Ciência Animal Brasileira
Ciência Animal Brasileira
Brazilian Animal Science
e-ISSN 1809-6891
DOI: 10.1590/1809-6891v23e-71852E

Section: Veterinary medicine
Research article

Influence of heat stress on *in vitro* oocyte and embryo production in high-yielding Holstein cows

Influência do estresse calórico na produção in vitro de oócitos e embriões de vacas Holandesas de alta produtividade

Francieli Berling[1*], Fernanda Cavallari de Castro[2], Ana Carolina dos Santos Oliveira[1]

[1]Universidade Regional de Blumenau (FURB), Blumenau, Santa Catarina, Brazil
[2]Koe Bio Embryo Laboratory LTDA, Carambeí, Paraná, Brazil
[*]Correspondent: francieliberling@gmail.com

Abstract
The objective of this study was to evaluate the influence of thermal shock on oocytes used in the production of *in vitro* embryos (IVP) of high productivity Holstein cows on the day of follicular aspiration (OPU; 0), 30, 60 and 90 days before the OPU. From the mean temperature on day 0 and on the previous 30, 60 and 90 days, they were classified into comfort group (TC; up to 15°C) and heat stress (HS; above 15°C) groups. A negative influence was observed on oocytes and viable embryos (total and grade I). The heat stress in the periods of 30 and 60 days prior to OPU resulted in lower production of viable oocytes (P=0.0028; P=0.0092, respectively). Under stress, on the day of OPU (HS-OPU), cows showed no reduction in the amount of viable oocytes (P=0.5497) and there was no influence of temperature for the group stressed 90 days before OPU (P=0.8287). For total embryos, the difference occurred only in the HS-30 group (P=0.0317), where the groups HS-OPU, HS-60, HS-90 presented, respectively, P=0. 1987, P=0.0596 and P=0.4580. Regarding the production of embryos of grade 1, there was no difference for the groups HS-OPU (P=0.2291) and HS-90 (P=0.2868), but there was a reduction for HS-30 (P=0.0143) and HS-60 (P=0.0253). In summary, heat stress had a negative impact when it occurred 30 or 60 days before follicular aspiration. In addition, 30 days seems to be the period of more susceptibility and that causes the greatest deleterious effects on oocyte viability and IVP.
Keywords: Hyperthermia; Thermal shock; Ovum Pick Up; Dairy cows.

Resumo
Objetivou-se avaliar a influência do estresse térmico em oócitos utilizados na produção *in vitro* de embriões (PIV) bovinos da raça Holandesa de alta produtividade no dia da aspiração folicular (OPU; 0), 30, 60 e 90 dias antes da OPU. A partir da temperatura média no dia 0 e aos 30, 60 e 90 dias anteriores, foram classificados nos grupos conforto (CT; até 15°C) e estresse por calor (ET - acima de 15°C). Observou-se influência negativa em oócitos e embriões viáveis (total e grau I). A submissão ao estresse térmico nos períodos de 30 e 60 dias anteriores à OPU resultou em menor produção de oócitos viáveis (P=0,0028; P=0,0092, respectivamente). Sob estresse, no dia da OPU (ET-OPU), as vacas não apresentaram redução na quantidade de oócitos viáveis (P=0,5497) e não houve influência da temperatura para o grupo estressado 90 dias antes da OPU (P=0,8287). Para embriões totais, a diferença ocorreu apenas no grupo ET-30 (P=0,0317), onde os grupos ET-OPU, ET-60, ET-90 apresentaram, respectivamente, P=0,1987, P=0,0596 e P=0,4580. Em relação à produção de embriões grau 1, não houve diferença para os grupos ET-OPU (P=0,2291) e ET-90 (P=0,2868), porém houve redução para ET-30 (P=0,0143) e ET- 60 (P=0,0253). Em resumo, o estresse por calor teve impacto negativo quando ocorreu 30 ou 60 dias antes da aspiração folicular. Além disso, 30 dias parece ser o período de maior suscetibilidade e que causa os maiores efeitos deletérios na viabilidade oocitária e na PIV.
Palavras-chave: Hipertermia; Estresse térmico; Aspiração Folicular; Vacas leiteiras.

Received: February 15, 2022. Accepted: June 7, 2022. Published: July 5, 2022.

Introduction

Considerable changes have typified the dairy production chain in recent years, although production has been expanded, it was possible to observe a significant drop in the number of producers and animals [1]. The number of cows milked in 2020 was 16.2 million, a decrease of 0.8% compared to the previous year, however, the productivity per animal increased and reached 2.192 liters/cow per year [2]. Presumably, this growth is mainly due to the genetic improvement of these animals through the introduction of technologies that increased productivity [3], including *in vitro* embryo production (IVP) [4].

IVP is widely applied in animals of high zootechnical value as it maximizes the reproductive potential of the herd [5] and increases productivity per animal by replacing the herd with genetically improved descendants within a short period of time [4]. Thus, it is possible for a female to generate an average of thirty-six offspring per year, while naturally she would only generate one [6].

European bovine breeds are highly selected for dairy production, among these, the Holsteins stand out for its productivity and for being cosmopolitan [7]. However, taurine cows are less resistant to hyperthermia when compared to *Bos taurus indicus* breeds, due to their lower heat dissipation capacity to the environment [8]. In this context, Brazilian climate may eventually expose these animals to high temperatures and relative humidity, in addition to the high incidence of solar radiation, in order to cause a breakdown of body homeostasis, negatively impacting both the productive and reproductive performance of the herd [9].

The climatic conditions imposed on the matrices influence the IVP, where embryonic development is compromised by the high sensitivity to high temperatures presented by both oocytes and embryos [10]. These conditions associated with the high metabolic levels of high-producing cows [8] make Holstein cows more susceptible to heat stress and, consequently, oocytes have a lower capacity to generate blastocysts [11].

Therefore, this study aimed to evaluate the influence of room temperature on the day of OPU (0) and the mean temperature 30, 60 and 90 days before the procedure on the viability of the produced oocyte. In addition, the *in vitro* production of total and grade I embryos from oocytes subjected to *in vivo* heat stress on day 0 and in the periods of 30, 60 and 90 days prior to the procedure was compared.

Material and methods

Animals

The present study used data collected from June 2018 to August 2019 on the *in vitro* production of embryos from a commercial laboratory. All data came from a single dairy farm, located in the city of Carambeí, state of Paraná, Brazil, at coordinates 24°47'02.0"S, 50°12'30.5W. The information regarding the classification of oocytes and embryos from 326 dairy cows, aged between 4 and 14 years old, were allocated in a Microsoft Excel 365® program spreadsheet, adopting as exclusion criteria cows whose average lactation in 305 days was less than 8,000 kg of milk. The herd remained confined in a Free Stall system with ventilation and water sprinkling and presented a general average production of 42 kg milk/day/animal and was homogeneous in terms of body condition, which was classified as excellent.

Regarding the number of oocytes collected, among the three hundred and twenty-six matrices classified as high production (n=326), 138 cows were allocated in the group called thermal comfort on the day of OPU (TC-OPU) and 186 in the group of heat stress (HS-OPU); thirty days before OPU 144 were in comfort group (TC-30) and 182 in heat stress (HS-30); at sixty days 142 (TC-60) and 183 (HS-60); and at 90 days 85 (TC-90) and 238 (HS-90). This is a retrospective study, so there was no need for approval by an ethics committee.

Temperature and humidity

From a report published by the National Institute of Meteorology of Brazil (NIMET), the averages of temperature and relative air humidity were obtained for the day of follicular aspiration (OPU), at 30, 60 and 90 days prior to the procedure. Based on this information, the matrices were classified into a control group and a heat stress group at 0, 30, 60 and 90 days before aspiration. The temperature range defined by Klein [12] as ideal for high-yielding Holstein cows is between 4 and 15°C. The average relative humidity limit cited as ideal for animal welfare is between 50-90% [13].

In the present study, since the average data of Relative Humidity, obtained on the day of the OPU (83.8ºC ± 6.9), at 30 (81.1ºC ± 4.5), 60 (81.5ºC ± 8, 2) and 90 (81.3 ºC ± 7.9) days prior to the process remained very close, and with a small standard deviation (<10%), only the average temperature variable was fixed for data analysis, calculated for each of the groups (0, 30, 60 and 90 days). Thus, animals exposed to an average temperature above 15°C were classified as the heat stress group, and those within the comfort range (up to 15°C) were called the control

group (thermal comfort).

Data analysis

Statistical analysis used the Anova Two Way test, with the aid of the Graphpad Prism 8® software. Firstly, for each period (day 0, 30, 60 and 90) a control group and a stress group were compared. In addition, simultaneous comparisons were established between all TC and HS groups with all periods (day 0, 30, 60 and 90). This methodology was applied to each variable studied (viable oocytes, total and grade I embryos).

Finally, to increase accuracy, the T-Student test was used, which compared the number of viable oocytes aspirated in the comfort versus heat stress group for each period (0, 30, 60 and 90). Furthermore, using the same methodology, total and grade I embryo production were compared in the control and stress groups.

Results

When applying the Anova Two Way test, the influence of temperature on the production of oocytes (P = 0.0012), total embryos (P = 0.0024) and grade I embryos (P = 0.0003) was observed. In the case of oocytes, the impact of temperature was pronounced for a period of thirty days; the TC-30 and HS-30 groups differed from each other and from the other groups by the Anova test. That is, cows subjected to heat stress thirty days before follicular aspiration produced viable oocytes in a significantly lower quantity than those observed in the other evaluated groups.

Regarding the Student T test, the submission to heat stress in the periods of 30 and 60 days prior to the OPU resulted in lower production of viable oocytes for the stressed group (P = 0.0028; P = 0.0092, respectively). When under heat stress on day 0 (HS-OPU), cows showed no reduction in the number of viable oocytes (P = 0.5497), as well as no influence of temperature for the stress group in the period of 90 days before OPU (P = 0.8287). The results on oocyte production analyzed by the T test are shown in Figure 1.

For total embryos (Figure 2), the difference occurred only in the HS-30 group (P = 0.0317), as the HS-OPU, HS-60, HS-90 groups showed, respectively, P = 0.1987, P = 0.0596, P = 0.4580.

Regarding the production of grade 1 embryos (Figure 3), there was no difference for the HS-OPU (P = 0.2291) and HS-90 (P = 0.2868) groups, however there was an expressive divergence for HS-30 (P = 0.0143) and HS-60 (P = 0.0253).

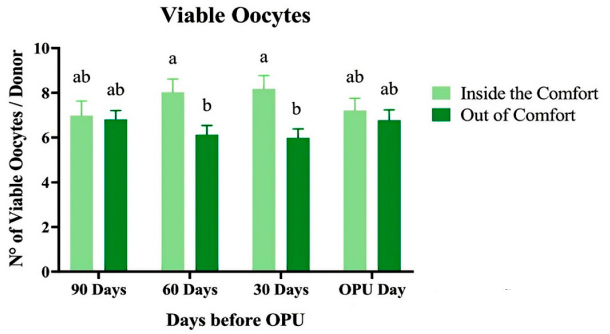

Figure 1. Number of viable oocytes produced by the comfort and heat stress groups on the day of OPU and at 30, 60 and 90 days prior to the procedure.

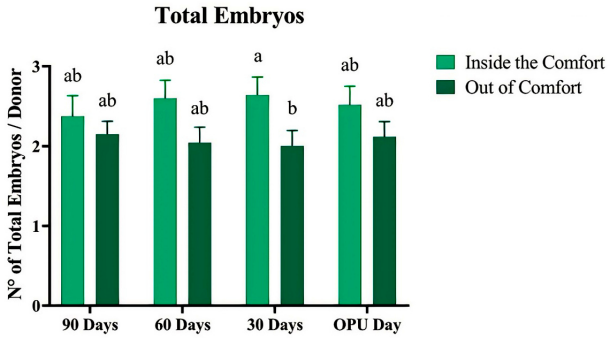

Figure 2. Number of total embryos produced by the groups in comfort and heat stress on the day of OPU and at 30, 60 and 90 days prior to the procedure.

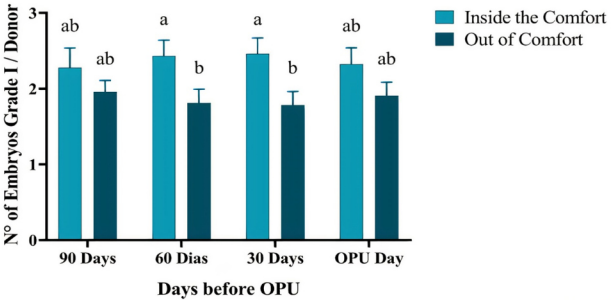

Figure 3. Number of Grade 1 embryos produced by the groups in comfort and heat stress on the day of OPU at 30, 60 and 90 days prior to the procedure.

Discussion

It is known that high temperatures negatively impact the oocyte quality of Bos taurus cows, which consequently hinders embryonic development *in vitro* [14,15]. During the period when temperatures decrease, it is possible to observe a greater quantity [14] and quality of oocytes, which in turn provides better embryonic development [15]. Furthermore, high-yielding Holstein cows are more prone to heat stress and when subjected to high temperatures they reduce the conception rate *in vivo* [16].

In the present study, when evaluating 326 high-yielding Holstein animals in excellent body condition, it was shown that temperature has an influence on oocyte viability as well as on *in vitro* embryo development and on the quality of the generated embryos. Thus, it was possible to identify the periods in which the dairy farmers, subjected to heat stress, suffered a negative impact on the *in vitro* production of embryos.

The group exposed to heat stress 90 days before follicular aspiration did not differ statistically from the comfort group for any of the evaluated classes. However, for the period of 60 days, oocyte viability was higher in the TC group. These data are in line with information generated by Britt [17], who indicated an estimated time of 60-80 days for the development of follicles from recruitment (primary phase) to maturation.

Therefore, the fact that there was no divergence between the HS-90 and TC-90 groups can be explained by the fact that exposure to high temperatures occurred in a time interval greater than that necessary for follicular growth, so that there was enough time for the emergence of healthy follicles. Despite this, there are reports of the residual effect during Autumn due to the stress suffered in Summer [18], which triggers a low rate of blastocyst production in up to 105 days post-stress [19].

When the follicle is exposed to adverse conditions in the initial period of growth, gene expression can be affected, causing developmental changes and generating dysfunctional mature follicles with poor quality oocytes and corpus luteum [17]. intense transcription and translation in oocytes [20]. Therefore, there are reports of downregulation during the summer in the expression of genes associated with oocyte maturation (BMP15; GDF9 and FGF 8, 10, 16 and 17), as well as those related to early embryonic development (GAPDH, GDF9 and POU5F1) [21].

Furthermore, the onset of follicular development is characterized by a high rate of granulosa cell mitosis, which remains high until the follicle reaches diameters between 0.68 – 1.52 mm and reaches the peak of mitotic activity [22]. Since then, follicular development is based on the exponential growth of the antrum [22].

In this context, high temperature reduces the viability of theca and granulosa cells, causing low production of androstenedione and estradiol [23, 18]. There is divergence of data in the literature on the alteration caused in gonadotropins, however most of them suggest that there is a reduction in plasma [24,19] and follicular inhibin levels [23], increase in FSH concentration [24] and blockade of the preovulatory wave of LH [25].

In contrast, an increase in LH concentrations has already been observed [26], and in relation to the pattern of secretion, a decrease in amplitude [27] and frequency [28] has also been reported for LH pulse. Perhaps these discrepancies are explained by preovulatory estradiol levels, since the amplitude of GnRH-induced preovulatory LH tonic and plasma pulses are decreased in cows with low plasma estradiol concentrations, but not in cows with high concentrations [27].

These endocrine changes can interrupt follicular development through immediate [29] or delayed effect, as a residual effect on steroidogenesis of up to 26 days in Holstein cows has already been found, even after brief exposure to HS for 5 days [30]. Furthermore, reduced androgen levels can result in early follicular atresia [30]. Ferreira et al. [11] reported a lower quantity of viable oocytes than those collected at the same time in the cold season, indicating lower competence of oocytes under heat stress.

The deleterious effects caused by high temperatures on the functioning of the reproductive system can last for months [31], however, for the follicle to develop from the appearance of the antrum to the preovulatory stage (0.13 mm to 8.56 mm), approximately 42 days are needed, that is, 2 estrous cycles [22].

Nevertheless, Al-katanani et al. [32], when studying cows under heat stress throughout summer, did not find any reduction in thermal effects on oocyte quality after the animals were cooled for a period of 42 days before colecting gametes, indicating that the damage to the oocyte occurs in a period longer than 42 days, that is, before the antral period. Thus, this information corroborates the dissimilarity found between the groups TC-60 and HS-60, showing that HS causes deleterious effects when there is exposure 60 days before OPU. These findings are in addition to those of Fialho et al., [33], who found a reduction in the viability of cumulus oocyte complexes with exposure for 60 days in animals of Pantaneira breed.

When the period of exposure coincides with the beginning of nuclear maturation, the generated embryos may fail in genomic activation and, consequently, in developing for the compact morula and expanded blastocyst stages [34]. The connection of gap junctions of cumulus cells is highly related to the chromatin state and, upon uncoupling these channels, degradation of the germinal vesicle is observed [35,36]. Thus, high temperatures during maturation have a negative impact on gap junctions, causing acceleration in oocyte chromatin condensation and damage to later development [37].

In this study, although the number of total embryos did not differ in cows in thermal comfort compared to those subjected to high temperatures 60 days before OPU, the number of grade I embryos was higher in the TC group, suggesting that the low oocyte competence caused by heat stress does not significantly influence the amount of embryos produced, but it does affect their quality.

In vivo studies show that under heat stress heifers

generate fewer embryos rated as excellent/good and more abnormal and development-retarded vesicles [38]. Abnormalities such as extruded or degenerating blastomeres from the cell mass, presenting irregular shape and a dark, granular appearance may be observed [39].

Furthermore, oocytes obtained in winter have dark and homogeneous cytoplasm, while those retrieved in summer may be dark and heterogeneous. These irregularities are caused by lipid modifications triggered by heat stress [40]. The authors observed that during summer, oocytes, granulosa cells and follicular fluid had a higher percentage of saturated fatty acids, while in winter, oocytes and granulosa cells had a higher percentage of polyunsaturated fatty acids.

Saturated fatty acids can increase cell membrane stability, while unsaturated ones cause a decrease in stability [41]. The saturated form still inhibits the survival and proliferation of granulosa cells in high-yielding cows[41]. Thus, temperature can influence the biochemical properties of the oocyte membrane and this, in turn, influence functionality and fertility, causing a negative impact on the ability of gametes to develop to the blastocyst stage during hot periods [40].

Perhaps, the dissimilarity observed between grade I embryos and not total embryos is due to the fact that oocytes submitted to HS *in vivo* remain able to be fertilized and undergo the initial cleavages *in vitro*, however, the quality is reduced. For heifers, the fertilization rate is similar in thermoneutral and heat-stressed groups [34], suggesting that, depending on the degree of stress, fertilization is not affected, or that no changes were observed because they are heifers, which are less susceptible to stress than lactating cows.

Matured oocytes [42,43], as well as those fertilized *in vitro* [44] under heat stress result in a lower cleavage rate and low capacity to develop to a blastocyst [42,44,43]. However, Ferreira et al. [11] when collecting oocytes from high-yielding Holstein cows from mid-summer, that is, from animals subjected to high temperatures for more than thirty days, found similar *in vitro* cleavage rates between the summer and winter groups, but the amount of blastocyst was reduced. Al-Katanani et al., [32] found results similar to Ferreira et al. [11], therefore, some component of the embryo formed from the oocyte was damaged by HS [32,29].

The group submitted to heat stress 30 days before follicular aspiration showed oocyte viability (grade I, II and III), as well as significantly lower total and grade I embryo production in the stressed groups. The data obtained in this study are in accordance with information found in literature, where the low competence of oocytes from Holstein cows during periods with high temperatures is known [32,29].

Although HS can lead the oocyte to apoptosis both in the maturation phase and in the early stages of development [45], the indication of the thermal influence on oocyte viability through the Anova test in a 30-day previous exposure suggests that this time interval is the most critical point and the one with the greatest susceptibility to heat stress, since the test has less sensitivity, that is, HS-30 and TC-30 differed significantly from each other and from the other groups, so that it was possible to detect the thermal impact within a period of thirty days.

By submitting Holstein heifers to HS for 10 hours before insemination, an increase in the incidence of abnormal or retarded embryos was observed, indicating the sensitivity of oocytes to HS in the periovulatory period [34]. Bovine oocytes subjected to heat shock during maturation undergo cellular changes that result in the delay and/or the interruption of embryonic development [45], as it coincides with the initial stages of nuclear and cytoplasmic maturation [46].

The exposure time of the HS-30 group is in accordance with the final growth period mentioned by Lussier et al. [20]. The decline in embryo productivity caused by exposure to hyperthermia in this period is possibly due to the inhibition of protein synthesis [47] and the resumption of meiosis to metaphase II [48], since the meiotic process is extremely sensitive to high temperatures [34]. *In vitro*, this disruption of nuclear maturation is seen in bovine oocytes under heat stress [49].

HS blocks the progression from meiosis I to meiosis II and increases the proportion of apoptotic oocytes, thus compromising fertilization rates [49]. Furthermore, in addition to the maternal genetic material, the oocyte provides the embryo with organelles, messenger RNA and other macromolecules essential for embryonic development [29]. Thus, the initial period of the embryo depends on the mRNA from the oocyte for protein synthesis [29] and any intervention in this transmission process causes irremediable damage to the embryo [14].

The group submitted to heat stress on the day of follicular aspiration did not differ from the control group in the production of viable oocytes, total embryos and grade I embryos. Similar study developed with Pantaneira cows by Fialho et al. [33], showed no change in the viability of cumulus oocyte complexes when stress occurred only on the OPU day. It is possible that the period of heat stress of just one day was not enough to trigger deleterious effects, or that there was no time necessary for the expression of negative effects, which was later manifested as it occurs in long-term stress during the summer. Roth et al. [50] demonstrated that under heat stress cows during the summer have a delayed effect during the fall with a decrease in oocyte quality and embryonic development.

Therefore, techniques to prevent heat stress in high-producing animals and reduce the negative impact

on *in vitro* embryo production must be thoroughly studied and analyzed for their feasibility for the introduction in preventive management. It is noteworthy that several weeks are required for the compromised follicles to ovulate or suffer atresia, thus fertility is not restored until damaged follicles are removed [29]. Genetic improvement, with the introduction of thermotolerant genes, is a possibility that should be studied further [31]. In addition, another option would be the use of antioxidant substances in animal feed [31] or their addition in the *in vitro* environment, since heat stress generates excessive production of reactive oxygen species (ROS's), compromising the function of the oocyte [51].

Conclusion

High-yielding Holstein cows are more susceptible to damage caused by high temperatures in reproductive performance. However, exposure to heat stress on the day of OPU and 90 days before did not impact oocyte viability, embryo quality and quantity. On the other hand, as the exposure period approaches follicular aspiration (60 and 30 days before), the damage becomes more evident. New studies must be developed in order to evaluate ways to reduce the thermal effects on embryo production in high-yielding Holstein cows. In this context, the use of animals bearing genotypes more resistant to the effects of heat, as well as the adoption of diets rich in antioxidants can be considered.

Declaration of interest conflict

Authors have no conflicts of interest to declare.

Author contributions

Conceptualization: F. Berling, F. C. de Castro and A. C. S. Oliveira; *Data curation:* F. C. de Castro; *Formal Analysis:* F. Berling and F. C. de Castro; *Investigation:* F. C. de Castro; *Methodology:* F. C. de Castro and A. C. S. Oliveira; *Supervision:* A. C. S. Oliveira; *Visualization:* F. Berling; *Writing (original draft):* F. Berling. *Writing (review & editing):* F. Berling and F. C. de Castro.

References

1. Rocha DT, Carvalho GR, Resende JC. Cadeia produtiva do leite no Brasil: produção primária [internet]. Embrapa; 2020 Aug [cited 2021 Sep 28]. Available from: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/215880/1/CT-123.pdf. Portuguese.

2. Ibge. Produção da Pecuária Municipal 2020 [internet]. Ibge; 2021 [cited 2022 May 21]; 48:1-12. Available https://biblioteca.ibge.gov.br/visualizacao/periodicos/84/ppm_2020_v48_br_informativo.pdf.

3. Embrapa. Anuário de Leite 2019: Sua excelência, o consumidor [internet]. Embrapa; 2019 May [cited 2021 Sep 28]. Available from: https://www.infoteca.cnptia.embrapa.br/handle/doc/1109959.

4. Mello RRC, Ferreira JE, Sousa SLG, Mello MRB, Palhano HB. Produção *in vitro* (PIV) de embriões em bovinos. Rev Bras Reprod Anim [internet]. 2016 [cited 2022 Feb 07]; 40(2):58-64. Available from: http://www.cbra.org.br/pages/publicacoes/rbra/v40/n2/p58-64%20(RB602).pdf.

5. Golçalves PBD, Figueiredo JR, Freitas VJF. Biotécnicas Aplicadas à Reprodução Animal. 2 ed. São Paulo: Roca; 2016. Portuguese.

6. Rumpf R. Avanços metodológicos na produção *in vitro* de embriões. R Bras Zootec. [internet]. 2007 [cited 2022 Feb 07]; 36. Available from: https://doi.org/10.1590/S1516-35982007001000021.

7. Alvim MJ, Paciullo DSC, Carvalho MM, Aroeira LJM, Carvalho LA, Novaes LP, Gomes AT, Miranda JEC, Ribeiro ACCL. Sistema de produção de leite com recria de novilhas em sistemas silvipastoris [internet]. Embrapa. 2005 Dec [Cited 2021 Sep 28]; (7). Available from: https://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Leite/LeiteRecriadeNovilhas/racas.htm#:~:text=Na%20pecu%C3%A1ria%20leiteira%2C%20considera%2Dse,%2C%20Guzer%C3%A1%2C%20Indubrasil%2C%20Sindi%20ou.

8. Lopes FFP, Lima RS, Risolia PHB, Ispada J, Assumpção MEOD, Visintin JA. Heat stress induced alteration in bovine oocytes: functional and cellular aspects. Anim Reprod. [internet]. 2012 [cited 2022 Feb 07]; 9(3):395-403. Available from: https://www.animal-reproduction.org/article/5b5a605af7783717068b46f6.

9. Pizzatto J, Vieira FMC, Macagnan R, Mayer LRR, Oliveira NS, Pilatti JA. Comparação da termorregulação de vacas holandesas em sistema compost barn. Bbiomet [internet]. 2017. Available from: https://cbbiomet.figshare.com/articles/journal_contribution/Compara_o_da_termorregula_o_de_vacas_holandesas_em_sistema_compost_barn/5193268.

10.Macedo GG, Silva EVC, Pinho RO, Assumpção TI, Jacomini JO, Santos RM, Martins LF. O estresse por calor diminui a fertilidade de fêmeas bovinas por afetar o desenvolvimento oocitário e o embrionário. Rev Bras Reprod Anim. [internet]. 2014 [cited 2022 Feb 07]; 38(2):80-85. Available from: http://www.cbra.org.br/pages/publicacoes/rbra/v38n2/pag80-85%20(RB486).pdf

11.Ferreira RM, Ayres H, Chiaratti MR, Ferraz ML, Araújo AB, Rodrigues CA, Watanabe YF, Vireque AA, Joaquim DC, Smith LC, Meirelles FV, Baruselli PS. 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. [internet]. 2011 [cited 2022 Feb 07]; 94(5):2383-2392. Available from: https://doi.org/10.3168/jds.2010-3904.

12.Klein BG. Cunningham Tratado de Fisiologia Veterinária. 5ed. Rio de Janeiro: Elsevier; 2014.

13.Lima MTV, Feitosa JV, Oliveira CW, Costa ANL. Influência da temperatura e umidade sobre o conforto térmico bovino em Barbalha, Ceará. Pubvet [internet]. 2019 [cited 2022 May 03]; 13(12): 477, 1-8. Available from: https://doi.org/10.31533/pubvet.v13n12a477.

14.Gendelman M, Aroyo A, Yavin S, Roth Z. Seasonal effects on expression, cleavage timing, and developmental competence of bovine preimplantation embryos. Reproduction [internet]. 2010 [cited 2022 Feb 07];140(1):73-82. Available from: https://doi.org/10.1530/REP-10-0055.

15.Rocha A, Randel RD, Broussard JR, Lim JM, Blair RM, Roussel JD, Godke RA, Hansel W. High environmental temperature and humidity decrease oocyte quality in Bos taurus but not in Bos indicus cows. Theriogenology [internet]. 1998

[cited 2022 Feb 07]; 49(3):657-65. Available from: https://doi.org/10.1016/S0093-691X(98)00016-8.

16.Pires MFA, Ferreira AM, Saturnino HM, Teodoro RL. Taxa de gestação em fêmeas da raça Holandesa confinadas em free stall, no verão e no inverno. Arq Bras Med Vet Zootec. [internet]. 2002 [cited 2022 Feb 07]; 54(1). Available from: https://doi.org/10.1590/S0102-09352002000100009.

17.Britt JH. Impacts of Early Postpartum on Follicular Development and Fertility. In: Proceedings of the Twenty-Fourth Annual Conference American Association of Bovine Practitioners [internet]. 1991 Sep [cited 2022 Feb 07]; 18-21. Available from: https://journals.tdl.org/bovine/index.php/AABP/article/view/6706.

18.Wolfenson D, Roth Z, Meidan R. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Anim Reprod Sci [internet]. 2000 [cited 2022 Feb 07]; 60-61:535-47. Available from: https://doi.org/10.1016/S0378-4320(00)00102-0.

19.Torres-Júnior JRS, Pires MFA, SÁ WF, Ferreira AM, Viana JHM, Camargo LSA, Ramos AA, Folhadella IM, Polisseni J, Freitas C, Clemente CAA, SÁ Filho MF, Paula-Lopes FF, Baruselli PS. Effect of maternal heat stress on follicular growth and oocyte competence in Bos indicus cattle. Theriogenology [internet]. 2008 [cited 2022 Feb 07]; 69(2):155-66. Available from: https://doi.org/10.1016/j.theriogenology.2007.06.023.

20.Roth Z. Effect of Heat Stress on Reproduction in Dairy Cows: Insights into the Cellular and Molecular Responses of the Oocyte. Annu Rev Anim Biosci [internet]. 2017 [cited 2022 Feb 07]; 5:151-170. Available from: https://doi.org/10.1146/annurev-animal-022516-022849.

21.Ferreira RM, Chiaratti MR, Macabelli CH, Rodrigues CA, Ferraza ML. The infertility of repeat-breeder cows during summer is associated with decreased mitochondrial DNA and increased expression of mitochondrial and apoptotic genes in oocytes. Biol Reprod [internet]. 2016 [cited 2022 May 03]; 94(3):66, 1-10. Available from: https://doi.org/10.1095/biolreprod.115.133017.

22.Lussier JG, Matton P, Dufour JJ. Growth rates of follicles in the ovary of the cow. J Reprod Fert [internet]. 1987 [cited 2022 Feb 07]; 81(2):301-7. Available from: https://doi.org/10.1530/jrf.0.0810301.

23.Lew BJ, Meidan R, Wolfenson D. Concentrações hormonais e desenvolvimento folicular de vacas leiteiras em hipertermia sazonal e aguda. Arq Bras Med Vet Zootec [internet]. 2006 [cited 2022 Feb 07]; 58(5):816-822. Available from: https://doi.org/10.1590/S0102-09352006000500017.

24.Roth Z, Meidan R, Braw-Tal R, Wolfenson D. Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows. J Reprod Fertil [internet]. 2000 [cited 2022 Feb 07]; 120(1):83-90. Available from: https://pubmed.ncbi.nlm.nih.gov/11006149/#:~:text=The%20enhanced%20growth%20of%20follicles,cycle%3B%20P%20%3C%200.05.

25.Armengol-Gelonch R, Mallo JM, Ponté D, Jimenez A, Valenza A, Souza AH. Impact of phase of the estrous cycle and season on LH surge profile and fertility in dairy cows treated with different GnRH analogs (gonadorelin vs. buserelin). Theriogenology [internet]. 2017 [cited 2022 Feb 07]; 91:121-126. Available from: https://doi.org/10.1016/j.theriogenology.2017.01.001.

26.Roman-Ponce H, Thatcher WW, Wilcox CJ. Hormonal interrelationships and physiological responses of lactating dairy cows to a shade management system in a subtropical environment. Theriogenology [internet]. 1981 [cited 2022 May 03]; 16(2): 139-154. Available from: https://doi.org/10.1016/0093-691X(81)90097-2.

27.Gilad E, Meidan R, Berman A, Graber Y, Wolfenson D. Effect of heat stress on tonic and GnRH-induced gonadotrophin secretion in relation to concentration of estradiol in plasma of cyclic cows. Journal of Reproduction and Fertility [internet]. 1993. [cited 2022 May 03]; 99(2):315-321. Available from: https://doi.org/10.1530/jrf.0.0990315.

28.Wise ME, Armstrong DV, Huber JT, Hunter, R, Wiersma F. Hormonal Alterations in the Lactating Dairy Cow in Response to Thermal Stress. J Dairy Sci [internet]. 1988. [cited 2022 May 03]; 71(9): 2480-2485. Available from: https://pubmed.ncbi.nlm.nih.gov/16725628/.

29.Hansen PJ. Antecedents of mammalian fertility: Lessons from the heat-stressed cow regarding the importance of oocyte competence for fertilization and embryonic development. Animal Frontiers [internet]. 2013 [cited 2022 Feb 07]; 3(4): 34–39. Available from: https://doi.org/10.2527/af.2013-0031.

30.Roth Z, Meidan R, Shaham-Albalancy U, Braw-Tal, R, Wolfenson D. Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles. Reproduction [internet]. 2001 [cited 2022 Feb 07]; 121(5):745-51. Available from: https://pubmed.ncbi.nlm.nih.gov/11427162/.

31.Hansen PJ. Reproductive physiology of the heat-stressed dairy cow: implications for fertility and assisted reproduction. Anim Reprod [internet]. 2019 [cited 2022 Feb 07]; 16(3):497-507. Available from: http://dx.doi.org/10.21451/1984-3143-AR2019-0053.

32.Al-katanani YM, Paula-Lopes FF, Hansen PJ. Effect of season and exposure to heat stress on oocyte competence in Holstein cows. J Dairy Sci [internet]. 2002 [cited 2022 Feb 07]; 85(2):390-6. Available from: https://doi.org/10.3168/jds.S0022-0302(02)74086-1.

33.Fialho ALL, Souza-cáceres MB, Silva WAL, Arruda EDS, Kischel H, Ribeiro-Ferreira MGC, Medeiros CF, Silva JR, Oliveira MVM, Ferraz ALJ, Melo-Sterz, F. A. Efeito do estresse térmico calórico agudo e crônico sobre a qualidade oocitária de bovinos de raças adaptadas. Arq Bras Med Vet Zootec [internet]. 2018 [cited 2022 Feb 07]; 70(1):64-72. Available from: https://doi.org/10.1590/1678-4162-9494.

34.Putney DJ, Mullins S, Thatcher WW, Drost M, Gross TS. Embryonic Development in Superovulated Dairy Cattle Exposed to Elevated Ambient Temperatures Between the Onset of Estrus and Insemination. Anim Reprod Sci [internet]. 1989 [cited 2022 Feb 07]; 19(1–2):37-51. Available from: https://doi.org/10.1016/0378-4320(89)90045-6.

35.Luciano AM, Franciosi F, Modina SC, Lodde V. Gap Junction-Mediated Communications Regulate Chromatin Remodeling During Bovine Oocyte Growth and Differentiation Through cAMP-Dependent Mechanism(s). Biology of Reproduction [internet]. 2011 [cited 2022 May 03]; 85(6): 1252-1259. Available from: https://doi.org/10.1095/biolreprod.111.092858.

36.Lodde V, Modina S, Galbusera C, Franciosi F, Luciano AM. Large-scale chromatin remodeling in germinal vesicle bovine oocytes: interplay with gap junction functionality and developmental competence. Mol Reprod Dev [internet]. 2007 [cited 2022 May 03]; 74(6):740-9. Available from: https://pubmed.ncbi.nlm.nih.gov/17075796/.

37.Campen KA, Abbott CR, Rispoli LA, Payton RR, Saxton AM, Edwards JL. Heat stress impairs gap junction communica-

tion and cumulus function of bovine oocytes. J Reprod Dev [internet]. 2018 [cited 2022 May 03]; 64(5):385-392. Available from: https://pubmed.ncbi.nlm.nih.gov/29937465/.

38.Putney DJ, Drost M, Thatcher WW. Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between Days 1 to 7 post insemination. Theriogenology [internet]. 1988 [cited 2022 Feb 07]; 30(2):195-209. Available from: https://doi.org/10.1016/0093-691X(88)90169-0.

39.Putney DJ, Drost M, Thatcher WW. Influence of summer heat stress on pregnancy rates of lactating dairy cattle following embryo transfer or artificial insemination. Theriogenology [internet]. 1989 [cited 2022 Feb 07]; 31(4):765-78. Available from: https://doi.org/10.1016/0093-691X(89)90022-8.

40.Zeron Y, Ocheretny A, Kedar O, Borochov A, Sklan D, Arav A. Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. Reproduction [internet]. 2001 [cited 2022 Feb 07]; 121(3):447-54. Available from: https://pubmed.ncbi.nlm.nih.gov/11226071/.

41.Vanholder T, Leroy JLMR, Van Soom A, Opsomer G, Maes D, Coryn M, Kruif A. Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation *in vitro*. Anim Reprod Sci [internet]. 2005 [cited 2022 Feb 07]; 87(1-2):33-44. Available from: https://doi.org/10.1016/j.anireprosci.2004.09.006.

42.Ispada J, Rodrigues TA, Risolia PHB, Lima RS, Gonçalves DR, Rettori D, Nichi M, Feitosa WB, Paula-Lopes FF. Astaxanthin counteracts the effects of heat shock on the maturation of bovine oocyte. Reprod Fertil Dev [internet]. 2018 [cited 2022 Feb 07]; 30(9):1169-1179. Available from: https://doi.org/10.1071/RD17271.

43.Rodrigues TA, Tuna KM, Alli AA, Tribulo P, Hansen PJ, Koh J, Paula-Lopes FF. Follicular fluid exosomes act on the bovine oocyte to improve oocyte competence to support development and survival to heat shock. Reprod Fertil Dev [internet]. 2019 [cited 2022 Feb 07]; 31(5):888-897. Available from: https://doi.org/10.1071/RD18450.

44.Rivera RM, Hansen PJ. Development of cultured bovine embryos after exposure to high temperatures in the physiological range. Reproduction [internet]. 2001 [cited 2022 Feb 07]; 121(1):107-15. Available from: https://pubmed.ncbi.nlm.nih.gov/11226033/.

45.Roth Z, Hansen PJ. Involvement of apoptosis in disruption of developmental competence of bovine oocytes by heat shock during maturation. Biol Reprod [internet]. 2004 [cited 2022 Feb 07]; 71(6):1898-906. Available from: https://doi.org/10.1095/biolreprod.104.031690.

46.Hyttel P, Callesen H, Greve T. Ultrastructural features of preovulatory oocyte maturation in superovulated cattle. J Reprod Fert [internet]. 1986 [cited 2022 Feb 07]; 76(2):645-56. Available from: https://doi.org/10.1530/jrf.0.0760645.

47.Edwards JL, Hansen P J. Differential responses of bovine oocytes and preimplantation embryos to heat shock.Mol Reprod Dev [internet]. 1997 [cited 2022 Feb 07]; 46(2):138-45. Available from: https://doi.org/10.1002/(SICI)1098-2795(199702)46:2<138::AID-MRD4>3.0.CO;2-R.

48.Baumgartner AP, Chrisman CL. Cytogenetic analysis of ovulated mouse oocytes following hyperthermic stress during meiotic maturation. Exp Cell Res [internet]. 1981 [cited 2022 Feb 07]; 32(2):359-366. Available from: https://doi.org/10.1016/0014-4827(81)90111-7.

49.Roth Z, Hansen PJ. Disruption of nuclear maturation and rearrangement of cytoskeletal elements in bovine oocytes exposed to heat shock during maturation. Reproduction [internet]. 2005 [cited 2022 Feb 07]; 129(2):235-44. Available from: https://doi.org/10.1530/rep.1.00394.

50.Roth Z, Arav A, Bor A, Zeron Y, Braw-Tal R, Wolfenson D. Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. Reproduction [internet]. 2001 [cited 2022 Feb 07]; 122(5):737-44. Available from: https://pubmed.ncbi.nlm.nih.gov/11690534/#:~:text=The%20results%20show%20a%20delayed,quality%20oocytes%20in%20the%20autumn.

51.Cavallari, F. C.; Leal, C. L. V.; Zvi, R.; Hansen, P. J. Effects of melatonin on production of reactive oxygen species and developmental competence of bovine oocytes exposed to heat shock and oxidative stress during in vitro maturation. Zygote [internet]. 2019 [cited 2022 May 03]; 27(4):262. Available from: https://pubmed.ncbi.nlm.nih.gov/31171044/.