term, visible, and invisible effects in dairy cows. Its effects on reproduction are evident in all stages, from oocyte development to birth. Disturbance in ovarian follicle development, follicular dominance deficiency, anoestrus, polyspermia, embryonic losses, decreased fetal growth, and abortion are some examples of responses to these effects. The aim of the present study was aimed to determine the effects of ambient temperature on oocyte quality and number and embryo production rates.

***Materials, Methods & Results*:** The animal material used in this study comprised 10 Holstein heifers. At the beginning of the study, the heifers were 13-15 months old. OPU was performed at different times of the year, and weather conditions were recorded. Grouping according to ambient temperature was done as < 10°C (group 1), 10-25°C (group 2), and > 25°C (group 3). The veterinary ultrasonography device and a set of compatible intravaginal OPU probe, catheter, and aspiration device were used for OPU application. All antral follicles with diameters of 2-8 mm in the ovaries were aspirated. The aspirated follicle fluids were examined under a stereo microscope, and the cumulus-oocyte complexes (COC) were collected and classified according to their structure. A, B, and C-quality oocytes were included in the *in vitro* embryo production process. After performing 69 OPUs on random days of the cycle, the number of oocytes per OPU was found to be 8.72, 6.32, and 6.85 in groups 1, 2, and 3, respectively ($P < 0.05$). The number of viable oocytes per OPU was 6.83, 4.64, and 4.65 in groups 1, 2, and 3, respectively ($P < 0.05$). The statistical difference between the first group and the other groups was significant for cleavage and blastocyst counts ($P < 0.05$).

***Discussion*:** All the negative effects of heat stress on animals resulted from the increased body temperature. Reproductive performance is adversely affected by high temperatures and humidity during periods of high ambient temperatures. Metabolic heat is released, and the heat load increases due to the metabolism of nutrients in cattle. Internal body temperature is regulated via the dissipation of metabolic heat to the environment. The amount of heat dissipated via conduction and convection depends on the unit body weight, surface area, skin and coat color, difference in temperature gradient of the animal and ambient temperature, and humidity. In the present study, it was determined that the blastocyst development rates of the oocytes obtained in the warm season (>25°C [group 3]) were lower than those of the other groups. It was concluded that this may be because the oocytes developed under chronic heat stress in the animals, and several cycles were required to enhance oocyte quality and developmental potential. Additional studies are needed to investigate the response of oocytes obtained with OPU to heat stress during embryonic developmental stages and to determine the sensitivity and effects of embryonic tissue damage according to developmental stages. Based on the results of the present study, it was concluded that performing OPU and *in vitro* embryo production (IVEP) when the ambient temperature is close to the thermoneutral limits may increase the blastocyst development rates.

Keywords: blastocyst, heat stress, heifer, *in vitro* embryo production, oocyte quality, ovum pick-up.

DOI: 10.22456/1679-9216.122371

Received: 7 February 2022 Accepted: 27 March 2022 Published: 29 April 2022

[1]Department of Obstetrics and Gyneacology & [3]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Selçuk, Selçuklu, Konya, Turkey. [2]Department of Obstetrics and Gyneacology, Faculty of Veterinary Medicine, University of Cumhuriyet, Sivas, Turkey. CORRESPONDENCE: S.U. Cizmeci [ulkum@selcuk.edu.tr]. Department of Obstetrics and Gyneacology, Faculty of Veterinary Medicine, Selcuk University. 42250 Selçuklu, Konya, Turkey.

INTRODUCTION

Due to the rapidly increasing global population, animal food deficit [20,49] and global climate change, livestock are adversely affected worldwide [11,33,39,45]. The currently dominant 1.5°C rise in global temperature is estimated to rise to 5°C in the 2050s [51]. The ambient temperature range in which animals do not use additional energy to maintain their body temperature is called the thermoneutral zone (TNZ). In general, the TNZ range is influenced by certain factors such as animal age, species, ration, ambient temperature, yield and animal behavior [2].

An increase in cortisol secretion during heat stress results in the disruption of GnRH secretion and reduction of blood FSH and LH levels [3,8,28,51]. Consequently, follicular development is suppressed, the estrous cycle becomes irregular [4,37,50], and oocyte quality deteriorates [7]. In addition, problems in the detection of estrus may occur due to decreased estradiol levels [3,50], low birth weight and growth retardation, and impaired placental development may be observed in calves due to the decrease in nutrient and oxygen intake [8,48].

It also includes problems such as the small diameter of the dominant follicle in the 1st and 2nd follicular waves, lack of dominance, and an increase of large follicles [14,29,42]. For this reason, the fertilization and blastocyst rates of oocytes collected in the summer months are lower than those collected in the winter months [19,41,44], and at least 2-3 should pass for the end of the heat stress [43]. The aim of the present study was aimed to determine the effects of ambient temperature on oocyte quality and number and embryo production rates.

MATERIALS AND METHODS

Animals

Ten Holstein heifers were used in this study. At the beginning of the study, the heifers were 13-15 months old. The heifers were fed a total mixed ration (TMR) at level of energy requirement of maintenance in a semi-open barn.

Experimental setup

Heifers selected as donors were not administered hormones for superovulation or synchronization before OPU. OPU procedures were performed at different times of the year and on random days of the cycle. The air temperatures on the days of OPU application were recorded. Grouping according to ambient temperature was done as < 10°C (group 1), 10-25°C (group 2), and > 25°C (group 3). The OPU-treated area was maintained at 24-26°C using air conditioning. The laboratory environment was climatized to > 30°C.

OPU application

The veterinary ultrasonography device[1] and a set of compatible intravaginal OPU probe, catheter, and aspiration device[2] were used for OPU application. For OPU, the animal was put into labor and anesthetized via the lower epidural route (4-6 cc of local anesthetic)[3]. All antral follicles with diameters of 2-8 mm in the ovaries were aspirated using a special convex vaginal probe (4.0-9.0 MHz, combined with a probe and a catheter with a 20 G needle at the tip) at 80-90 mmHg aspiration pressure. The aspiration line was rinsed continuously with an ovum pick-up (OPU) solution during follicular aspiration. During the procedure, the aspirated follicular fluid and COCs were maintained at 37°C in a special section connected to the pump.

In vitro *embryo production (IVEP) application*

Bovine IVF (OPU, BO-Wash, BO-IVM, BO-IVF, Semenprep, BO-IVC, and BO-Oil)[4] medias were used in this study.

The COCs from the follicle fluids were collected under a stereomicroscope and classified according to the structure of the COC (i.e., A, B, C, and D-quality COCs). The following criteria were used to evaluate the collected COCs:

Grade A: Oocytes with ≥ 5 compact cell layers and homogeneous cytoplasm.

Grade B: Oocytes with few inhomogeneous areas in the cytoplasm and 3-5 layers of compact cell layers or ≥ 5 layers of compact cells and dense inhomogeneous areas.

Grade C: Oocytes with few cell layers (> 3) and few inhomogeneous areas in the cytoplasm or localized loss of cumulus and little homogeneous area.

Grade D: Oocytes with small, granular, inhomogeneous cytoplasm, with the cumulus layer completely lost [36].

A, B, and C-grade oocytes were used in the *in vitro* embryo production process. The oocytes were washed with the BO WASH (with HEPES) solution. Then, 10 oocytes were placed in 100 µL HEPES-free BO-IVM (*in vitro* maturation) medium equilibrated

for at least 4 h in petri dishes covered with mineral oil. Next, the oocytes were incubated in a mono gas incubator[5] (5.5% CO_2, 38.8°C, humidified air) for 22 h. Maturation controls were established according to the expansion of cumulus cells and the presence of a polar body. The presence of one of these parameters was considered indicative of maturation. After centrifugation with the Semenprep solution at 328 *g* twice for 5 min, semen diluted with equilibrated BO-IVF (*in vitro* fertilization) medium to 10,000 (100,000 spermatozoa/30 µL) spermatozoa per oocyte was incubated in a mono gas incubator. Mature oocytes were placed in IVF drops and incubated for 20-22 h. Cumulus cleaning processes were performed via pipetting, assuming that sample losses may occur after vortexing. Fertilization control was carried out, after cleaning the cumulus cells, by observing the fertilization gap between the cytoplasm of the oocyte and Zona Pellucida (ZP) in the area where the sperm enters, or the presence of sperm bound to ZP [16]. After the fertilization stage, possible zygotes, which were removed from the cumulus cells via pipetting, were transferred to 100 µL *in vitro* culture (IVC) drops equilibrated overnight and cultured in a tri-gas incubator[5] (6% O_2, 6% CO_2, 88% N, 38.8°C) for 7 days [38]. The rates of 2-16-cell stage embryo formation on the 3rd day and blastocyst formation on the 6th and 7th days in the culture medium were evaluated.

The following formulae were used in the calculations [15,17]:

Maturation rate = (Number of oocytes with cumulus expansion or polar bodies detected (mature oocyte))/(Number of oocytes left to mature)

Cleavage rate = (Number of embryos with 2 cells and division)/(Number of mature oocytes)

Blastocyst formation rate = (Blastocyst count)/(Mature oocyte count)

Statistical analysis

The SPSS-Statistics-22[6] package program was used for statistical analysis. Differences among IVEP stages were evaluated using Duncan's test.

RESULTS

A total of 69 OPUs were performed on 10 animals on random days of the cycle. It was found that the air temperature was < 10°C (group 1), between 10 and 25°C (group 2), and > 25°C (group 3) in 18, 25, and 26 of these 69 applications, respectively. Furthermore, it was determined that 123 of the 157 oocytes collected in the 1st group, 116 of the 158 oocytes collected in the 2nd group, and 121 of the 178 oocytes collected in the 3rd group were alive. Oocytes evaluated as viable were used in the *in vitro* embryo production stages. After OPU, the number of oocytes per OPU was 8.72, 6.32, and 6.85 in groups 1, 2, and 3, respectively. The number of viable oocytes per OPU was 6.83, 4.64, and 4.65 in groups 1, 2, and 3, respectively. There was a statistically significant difference in the number of oocytes per OPU among the groups ($P < 0.05$). There was a statistically significant difference in the number of live oocytes per OPU between the first group and the other groups [Table 1] ($P < 0.05$).

All oocytes used for IVEP application were transferred to the fertilization stage regardless of their maturation response, followed by cleavage. After *in vitro* fertilization, 90 of 123 oocytes were cleaved in group 1; the number of cleavages per OPU was 5, and the cleavage rate was 73.17%. In the second group, 66 of 116 oocytes were cleaved, and the cleavage number per OPU was 2.64 (56.90%). In the third group, 74 of 121 oocytes were cleaved, and the number of cleavages per OPU was 2.85 (61.16%). The blastocyst counts were 1.39 (20.33%), 0.64 (13.79%), and 0.81 (17.36%) per OPU in groups 1, 2, and 3, respectively. The statistical difference between the first group and the other groups was significant for cleavage and blastocyst counts ($P < 0.05$).

DISCUSSION

In lactating Holstein cows, the TNZ is in the range of 4-24°C. The effects of heat stress on cows begin to be observed above 24°C, and milk yield decreases significantly above 27°C [27]. The average TNZ in Turkey is 16°C [10].

Owing to the metabolism of nutrients in cattle, metabolic heat is released, and the heat load increases [26]. Internal body temperature is regulated by dissipating metabolic heat to the environment. Heat loss in dairy cows occurs through convection, radiation, and evaporative cooling [5]. The amount of heat dissipated via conduction and convection depends on the unit body weight, surface area, skin and coat color, the difference in temperature gradient of the animal and ambient temperature, and humidity [18,23,24]. The physiological response to heat stress includes reduced feed intake, milk production, and secretion of thyroid hormones [13].

Table 1. Ovum Pick Up (total oocytes, viable oocytes) and *in vitro* embryo production (fertilization, cleavage and blastocysts) results.

Ambient temperature	< 10°C (Group 1)	10-25°C (Group 2)	>25°C (Group 3)
Number of OPU applications	18	25	26
Total Oocyte	157	158	178
A+B+C (Viable oocyte)	123	116	121
Number of oocytes per OPU	8.72[c]	6.32[e]	6.85[d]
Number of viable oocytes per OPU	6.83[a]	4.64[b]	4.65[b]
Number of fertilized oocytes	90	66	74
Number of cleavages per OPU	5.00[f]	2.64[g]	2.85[g]
Number of blastocysts per OPU	1.39[h]	0.64[i]	0.81[i]
Cleavage rate %	73.17	56.90	61.16
Blastocyst rate %	20.33	13.79	17.36

All negative effects of heat stress on animals are the result of increased body temperature. The increase in body temperature results from disruption of the balance between heat production and heat loss [5]. In cows exposed to heat stress, the mechanism of GnRH release from the hypothalamus is disrupted, and the blood levels of FSH and LH decrease. These neuroendocrine and endocrine events negatively affect the selection and development of follicles, which may result in the disruption of ovulation and functional corpus luteum development [9,52]. Reproductive performance is adversely affected by high temperature and humidity during high ambient temperature periods [25]. There is no direct evidence for the effect of heat stress on primary follicles. However, the observation that reproductive parameters return to normal 40-60 days after exposure to heat stress suggests that heat stress exerts its effects during the very early stages of follicle development. The fact that the time from the primary follicle stage to the Graafian follicle stage is approximately 60 days, and oocyte development is the most sensitive period in the primary and secondary follicle stages before the zona pellucida has formed, explains this mechanism [12]. In Brazil, where the annual average temperature is >25°C, OPU has been applied at different times of the year in cattle breeds with different TNZ ranges; the results have shown that the blastocyst development rates are low in oocytes obtained under chronic exposure during the hot months of the year [47]. Similarly, in the present study, the blastocyst development rates were lower in the 3rd group than in the other groups.

It has been reported that the cleavage, maturation, and blastocyst formation rates of oocytes obtained from animals exposed to heat stress are low [40]. Furthermore, the oocytes obtained from follicles that develop under heat stress are of poor quality [1]. It has been reported that upon the short-term exposure of preantral follicles and the COCs to a high temperature (41°C for 12 h), preantral follicles are less sensitive to heat stress than the COCs, primordial follicles develop early, ROS production from preantral follicles and their COCs increases, estradiol production by the COCs decreases, and progesterone secretion increases [35]. It has been reported that the animals exposed to heat stress show changes in steroid concentrations in the follicular fluid [34]. Furthermore, heat stress results in disruption of the cytoskeletal structure of COCs during the first 12 h of *in vitro* maturation, reduction in the nuclear maturation of oocytes, and death of oocytes due to apoptosis [42]. In the present study, we determined that the number of viable oocytes per OPU was low in applications performed at high ambient temperatures. The reduction in the fertilization, cleavage, and blastocyst formation rates of the collected oocytes was statistically significant. In our study, wherein IVEP was applied, it was concluded that the lower blastocyst formation rates obtained when OPUs (>25°C [group 3]) were performed during the period of heat stress may be because of the oocyte death due to apoptosis or poor oocyte quality.

Previous studies have shown that the follicles are 0.1°C to 2.5°C cooler than the adjacent

ovarian tissues and nearby organs before ovulation [21,22]. Furthermore, the follicle is 1.0°C or colder than the neighboring tissues, while the maturation, fertilization, and embryonic development stages are normal, and the oocytes that result in a successful pregnancy originate from colder follicles [30-32]. Therefore, it was concluded that the better rates in group 1 outside the thermoneutral area may be due to that the cattle are not affected by cold as much as by hot conditions.

High ambient temperature, which continues during *in vitro* fertilization, adversely affects the anti-polyspermy systems of oocytes, thus increasing fertilization loss [46]. In this study, it was considered that the differences in the number of cleavages and blastocysts among the groups were related to the environmental conditions to which the oocytes obtained via OPU were exposed during development, since fertilization was provided under the same conditions after the oocytes were obtained. Consistent with the results of the present study, it has been reported that heat stress causes deterioration of ovarian function; additionally, reduction in the embryo retrieval rates has been observed in *in vivo* embryo production studies [6].

CONCLUSION

Additional studies are needed to investigate the response of oocytes obtained with OPU to heat stress during embryonic developmental stages and to determine the sensitivity and effects of embryonic tissue damage according to developmental stages. In this study, it was concluded that when the ambient temperature is close to the TNZ, providing shelter conditions to animals to protect them from heat stress during OPU and IVEP application can increase the blastocyst development rates.

MANUFACTURERS

[1]Esaote Biomedica. Genova, Italy.

[2]Minitübe GmbH. Tiefenbach, Germany.

[3]Sanovel Ilac. Istanbul, Turkey.

[4]IVF Bioscience, Cornwall, UK.

[5]Thermo Fisher Scientific. Waltham, MA,USA.

[6]IBM - International Business Machines Corporation. Armonk, NY, USA.

Ethical approval. The study was conducted with approval of the Selcuk University Veterinary Faculty Experimental Animals Production and Research Center Ethics Committee (SÜVDAMEK) (2019/28).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

REFERENCES

1 **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. *International Journal of Veterinary Science and Medicine*. 6: 1-5.

2 **Aggarwal A. & Upadhyay R. 2013.** Thermoregulation. In: *Heat Stress and Animal Productivity*. London: Springer, pp.2-20.

3 **Altınccekic S. & Koyuncu M. 2012.** Farm animals and stress. *Journal of Animal Production*. 53(1): 27-37.

4 **Ari U.C. 2015.** Physiologic and Hormonal Effects of Heat Stress on Reproduction in Cattle. In: *Turkiye Klinikleri Reproduction and Artificial Insemination - Special Topics*. 1(1): 1-10.

5 **Atrian P. & Shahryar H.A. 2012.** Heat Stress in Dairy Cows (A Review). *Research in Zoology*. 2(4): 31-37

6 **Bényei B., Gáspárdy A. & Barros C.W.C. 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. *Animal Reproduction Science*. 68(1-2): 57-68.

7 **Bridges P.J., Brusie M.A. & Fortune J.E. 2005.** Elevated (heat stress) *in vitro* reduces androstenedione and estradiol and increases progesterone secretion by follicular cells from bovine dominant follicles. *Domestic Animal Endocrinology*. 29: 508-522.

8 **De Rensis F. & Scaramuzzi RJ. 2003.** Heat stress and seasonal effects on reproduction in the dairy cow-a review. *Theriogenology*. 60: 1139-1151.

9 **De Rensis F., Saleri R., Garcia-Isierto I., Scaramuzzi R. & Lopes-Gaitus F. 2021.** Effects of Heat Stress on Follicular Physiology in Dairy Cows. *Animals*. 11(3406): 1-9.

10 **Durmuş M. & Koluman N. 2019.** Hormonal Changes caused on Ruminant Animals exposed to High Environment Temperature. *Journal of Animal Production*. 60 (2): 159-169.

11 El-Sayed A. & Kamel M. 2020. Climatic changes and their role in emergence and re-emergence of diseases. *Environmental Science and Pollution Research*. 27(18): 22336-22352.

12 Fair T. 2003. Follicular oocyte growth and acquisition of developmental competence. *Animal Reproduction Science*. 78: 203-216.

13 Farooq U., Samad H.A., Shehzad F. & Qayyum A. 2010. Physiological responses of cattle to heat stress. *World Applied Sciences Journal*. 8: 38-43.

14 Gendelman M., Aroyo A., Yavin S. & Roth Z. 2010. Seasonal effects on gene expression, cleavage timing, and developmental competence of bovine preimplantation embryos. *Reproduction*. 140(1): 73-82.

15 Grondahl M.L., Christiansen S.L., Kesmodel U.S., Agerholm I.E., Lemmen J.G., Lundstrom P., Bogstad J., Raaschou-Jensen M. & Ladelund S. 2017. Effect of women's age on embryo morphology, cleavage rate and competence- A multicenter cohort study. *PLoS One*. 12: 1722456.

16 Gürer F. 2003. Oocyte maturation *in vivo* and *in vitro*. In: Hassa H. (Ed). *Clinical Approach and IVF Laboratory Practices in Infertile Cases*. Eskisehir: Osmangazi Publishing, pp.229-240.

17 Hammond E.R. & Morbeck D.E. 2019. Tracking Quality: can embryology key performance indicators be used to identify clinically relevant shifts in pregnancy rate? *Human Reproduction*. 34: 37-43.

18 Hansen P.J. & Arrchiga C.F. 1999. Strategies for managing reproduction in the heat-stressed dairy cows. *Journal of Animal Science*. 77(2): 36-50.

19 Hansen P.J., Drost M., Rivera R.M., Paula-Lopes F.F., Al-Katanani Y.M., Krininger III C. E. & Chase Jr. C.C. 2001. Adverse impact of heat stress on embryo production: causes and strategies for mitigation. *Theriogenology*. 55(1): 91-103.

20 Hill D.L. & Wall E. 2017. Weather influences feed intake and feed efficiency in a temperate climate. *Journal of Dairy Science*. 100(3): 2240-2257.

21 Hunter R.H.F. & López-Gatius F. 2020. Intra-follicular temperature acts to regulate mammalian ovulation. *Acta Obstetricia et Gynecologica Scandinavica*. 99: 301-302.

22 Hunter R.H.F. 2012. Temperature gradients in female reproductive tissues. *Reproductive BioMedicine Online*. 24: 377-380.

23 Hutchinson J.C.D & Brown G.D. 1969. Penetrance of cattle coats by radiation. *Journal of Applied Physiology*. 26(4): 1-11.

24 Hutchinson J.C.D & Sykes A.H. 1953. Physiological acclimatization of fowls to a hot humid environment. *The Journal of Agricultural Science*. 43(03): 294-322.

25 Ju J.C. 2005. Cellular responses of oocytes and embryos under thermal stress: hints to molecular signaling. *Animal Reproduction*. 2(2): 79-90.

26 Kadzere C.T., Murphy M.R., Silanikove N. & Maltz E. 2002. Heat stress in lactating dairy cows: a review. *Livestock Production Science*. 77: 59-91.

27 Krihara M. & Shioya S. 2002. Dairy cattle management in a hot environment. 1-9. Access: https://www.fftc.org.tw/htmlarea_file/library/20110801153254/eb529.pdf.

28 Kutlu B. & Varisli O. 2012. The Pregnancy rate of cattle, which was inseminated in different season in Sanliurfa. *Harran Üniversitesi Üniversitesi Veteriner Fakültesi Dergisi*. 1(2): 97-102.

29 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. *Animal Reproduction*. 9(3): 395-403.

30 López-Gatius, F. & Hunter, R.H.F. 2017. Clinical relevance of pre-ovulatory follicular temperature in heat-stressed lactating dairy cows. *Reproduction in Domestic Animals*. 52: 366-370.

31 López-Gatius F. & Hunter R.H.F. 2019. Pre-ovulatory follicular temperature in bi-ovular cows. *Journal of Reproduction and Development*. 65: 191-194.

32 López-Gatius F. & Hunter R.H.F. 2019. Pre-ovulatory follicular cooling correlates positively with the potential for pregnancy in dairy cows: Implications for human IVF. *Journal of Gynecology Obstetrics and Human Reproduction*. 48: 419-422.

33 Naranjo-Gómez J.S., Uribe-García H.F., Herrera-Sánchez M.P., Lozano-Villegas K.J., Rodríguez-Hernández R. & Rondón-Barragán I.S. 2021. Heat stress on cattle embryo: gene regulation and adaptation. *Heliyon*. 7(3): e06570.

34 Negron-Perez V.M., Fausnacht D.W. & Rhoads M.L. 2019. Invited review: Management strategies capable of improving the reproductive performance of heat-stressed dairy cattle. *Journal of Dairy Science*. 102: 10695-10710.

35 **Paes V.M., Vieira L.A., Correira H.H.V., Sa N.A.R., Moura A.A.A., Sales A.D., Rodrigues A.P.R., Magalhaes-Padilha D.M., Santos F.W., Apgar G.A., Campello C.C., Camargo L.S.A. & Figueiredo J.R. 2016.** Effect of heat stress on the survival and development of *in vitro* cultured bovine preantral follicles and on *in vitro* maturation of cumulus-oocyte complex. *Theriogenology*. 86: 994-1003.

36 **Petyim S, Bage R, Forsberg M, Rodriguez-Martinez, H. & Larsson B. 2001.** Effects of repeated follicular puncture on ovarian morphology and endocrine parameters in dairy heifers. *Journal of Veterinary Medicine Series A*. 48: 449-463.

37 **Polsky L. & von Keyrerlink M.A.G. 2017.** Invited review: effects of heat stress on dairy cattle welfare. *Journal of Dairy Science*. 100: 8645-8657.

38 **Razza E.M., Pedersen H.S., Stroebech L., Fontes P.K., Kadarmideen H.N., Callesen H., Pihl M., Nogueira F.G. & Hyttel P. 2018.** Simulated physiological oocyte maturation has side effects on bovine oocytes and embryos. *The Journal of Assisted Reproduction and Genetics*. 36(3): 413-424.

39 **Renaudeau D., Collin A., Yahav S., De Basilio V., Gourdine J.L. & Collier R.J. 2012.** Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal*. 6(5): 707-728.

40 **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.

41 **Rocha A., Randel R.D., Broussard J.R., Lim J.M., Blair R.M., Roussel J.D., Godke R.A. & Hansel W. 1998.** High environmental temperature and humidity decrease oocyte quality in *Bos taurus* but not in *Bos taurus* cows. *Theriogenology*. 49(3): 657-665.

42 **Roth Z. 2008.** Heat stress, the follicle, and its enclosed oocyte: mechanisms and potential strategies to improve fertility in dairy cows. *Reproduction in Domestic Animals*. 43: 238-244.

43 **Roth Z., Arav A., Bor A., Zeron Y., Braw-Tal R. & Wolfenson D. 2001.** Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. *Reproduction*.122(5): 737-744.

44 **Roth Z. 2018.** Symposium review: Reduction in oocyte developmental competence by stress is associated with alterations in mitochondrial function. *Journal of Dairy Science*. 101(4): 3642-3654.

45 **Rust J.M. 2019.** The impact of climate change on extensive and intensive livestock production systems. *Animal Frontiers*. 9(1): 20-25.

46 **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. *Molecular Reproduction and Development*. 82(1): 36-47.

47 **Souza-Cácares M.B., Fialho A.L.L., Silva W.A.L., Cardoso C.J.T., Pöhland R., Martins M.I.M. & Melo-Sterza F.A. 2019.** Oocyte quality and heat shock proteins in oocytes from bovine breeds adapted to the tropics under different conditions of environmental thermal stress. *Theriogenology*. 130: 103-110.

48 **Tao S. & Dahl G.E. 2013.** Invited review: Heat stress effects during late gestation on dry cows and their calves. *Journal of Dairy Science*. 96(7): 4079-4093.

49 **Van Zanten H.H., Herrero M., Van Hal O., Röös E., Muller A., Garnett T., Pierre J.G., Schader C. & De Boer I.J. 2018.** Defining a land boundary for sustainable livestock consumption. *Global Change Biology*. 24(9): 4185-4194.

50 **Wang J., Li J., Wang F., Xiao J., Wang Y., Yang H., Li S. & Cao Z. 2020.** Heat stress on calves and heifers: a review. *Journal of Animal Science and Biotechnology*. 11: 79.

51 **Wankar A.K., Rindhe S.N. & Doijad N.S. 2021.** Heat stress in dairy animals and current milk production trends, economics, and future perspectives: the global scenario. *Tropical Animal Health and Production*. 53(70): 1-12.

52 **Wilson S. J., Mairon R.S., Spain J.N. Spe,iers D.E. Keisler D.H. & Lucy M.C. 1998.** Effects of controlled heat stress on ovarian function of dairy cattle. Lactating cows. *Journal of Dairy Science*. 81(8): 2124-2131.