InterSciencePlace
International Scientific Journal – ISSN: 1679-9844
Nº 1, volume 18, article nº 13, January/March 2023
D.O.I: http://dx.doi.org/10.6020/1679-9844/v18n1a13
Accepted: 02/12/2022 Published: 26/03/2023

REPRODUCTIVE EFFICIENCE AND IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THERMOTOLERANT HOLSTEIN COWS

EFICIÊNCIA REPRODUTIVA E IDENTIFICAÇÃO DE POLIMORFISMOS DE BASE ÚNICA EM VACAS HOLSTEIN TERMOTOLERANTES

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Abstract

The aim of this study was to identify novel SNPs in thermotolerant Holstein cows and to evaluate the reproduction of these animals. Vaginal temperatures of 110 Holstein animals were measured with an automatic thermometer during the summer. Animals were classified as thermotolerant (TT), animals in which no temperature event (at least 30 min) occurred above 39 °C; intermediate (INT), animals in which one or more half-hour temperature events occurred between 39.1 and 39.5 °C; and thermosensitive/heat stressed (HS), animals in which the temperature exceeded 39.5 °C in at least one half-hour event. Tail hair was collected for DNA extraction, PCR, and DNA sequencing. Primers from ATP1A1 gene and HSP90AB1 gene were used to detect polymorphism. Temperature [mean, maximum, and coefficient of variance (CV)], milk production data, and number of artificial inseminations required for pregnancy were analyzed using the GLM procedure and PDIFF of SAS. Seventy-one cows were placed in the HS group, 14 in the INT group, and 25 in the TT group. Mean, maximum and CV temperatures were higher in animals in the HS group ($P < .01$). Milk production was not different within the groups. The number of inseminations required for the cow to become pregnant was higher ($P = 0.05$) in the HS group. There was a moderate and positive correlation (0.33; $P < 0.004$) between temperature CV and number of inseminations. One SNP was identified in the gene ATP1A1; this mutation represents a change from aspartate to asparagine. Four SNPs were identified in the HSP90AB1 gene. In summary, animals that can maintain a lower body temperature under heat stress have better fertility because they require less artificial insemination to become pregnant, regardless of milk production. Novel SNPS have been found in both ATP1A1 and HSP90AB1 genes, but further studies are needed to link them to thermoregulation.

Key words: cow, heat stress, reproduction, single nucleotide polymorphisms.

INTRODUCTION

Heat stress is a major problem affecting the cattle industry worldwide (SILVA et al., 2013). In tropical regions where average temperatures are high throughout the year, this is a problem that massively affects animals (ROCHA et al., 2012). Reproductive disorders due to heat stress can occur through two general mechanisms: 1) homeokinetic changes that occur to regulate body temperature, such as. redistribution of blood flow to the body periphery to increase sensory heat loss, and decreased feed intake during heat stress, and 2) failure of the homeokinetic system to regulate reproduction; elevated body temperature can impair the function of germ cells, the embryo in early development, and other reproductive cells (LUCY, 2001; WEST, 2002; HANSEN, 2007; HANSEN, 2009; SENOSY et al, 2011).

The effects of heat stress on reproductive functions are exacerbated by the increase in metabolic heat production associated with lactation (SARTORI et al.,

2002) and increased humidity (WEST, 2003). Reproductive ability is thought to be negatively correlated with milk yield due to reductions in body thermoregulation and genetic selection of animals for higher production (ROTH, 2008). However, studies with Holstein cows (Bos taurus) have shown that some animals of this breed can suffer from heat stress without seriously affecting their fertility (VASCONCELOS et al., 2011). The ability to maintain a low body temperature during heat stress is moderately heritable (SEATH 1947; DIKMEN et al., 2012); therefore, selection for thermoregulation should reduce the consequences of heat stress (DIKMEN et al., 2015).

Heat tolerance has been linked to single nucleotide polymorphisms (SNPs) in genes involved in the response to heat stress or responsible for homeostasis (RAVAGNOLO & MISZTAL, 2002). Studies have demonstrated the relationship between genetic polymorphisms and heat stress response in dairy cows (CHRENCK et al. 2003; LIU et al., 2011), as well as their relationship with reproduction (ORTEGA et al., 2016) and production (COCHRAN et al., 2013). The stress response-related genes HSP90AB1 and ATP1A1 have been linked to thermotolerance in Holstein heifers and cows (LIU et al., 2011; CHAROENSOOK et al., 2012).

The ability to maintain body temperature under thermal stress is a valuable resource for dairy cattle in tropical and subtropical countries (GANAIE et al., 2013). Although differences in thermotolerance between breeds and between crosses have been studied (BEATTY et al., 2006), few studies have attempted to understand the mechanism of thermotolerance and the heritability of this trait. In addition, there are no studies that link thermotolerance, SNPs, and reproductive ability in these animals.

therefore, the aim of the present study was to identify novel SNPs in ATP1A1 and HSP90AB1 in Holstein cows considered thermotolerant and sensitive to heat stress and to evaluate the reproduction of these animals by artificial insemination.

MATERIAL AND METHODS

Classification of animals with respect to thermoregulation

The experiment was conducted during the months of January to March (summer in Brazil) with 110 lactating Holstein cows with a body condition score between 3 and 3.5 (WILDMAN et al., 1982). The animals came from two different farms in southeastern Brazil (20º 20' 23'' S 41º 08' 05'' W and 19º 56' 08'' S 40º 36' 01'' W). All animals were kept in a free-range barn under the same conditions, in stalls with fans and sprinklers. An automatic thermometer (iButton Maxim, California, USA) connected to an intravaginal placebo implant was used to measure the body temperature of each cow. The thermometers were left in each cow for 3 days, with temperature measured every 5 minutes. Between each insertion, the implants were washed with a chlorhexidine solution and autoclaved.

Animals were classified as follows: thermotolerant group (TT), i.e., animals in which no temperature event (at least 30 min) above 39 °C occurred; intermediate group (INT), i.e., animals in which one or more half-hour temperature events between 39.1 and 39.5 °C occurred; and thermosensitive/heat-stressed group (HS), i.e., animals in which temperature exceeded 39.5 °C in at least one half-hour event.

In addition, the coefficient of variation (CV) of temperatures within all measurements of each animal was calculated as the ratio of the standard deviation to the mean. The highest temperature of each animal within the three days was also recorded.

The average milk production on the day of implant insertion was recorded for each animal.

A thermometer was kept in the shade of the cow pen throughout the experiment to check the ambient temperature.

Animals participating in the experiment were inseminated 12 hours after the appearance of estrus signs without hormonal synchronization (AI). Semen from 29 bulls of Holstein and Gir breeds was used. After 30 days, pregnancy diagnosis was performed by ultrasonography. All animals diagnosed as non-pregnant or showing oestrus before ultrasonography were inseminated again until pregnancy was detected. For each animal, the number of inseminations needed since the last calving to diagnose pregnancy was calculated.

DNA extraction, PCR and sequencing

Tail hair samples were collected from all cows participating in the experiment. Genomic DNA extraction was performed using the DNeasy® Blood & Tissue Kit (Qiagen, Venlo, The Netherlands). First, hair samples were cut so that only the hair bulbs were used (0.1 mm). They were placed in 1.5-ml microtubes and 180 µl of ATL buffer and 20 µl of proteinase K were added, followed by vortexing for 15 seconds and centrifugation at 8000 rpm for 1 minute. These samples were placed in an incubator at 56 °C. After 2 hours, manual maceration was performed using a coneended polypropylene punch, followed by vortex and centrifugation, and again left in the incubator overnight. The next day, hair samples were macerated again and 5 µl of proteinase K was added to each sample. They were then vortexed for 15 seconds followed by centrifugation at 8000 rpm for 1 minute and placed back in the incubator for 2 hours at 56 °C. The following steps to perform the extraction were performed according to the manufacturer's instructions. The quality and quantity of the extracted DNA was checked on a 1% agarose gel, stained with SYBR® Safe (Invitrogen, São Paulo, Brazil), and visualized under ultraviolet light.

Primers used for polymorphism detection are listed in Table 1. Polymerase chain reactions (PCR) were performed using a total volume of 25 µl (genomic DNA, dNTP mix, Taq polymerase enzyme (LongRange PCR enzyme mix - Qiagen), MgCl2 and specific primers for each gene). Amplification was performed in an Apollo ATC 201 (Nyx Technique) thermal cycler consisting of an initial denaturation at 93 °C for 3 minutes, followed by 35 cycles of denaturation at 93 °C for 15 seconds, annealing at 55 °C for 30 seconds (HSP90AB1) or 50 °C for 30 seconds (ATP1A1), and extension at 68 °C for 3 minutes. PCR products were electrophoresed on a 1% agarose gel with SYBR® safe (Invitrogen) for visualization of fragment sizes. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). The purified products were visualized and quantified on a 1% agarose gel and finally sent to the Multiuser Genotyping and Sequencing Laboratory - LMGs of the University of Campinas for sequencing.

The sequences obtained were analyzed and edited using the BioEdit software program (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html). Alignment of the sequences was performed using the Clustal Omega virtual tool (http://www.ebi.ac.uk/Tools/msa/clustalo/). The sequences obtained from the experimental animals were aligned with those available in the NCBI database for the species Bos taurus (GenBank accession number NM _001079637.1 and NM _001076798.1) considering the genes HSP90AB1 and ATP1A1, respectively.

Statistical analysis

The effects of group (HS, IN, or TT) on animal mean and maximum temperature, milk production, and number of IA were analyzed by least-squares analysis of variance using SAS General Linear Models (SAS for Windows, version 9.2, Cary, NC). Differences between individual means were analyzed by pairwise comparisons

(Probability of Difference Analysis [PDIFF]; SAS). To exclude the influence of sire on pregnancy rate, logistic regression analysis was performed using the logistic procedure of SAS. Because there was no effect of sire or breed on pregnancy, this effect was excluded from the analysis.

The use of temperature CV as a parameter to analyze animal thermoregulation has been reported in the literature only in two articles (KOGA et al., 2004; GOURDINE et al., 2016), both of which use correlation analyzes to evaluate the effects on specific traits. Here, we analyzed the Pearson correlation coefficient between temperature CV and number of inseminations using the correlation procedure of SAS.

All values are expressed as least squares means ± SEM. Differences were considered significant when $P < 0.05$, and values of $P \geq 0.05$ and ≤ 0.1 were considered indicative of a trend.

RESULTS

Ambient temperatures

The minimum and maximum temperatures in the pens where the animals were kept throughout the study period were 19.7°C and 36.1°C, respectively, with a mean temperature of 28.2°C.

Reproductive and production traits

Of the 110 animals used in this study, 71 were classified in the HS group, 14 in the INT group, and 25 in the TT group.

The mean temperature measured during the three days that the thermometers were kept in the animals and the maximum temperature recorded in each group were higher ($P <.0001$) in the animals of the HS group than in the INT and TT groups. The INT group did not differ from the TT group (Figure 1 and 2).

The temperature CV measured in each animal was higher ($P=0.01$) in the HS group than in the TT group. There was a tendency ($P=0.064$) for the INT group to be higher than the TT group (Figure 3).

The mean value of milk production was not different within the groups (Figure 4).

The number of inseminations a cow needed to become pregnant was higher ($P=0.05$) for animals in the HS group than in the INT and TT groups. The INT group did not differ from the TT group (Figure 5). There was a moderate and positive correlation (0.33; $P < 0.004$) between temperature CV and number of inseminations.

Polymorphisms

ATP1A1

The sequences of sixty animals, 36 of which were classified as HS, 10 as INT, and 14 as TT, were used to compare exon 18-19 of the ATP1A1 gene with Bos taurus sequences available in the NCBI database.

A new polymorphism was identified in exon 19 of the ATP1A1 gene when the animals in the experiment were compared with the Bos taurus sequence. The polymorphism was a G-A mutation at nucleotide position 116,400,962 of the gene's mRNA. This polymorphism was identified in five animals; four were classified as HS and one as INT. This mutation represents a change from the amino acid aspartate to asparagine.

HSP90AB1

Sequences from 63 animals were used to compare exons 1 - 3 and 6 - 8 of the HSP90AB1 gene with Bos taurus sequences available in the NCBI database.

In nucleotide position 646, intron 2, an A-G mutation was identified in 1 HS and 1 INT cow. In intron 6, at nucleotide position 1918, nine individuals (5 HS and 4 TT) had a G-A mutation. Two additional mutations, both T-C, were observed in nucleotides 2017 and 2968, the first occurring in 2 HS animals and the second (nucleotide 2968) in one TT animal.

DISCUSSION

A dairy cow that maintains its body temperature below 39.1 °C is considered thermotolerant (WEST, 2003); when the ambient temperature exceeds 25-28.4 °C, the animal’s body temperature begins to rise (BERMAN et al., 1985; DIKMEN & HANSEN, 2009). The cows in this study were kept in a barn with fans and sprinklers to keep the animals’ body temperature in a comfortable range; however, during summer, the ambient temperature in the barn varied from 19.7 °C to 36.1 °C.

In this study, vaginal temperature was used to classify cows as TT, INT, or HS. Cows that had at least one event (at least 30 minutes) with temperatures above 39.5 °C were classified as HS. These cows also had a higher average temperature and a higher maximum temperature during the three days the thermometer remained in the animal.

Body temperature is controlled by modulation of metabolic heat produced by the body and heat release (SAILO et al., 2015). Small fluctuations in body temperature, expressed in this study as CV, even during periods of high milk production, result in low energy requirements for body temperature regulation (GOURDINE et al., 2016). The CV results presented here suggest that animals classified as TT require less energy for thermoregulation than animals classified as HS. KOGA et al. (2004) showed that Brahman breed cattle had a lower coefficient of variance and lower skin and rectal temperatures than buffalo. In sows, those that exhibited low variance in thermoregulatory responses showed high lactation performance (GOURDINE et al., 2016).

In the present study, cows classified as HS were found to require more AI to become pregnant than INT and TT cows. Similarly, cows with a higher coefficient of variation with respect to temperature showed a positive correlation with the number of artificial inseminations needed to become pregnant. It is well known that cows suffering from heat stress prior to artificial insemination have low pregnancy rates (PUTNEY et al., 1988). This is particularly notable in lactating dairy cows where lactating metabolic needs exacerbate fertility loss (AL-KATANANI et al., 1999; SARTORI et al., 2002; ROTH, 2008)).

Identifying thermotolerant animals that exhibit improved fertility in summer could be an alternative for dairy cows. In these animals, heat stress may affect oocyte quality (HANSEN & ARECHIGA, 1999), alter follicular function, affect corpus luteum development, and thus lower progesterone concentrations (WOLFENSON et al., 1995; RENSIS & SCARAMUZZI, 2003). A likely effect of low progesterone concentrations is premature oocyte maturation, which affects embryonic development after fertilization (DISKIN et al., 2012).

The ability of the animal to maintain its body temperature may affect both pregnancy rate and pregnancy maintenance (VASCONCELOS et al., 2006). In a recent study, VASCONCELOS et al. (2011) showed that high producing Holstein cows (> 35 kg/day) that could maintain a rectal temperature of less than 39 °C had equally good pregnancy rates after embryo transfer as animals with low milk production (< 35 kg/day). The cows used in the present study were high-producing animals with an average milk production of 31 kg/day. However, milk production did not differ between the TT, INT and HS groups and did not show any correlation with the variables studied, so we can assume that thermoregulation is not due to low milk production in the TT group.

ATP1A1, a Na+,K+-ATPase, is responsible to maintaing Na/K electrolyte balance, while HSP90AB1 is a heat shock protein. For both genes, novel SNPs were found in coding and noncoding regions. When found in the coding regions, these SNPs can alter DNA transcription, resulting in protein modification (KIM & MISRA, 2007) and consequently altering gene function (COLLINS et al., 1998). The SNPs identified in the coding regions in the present study all resulted in amino acid exchanges.

In Jersey crossbred cows, four SNPs were found in the HSP90AB1 gene (targeting intron 7 to exon 11) and one of the polymorphisms found was associated with thermotolerance (SAILO et al. 2015). In Holstein cows, a novel C-A polymorphism was found at nucleotide position 2789 of the ATP1A1 gene, but this SNP did not alter any amino acid (LIU et al. 2011). In another study, Holstein heifers with a polymorphism in intron 3 of the HSP90AB1 gene showed an improvement in thermotolerance (CHAROENSOOK et al., 2012). When located in the noncoding regions, these SNPs do not affect the encoded proteins, but they may alter gene splicing and thus gene expression.

Other genes have been identified in Holstein cows as genetic markers associated with genetic variation in thermoregulation during heat stress. DIKMEN et al. (2015) found six genes associated with rectal temperature, respiration rate, and sweating rate. Sahiwal cows with a GG genotype for HSP90AA1 had higher heat tolerance coefficients, suggesting that these animals are more sensitive to heat stress (KUMAR et al., 2015). Chinese Holstein cows carrying a mutant allele C on HSPAA1 had higher mRNA expression of this gene in the hot season. The authors associated this genetic variation in Hsp90AA1 with thermoresistance. (BADRI et al., 2018).

In the present study, novel SNPs were identified in both the ATP1A1 and HSP90AB1 genes. However, because cows from all three groups (HS, INT, and TT) had these polymorphisms, an association with thermotolerance was not possible. Further studies are needed to evaluate the effects of these SNPs on thermoregulation.

In conclusion, animals that can maintain a lower body temperature under heat stress conditions have better fertility because they require less artificial insemination to become pregnant, regardless of milk production. Novel SNPS have been found in both ATP1A1 and HSP90AB1 genes, but further studies are needed to associate them to thermoregulation.

REFERENCES

AL-KATANANI, Y.M.; RIVERA, R.M.; HANSEN, P.J. Seasonal variation in development of *in vitro* produced bovine embryos. The Veterinary Record. V. 150, p. 486–487, 2002.

AL-KATANANI, Y.M.; WEBB, D.W.; HANSEN, P.J. Factors affecting seasonal variation in 90-day nonreturn rate to first service in lactating Holstein cows in a hot climate. J. Dairy Sci. V. 82, p. 2611–2616, 1999.

BADINGA, L.; THATCHER, W.W.; DIAZ, T.; DROST, M.; WOLFENSON, D. Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. Theriogenology. V. 39(4), p. 797– 810, 1993.

BADRI TM, CHEN KL, ALSIDDIG MA, LI L, CAI Y, WANG GL. Genetic polymorphism in Hsp90AA1 gene is associated with the thermotolerance in Chinese Holstein cows. Cell Stress Chaperones. 2018 Jul;23(4):639-651. doi: 10.1007/s12192-017-0873-y.

BASIRICÒ, L.; MORERA, P.; PRIMI, V.; LACETERA, N.; NARDONE, A.; BERNABUCCI, U. Cellular thermotolerance is associated with heat shock protein 70.1 genetic polymorphisms in Holstein lactating cows. Cell Stress and Chaperones. V.16, p.441–448, 2011.

BEATTY, D.T; BARNES, A.; TAYLOR, E.; PETHICK, D.; MCCARTHY, M.; MALONEY, S.K. Physiological Responses of Bos Taurus and Bos Indicus Cattle to Prolonged, Continuous Heat and Humidity. J. Anim. Sci. V.84, p. 972-985, 2006.

BERMAN, A.; FOLMAN, Y.; KAIM, M.; MAMEN, M.; HERZ, Z.; WOLFENSON, D.; GRABER, Y. Upper Critical Temperatures and Forced Ventilation Effects for High-Yielding Dairy Cows in a Subtropical Climate. J. Dairy Sci. V.68(6), p.1488–1495, 1985.

BÓ, G.A.; BARUSELLI, P.S.; MARTINEZ, M.F. Pattern and Manipulation of Follicular Development in Bos Indicus Cattle. Animal Reproduction Science. V. 78, p. 307-326, 2003.

BOHMANOVA, J.; MISZTAL, I.; TSURUTA, S. National genetic evaluation of milk yield for heat tolerance of United States Holsteins. Interbull Bull. V. 33, p. 160–162, 2005.

CAETANO, A.R. Marcadores SNP: Conceitos Básicos, Aplicações no Manejo e no Melhoramento Animal e Perspectivas Para o Futuro. Revista Brasileira de Zootecnia. V. 38, p.64-71, 2009.

CAI, Y.; LIU, Q.; XING, G.; ZHOU, L. Polymorphism of the promoter region of Hsp70 gene and its relationship with the expression of HSP70mRNA, HSF1mRNA, Bcl-2mRNA and Bax-αmRNA in lymphocytes in peripheral blood of heat shocked dairy cows. Asian-Aust. J. Anim. V. 18(5), p. 734–740, 2005.

CARROLL, J.A.; BURDICK, N.C.; CHASE JR, C.C.; COLEMAN, S.W.; SPEIRS, D.E. Influence of environmental temperature on the physiological, endocrine, and immune responses in livestock exposed to a provocative immune challenge. Domestic Animal Endocrinology. V. 43, p. 146-153, 2012.

CHAPINAL, N.; LEBLANC, S.J.; CARSON, M.E.; LESLIE, K.E.; GODDEN, S.; CAPEL, M.; DUFFIELD, T.F. Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. J. Dairy Sci. V. 95(10), p. 5676–5682, 2012.

CHAROENSOOK, R.; GATPHAYAK, K.; SHARIFI, A.R.; CHAISONGKRAM, C.; BRENIG, B.; KNORR, C. Polymorphisms in the bovine HSP90AB1 gene are associated with heat tolerance in Thai indigenous cattle. Trop. Anim. Health Prod. V.44, p. 921-928. 2012.

CHEBEL, R.C.; SANTOS, J.E.; REYNOLDS, J.P.; CERRI, R.L.; JUNCHEM, S.O.; OVERTON, M. Factors Affecting Conception Rate After Artificial Insemination and Pregnancy Loss in Lactating Dairy Cows. Anim. Reproduction Science. V. 84, p. 239-255, 2004.

CHRENEK, P.; HUBA, J.; VASICEK, D.; PESKOVICOVÁ, D.; BULLA, J. The Relation between Genetic Polymorphism Markers and Milk Yield in Brown Swiss Cattle Imported to Slovakia. Asian-Aust. J. Anim. Sci. V. 16(10), p.1397–1401, 2003.

COCHRAN, S.D.; COLE, J.B.; NULL, D.J.; HANSEN, P.J. Discovery of single nucleotide polymorphisms in candidate genes associated with fertility and production traits in Holstein cattle. Genetics. V. 14(49), p. 1-23, 2013.

COLLINS, F.S.; BROOKS, L.D.; CHAKRAVARTI, A. A DNA Polymorphism Discovery Resource for Research on Human Genetic Variation. Genome Res. V. 8, p. 1229-1231, 1998.

DE VRIES, A. Economic value of pregnancy in dairy cattle. J Dairy Sci. V. 89(10), p. 3876-85, 2006.

DEMETRIO, D.G.B.; SANTOS, R.M.; DEMETRIO, C.G.B.; VASCONCELOS, J.L.M. Factors affecting conception rates following artificial insemination or embryo transfer in lactating Holstein cows. J. Dairy Sci. V. 90(11), p. 5073–5082, 2007.

DIKMEN, S.; COLE, J. B.; NULL, D. J.; HANSEN, P. J. Heritability of rectal temperature and genetic correlations with production and reproduction traits in dairy cattle. J. Dairy Sci. V.95, p. 3401-3405, 2012.

DIKMEN, S.; HANSEN, P.J. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J. Dairy Sci. V. 92, p.109–116, 2009.

DIKMEN, S.; WANG, X.; ORTEGA, M.S.; COLE, J.B.; NULL, D.J.; HANSEN, P.J. Single nucleotide polymorphisms associated with thermoregulation in lactating dairy cows exposed to heat stress. J. Anim. Breeding and Genetics. V.132, p. 409-419, 2015.

DISKIN, M.G.; MURPHY, J.J.; SREENAN, J.M. Embryo Survival in Dairy Cows Managed Under Pastoral Conditions. Animal Reproduction Science. V. 96, p. 297-311, 2006.

DISKIN, M.G.; PARR, M.H.; MORRIS, D.G. Embryo Death in Cattle: an update. Reproduction, Fertility and Development. V. 24, p. 244-251, 2012.

EALY, A.; HOWELL, J.; MOTERROSO, C.; ARECHIGA, C.F.; HANSEN, P.J. Developmental changes in sensitivity of bovine embryos to heat shock and use of antioxidants as thermoprotectants. J. of Animal Sci. V. 73, p. 1401–1407, 1995.

EALY, A.D.; DROST, M.; HANSEN, P. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. J. Dairy Sci. V. 76(10), p. 2899– 2905, 1993.

EDWARDS, J.; HANSEN, P.J. Differential responses of bovine oocytes and preimplantation embryos to heat shock. Molecular Reproduction and Development. V. 46, p. 138–145, 1997.

GANAIE, A.H.; GHASURA, R.S.; MIR, N.A.; BUMLA, N.A.; SANKAR, G.; WANI, S.A. Biochemical and Physiological Changes during Thermal Stress in Bovines: A Review. Iranian Journal of Applied Animal Science. V. 3(3), p. 423-430, 2013.

GOURDINE, J.L.; MANDONNET, N.; GIORGI, M.; RENAUDEAU, D. Genetic parameters for thermoregulation and production traits in lactating sows reared in tropical climate. Animal. V. jul 5, p. 1-10, 2016.

GUZELOGLU, A.; AMBROSE, J.D.; KASSA, T.; DIAZ, T.; THATCHER, M.J.; THATCHER, W.W. Long-term follicular dynamics and biochemical characteristics of dominant follicles in dairy cows subjected to acute heat stress. Animal Reproduction Science. V. 66, p. 15–34, 2001.

HANSEN, P.J.; ARÉCHIGA, C. F. Strategies for Managing Reproduction in the Heat-Stressed Dairy Cow. J. Anim Sci. V.77, p. 36-50, 1999.

HANSEN, P.J. Effects of heat stress on mammalian reproduction. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. V.364, p. 3341–3350, 2009.

HANSEN, P.J. To be or not to be—Determinants of embryonic survival following heat shock. Theriogenology. V. 68, p. 40-48, 2007.

HANSEN, P.J. Genetic variation in resistance of the preimplantation bovine embryo to heat shock. Reproduction, Fertility and Development. V.27, p. 22–30, 2015.

HERNANDEZ, J.A.; RISCO, C.A.; LIMA, F.S.; SANTOS, J.E.P. Observed and expected combined effects of clinical mastitis and low body condition on pregnancy loss in dairy cows. Theriogenology. v. 77, p.115–121, 2012.

HERNÁNDEZ-CERÓN, J.; CHASE, C.C.; HANSEN, P.J. Differences in heat tolerance between preimplantation embryos from Brahman, Romosinuano, and Angus breeds. J. Dairy Sci. V. 87, p. 53–58, 2004.

HOWELL, J.L.; FUQUAY, J.W.; SMITH, A.E. Corpus Luteum Growth and Function in Lactating Holstein Cows During Spring and Summer. J. Dairy Sci. V. 77(3), p. 735–739, 1994.

IBEAGHA-AWEMU, E.M.; KGWATALALA, P.; IBEAGHA, A.E.; ZHAO, X. A critical analysis of disease-associated DNA polymorphisms in the genes of cattle, goat, sheep, and pig. Mamm. Genome.V.19, p. 226–245, 2008.

JAINUDEEN, M.R.; HAFEZ, S.E. Reprodutive failure in females. In: HAFEZ, EE.; HAFEZ, B. Reproduction in farm animals. 7th ed. Philadelphia: Lippincott Williams & Wilkins, 2000. Cap. 17. p. 261-278.

KADOKAWA, H.; SAKATANI, M.; HANSEN, P.J. Perspectives on improvement of reproduction in cattle during heat stress in a future Japan. Animal Science Journal. V. 83(6), p. 439–445, 2012.

KIM, S. & MISRA, A. SNP Genotyping: Technologies and Biomedical Applications. Annu Rev Biomed Eng.V. 9, p. 289-320, 2007.

KOGA, A.; SUGIYAMA, M.; DEL BARRIO, A.N.; LAPITAN, R.M.; ARENDA, B.R.; ROBLES, A.Y.; CRUZ, L.C.; KANAI, Y. Comparison of the thermoregulatory response of buffaloes and tropical cattle, using fluctuations in rectal temperature, skin temperature and haematocrit as an index. J. of Agric. Sci. V.142, p. 351–355, 2004.

KREGAL, K.C. Molecular Biology of Thermoregulation Invited Review: Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J. Appl. Physiol. V. 92, p. 2177-2186, 2002.

KRININGER III, C.; STEPHENS, S.H.; HANSEN, P.J. Developmental Changes in Inhibitory Effects of Arsenic and Heat Shock on Growth of Pre-Implantation Bovine Embryos. Molecular Reproduction and Development. V. 340, p.335–340, 2002.

KUMAR, R.; GUPTA, I.D.; VERMA, V.; VERMA, N.; VINEETH, M. R. Genetic polymorphisms within exon 3 of heat shock protein *90AA1* gene and its association with heat tolerance traits in Sahiwal cows. Veterinary World. V. 8, p. 932-936, 2015.

LACERDA, T.; LOUREIRO, B. Selecting Thermotolerant Animals as a Strategy to Improve Fertility in Holstein Cows. Global Journal of Animal Scientific Research. V. 3(1), p. 119-127, 2015.

LI, Q.L.; ZHANG, Z.F.; XIA, P.; WANG, Y.J.; WU, Z.Y.; JIA, Y.H.; CHANG, S.M.; CHU, M.X. A SNP in the 3'-UTR of *HSF1* in dairy cattle affects binding of target *bta-miR-48.* Genetics and Molecular Research. V. 14(4), p. 12746-12755, 2015.

LIU, Y.; LI, D.; LI, H.; ZHOU, X.; WANG, G. A novel SNP of the ATP1A1 gene is associated with heat tolerance traits in dairy cows. Mol. Biol. Rep. V.38, p. 33-38, 2011.

LIU, Y.X.; ZHOU, X.; LI, D.Q.; CUI, Q.W.; WANG, G.L. Association of *ATP1A1* gene polymorphism with heat tolerance traits in dairy cattle. Genetics and Molecular Research. V. 9(2), p. 891-896, 2010.

LONERGAN, P. Influence of Progesterone on Oocyte Quality and Embryo Development in Cows. Theriogenology. V. 76, p. 1594-1601, 2011.

LUCY, M.C. Reproductive Loss in High-Producing Dairy Cattle: Where Will it End? Journal of Dairy Science. V. 84(6), p. 1277-1293, 2001.

ORTEGA, M.S.; DENICOL, A.C.; COLE, J.B.; NULL, D.J.; HANSEN, P. J. Use of single nucleotide polymorphisms in candidate genes associated with daughter pregnancy rate for prediction of genetic merit for reproduction in Holstein cows. Stichting International Foundation for Animal Genetics. V. 47, p. 288–297, 2016.

PUTNEY, D.J.; DROST, M.; THATCHER, W.W. Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between Days 1 to 7 post insemination. Theriogenology. V. 30(2), p. 195–209, 1988.

RAVAGNOLO, O.; MISZTAL, I. Studies on genetics of heat tolerance in dairy cattle with reduced weather information via cluster analysis. J Dairy Sci. V. 85(6), p.1586-1589, 2002.

RENSIS, F.; SCARAMUZZI, R.J. Heat stress and seasonal effects on reproduction in the dairy cow—a review. Theriogenology. v. 60, p. 1139-1151, 2003.

ROCHA, D.R.; SALLES, M.G.F.; MOURA, A.A.A.N.; ARAÚJO, A.A. Impacto do estresse térmico na reprodução da fêmea bovina. Rev. Bras. Reprod. Anim. V. 36(1), p. 18-24, 2012.

ROMAN-PONCE, H.; THATCHER, W.W.; CATON, D.; BARRON, D.H.; WILCOX, C.J. Thermal Stress Effects on Uterine Blood Flow in Dairy Cows. J. of Animal Sci. V. 46, p.175–180, 1978.

ROTH, Z. Heat Stress, the Follicle, and Its Enclosed Oocyte: Mechanisms and Potential Strategies to Improve Fertility in Dairy Cows. Reprod. Dom. Anim. V. 43(2), p. 238-244, 2008.

ROTH, Z.; ARAV, A.; BRAW-TAI, R.; BOR, A.; WOLFENSON, D. Effect of treatment with follicle-stimulating hormone or bovine somatotropin on the quality of oocytes aspirated in the autumn from previously heatstressed cows. J. Dairy Sci. V. 85(6), p.1398–1405, 2002.

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. Journal of Reproduction and Fertility. V. 120, p. 83-90, 2000.

ROTH, Z.; MEIDAN, R.; SHAHAM-ALBALANCY, A.; BRAW-TAL, R.; WOLFENSON, D. Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles. Reproduction (Cambridge, England). V. 121(5), p. 745–751, 2001.

SAILO, L.; GUPTA, I.D.; VERMA, A.; SINGH, A.; CHAUDHARI, M.V.; DAS, R.; UPADHYAY, R.C.; GOSWAMI, J. Single Nucleotide Polymorphism in HSP90AB1 Gene and its Association with Thermo-tolerance in Jersey Crossbred Cows. Animal Science Reporter. V. 9 (2), p.43-49, 2015.

SAKATANI, M.; KOBAYASHI, S.I.; TAKAHASHI, M. Effects of heat shock on *in vitro* development and intracellular oxidative state of bovine preimplantation embryos.

Molecular Reproduction and Development. V. 67, p.77–82, 2004.

SARTORI, R. Manejo Reprodutivo da Fêmea Leiteira. Reprod Anim. V.31, p.153-159, 2007.

SARTORI, R.; SARTOR-BERGFELT, R.; MERTENS, S.A.; GUENTHER, J.N.; PARRISH, J.J.; WILTBANK, M.C. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J. Dairy Sci. V. 85(11), p. 2803–2812, 2002.

SEATH, D.M.; MILLER, G.D. Heat tolerance comparisons between Jersey and Holstein cows. J. of Animal Sci. V. 6, p. 24–34, 1947.

SENOSY, W.; ZAIN, A.E.; ABDEL-RAZEK, A.K.; UCHIZA, M.; TAMEOKA, N.; IZAIKE, Y.; OSAWA, T. Association between Energy Status, Early Postpartum and Subsequent Embryonic Mortality in High- Yielding Recipient Cows. Animal Science Journal. V.83, p.284-290, 2011.

SHIMIZU, T.; OHSHIMA, I.; OZAWA, M.; TAKAHASHI, S.; TAJIMA, A.; SHIOTA, M.; KANAI, Y. Heat stress diminishes gonadotropin receptor expression and enhances susceptibility to apoptosis of rat granulosa cells. Reproduction (Cambridge, England). V. 129(4), p. 463–472, 2005.

SILVA, C.F.; SARTORELLI, E.S.; CASTILHO, A.C.S.; SATRAPA, R.A.; PUELKER, R.Z.; RAZZA, E.M.; TICIANELLI, J.S.; EDUARDO, H.P.; LOUREIRO, B.; BARROS, C.M. Effects of heat stress on development, quality and survival of Bos indicus and Bos taurus embryos produced in vitro. Theriogenology. v. 79, p. 351-357, 2013.

SILVA, R.; MAIA, A.; COSTA, L.; QUEIROZ, J. Latent heat loss of dairy cows in an equatorial semi-arid environment. International Journal of Biometeorol. V. 56, p. 927–932, 2012.

SUGIYAMA, S.; MCGOWAN, M.; KAFI, M.; PHILLIPS, N.; YOUNG, M. Effects of increased ambient temperature on the development of *in vitro* derived bovine zygotes. Theriogenology. V. 60, p.1039–1047, 2003.

THATCHER, W.W.; GUZELOGLU, A.; MATTOS, R.; BINELLI, M.; HANSEN, T.R..; PRU, J.K. Uterine-conceptus interactions and reproductive failure in cattle. Theriogenology. V. 56(09), p. 1435–1450, 2001.

VASCONCELOS, J.L.M.; COOKE, R.F.; JARDINA, D.T.G.; ARAGON, F.L.; VERAS, M.B.; SORIANO, S.; SCARPA, A.B. Associations among milk production and rectal temperature on pregnancy maintenance in lactating recipient dairy cows. Animal Reproduction Science. V. 127, p.140–147, 2011.

VASCONCELOS, J.L.M.; DEMÉTRIO, D.G.B.; SANTOS, R.M.; CHIARI, J.R.; RODRIGUES, C.; SÁ FILHO, O.G. Factors potentially affecting fertility of lactating dairy cow recipients. Theriogenology. V. 65, p.192–200, 2006.

WEST, J.W. Effects of heat-stress on production in dairy cattle. J. Dairy Sci. V. 86(6) p. 2131–2144, 2003.

WEST, J.W. Physiological Effects of Heat Stress on Production and Reproduction. Tri-State Dairy Nutrition Conference. P.1-10, 2002.

WILDMAN, E. E.; JONES, I G. M.; WAGNER, P. E; BOMAN, R. L. A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. J. Dairy Sci. V. 65, p. 495-501, 1982.

WILSON, S.J.; MARION, R.S.; SPAIN, J.N.; SPIERS, D.E.; KEISLER, D.H.; LUCY, M.C. Effects of controlled heat stress on ovarian function of dairy cattle. J. Dairy Sci. V. 81(8), p. 2124–2131, 1998.

WOLFENSON, D.; LEW, B.J.; THATCHER, W.W.; GRABER, Y.; MEIDAN, R. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. Animal Reproduction Science. V. 47, p. 9-19, 1997.

WOLFENSON, D.; ROTH, Z.; MEIDAN, R. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Animal Reproduction Science. V. 60-61, p. 535–547, 2000.

WOLFENSON, D.; THATCHER, W.W.; BADINGA, L.; SAVIO, J.D.; MEIDAN, R.; LEW, B.J.; BRAW-TAL, R.; BERMAN, A. Effect of Heat Stress on Follicular Development during the Estrous Cycle in Lactating Dairy Cattle. Biology of Reproduction. V. 52, p. 1106-1113, 1995.

Gene	**Sequence (5’ – 3’)**	**Exon**	**Reference**
ATP1A1	F: AGT GCT GCG TGA AAC CTG	16	Liu et al., 2011
	R: GTG ATG TGT GGA ATG TGT GC	16	Liu et al., 2011
HSP90AB1	F1: CCTGGATTGGAATGCCTAAC	2	Charoensook et al., 2012
	R1: TCAGGCTCTCATAGCGAATC	2	Charoensook et al., 2012
	F2: TCACCCAGGAGGAATATGGAG	6	Charoensook et al., 2012
	R2: AGAAGGACCGATTTTCTCACC	8	Charoensook et al., 2012

Table 1. Primers used for polymorphism detection of ATP1A1 and HSP90AB1

Figures

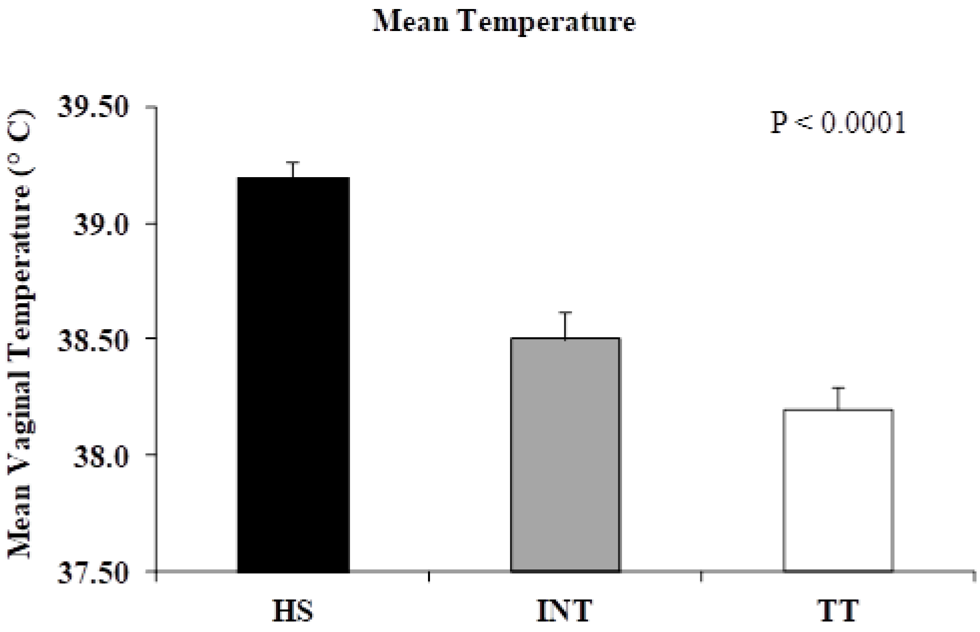

Figure 1. Mean temperature (°C) observed during the three days when thermometers were kept at the animals of each group (HS, INT and TT). The mean temperature was higher (P <.0001) in the animals of the HS group than in the animals of the INT and TT groups. The INT group did not differ from the TT group. Differences were considered statistically significant when P <

0.05.

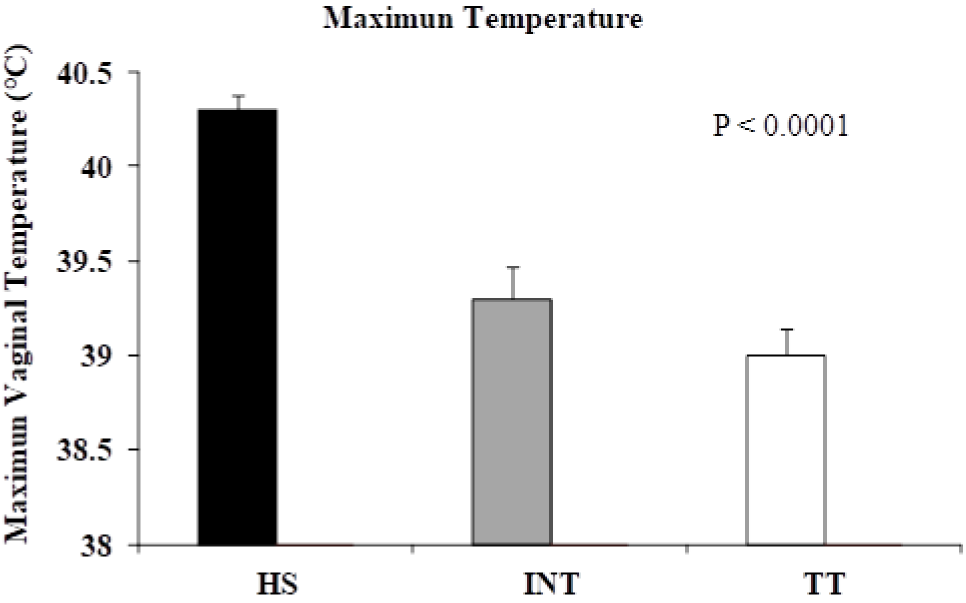

Figure 2. Mean maximum temperatures (°C) observed during the three days that thermometers were kept on animals in each group (HS, INT, and TT). Maximum temperatures were higher for animals in the HS group compared to the INT and TT groups (P <0001). The INT group did not differ from the TT group. Differences were considered statistically significant when P < 0.05.

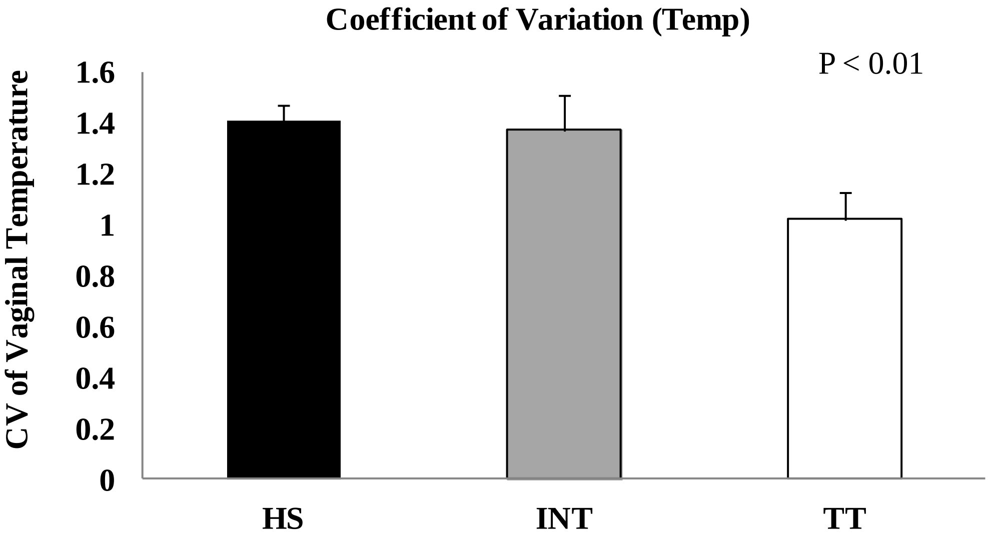

Figure 3. Coefficient of variance of the temperatures (°C) observed during the three days that thermometers were kept on animals in each group (HS, INT, and TT). Variations were higher for animals in the HS and INT group compared to the TT groups (P <0.01). The HS group did not differ from the INT group. Differences were considered statistically significant when P < 0.05.

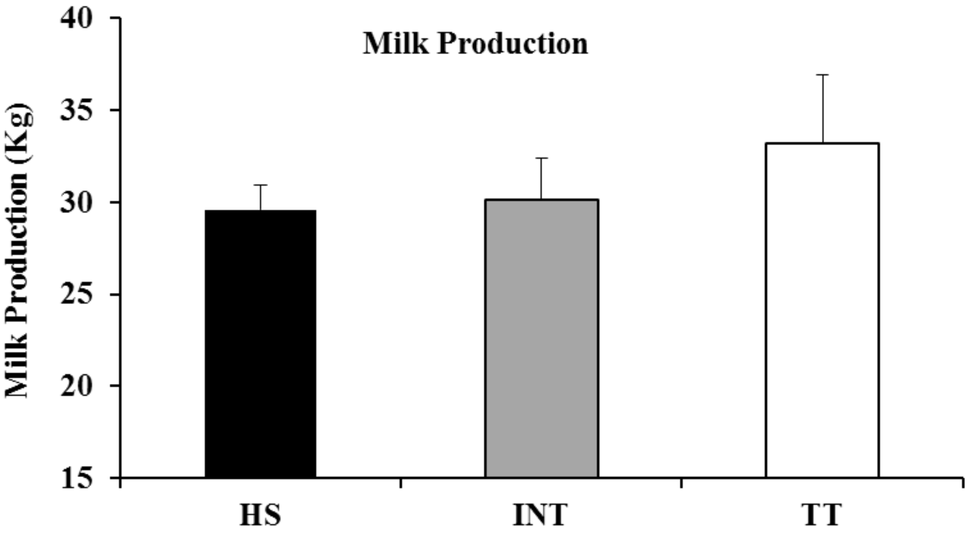

Figure 4. Mean milk production (kg) with respect to each classified group (HS, INT, and TT). No statistical difference was found within groups. P > 0.05.

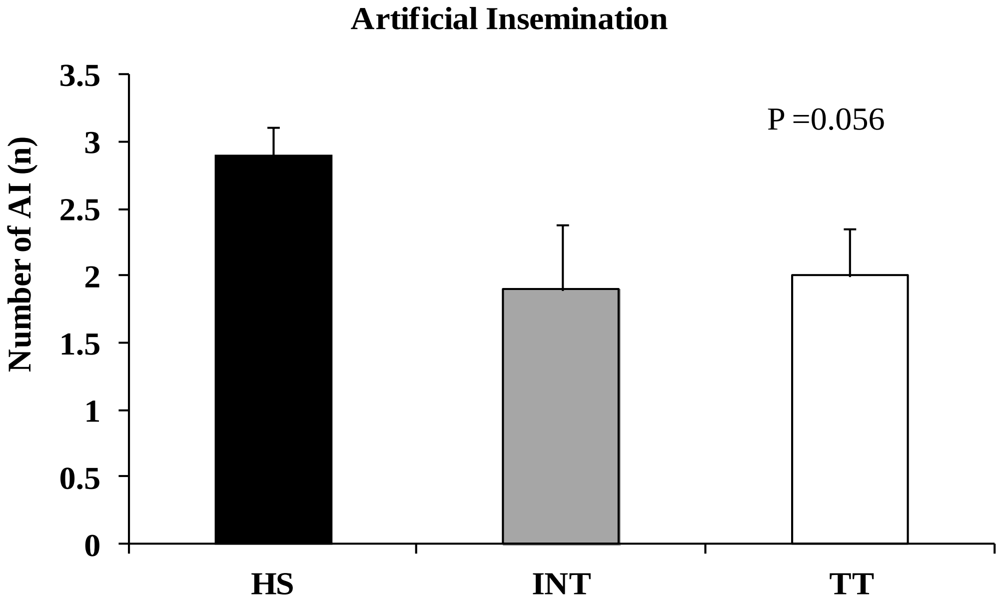

Figure 5. Number of inseminations required to diagnose pregnancy in each classified group (HS, INT, and TT). P = 0.052.