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Research Article

Effect of Heat Stress on Bovine Embryonic Development

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Abstract

To determine the effect of heat stress initiating at the 4-8 cells stage on bovine embryo to blastocyst stage and sire effect on embryonic development under heat stress conditions. Frozen semen from four Charolais sires were used to produce bovine embryos. Four to eight cells stage embryos that were produced from oocytes collected from slaughterhouse ovaries were submitted to two heat stress treatments. Experimental design in this study was the randomized complete design (CRD) with 7 replications per sire. The experiments were conducted at the Faculty of Veterinary Medicine, Khon Kaen University. At day 3 after fertilization, 4-8 cells stage embryos were selected and divided into 3 groups; group1: culture at 38.5^{O}C (control), group 2: culture at 41°C for 8 h once (heat 8h), and group3: culture at 41°C for 8 h every day until day 7 after fertilization (heat 8h/d). Embryos developed to the blastocyst stage were recorded at day 8 after fertilization and were determined for the number of cells by differential staining technique.

The results of this study were showed that daily exposure to heat stress (heat 8h/d) reduced development of 4-8 cell stage embryos to blastocyst stage compared with control ($P<0.05$). When the% of blastocyst development from heat stress groups were normalized to control (control = 100%) the total blastocyst development in heat 8h/d group from sire 3 (62.9%) was lower than that of control group ($P<0.05$). However, the% of expanded blastocyst development in heat 8h/d group were also significantly reduced ($P<0.05$) in all sires.The numbers total cell and trophectoderm of blastocysts in heat 8h/d group were lower than those of heat 8h and control groups($P<0.05$).The numbers of inner cell mass among groups were not different.

In conclusion, this study was suggested that there was no distinct effect of sires, within the same breed, on development to blastocyst stage under heat stress condition. Heat stress reduced the number of total cell in blastocyst stage through the reduction in the number of trophectoderm.

Keywords: Heat stress, Bovine embryo and Cell number

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ผลของความเครียดเนื่องจากความร้อนต่อการพัฒนาการในเอ็มบริโอโค

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บทคัดย่อ

วัตถุประสงค์การศึกษาครั้งนี้เพื่อศึกษาผลของความเครียดเนื่องจากความร้อนในเอ็มบริโอโคระยะ 4-8 เซลล์ต่อการพัฒนาการถึงระยะบลาสโตซีสต์ และผลของพ่อพันธุ์ต่อการพัฒนาการของเอ็มบริโอภายใต้สภาวะความเครียดเนื่องจากความร้อน โดยใช้น้ำเชื้อแช่แข็งจากโคพ่อพันธุ์ชาโรเล่ห์จำนวน 4 ตัวในการผลิตเอ็มบริโอ จากรังไข่โรงฆ่าสัตว์ในการผลิตเอ็มบริโอระยะ 4-8 เซลล์ จากนั้นนำมาทำการเหนี่ยวนำให้อยู่ภายใต้สภาวะความเครียดเนื่องจากความร้อน 2 รูปแบบ วางแผนการทดลองแบบ randomized complete design (CRD) ทำการทดลองจำนวน 7 ครั้งต่อพ่อพันธุ์ ทำการศึกษาที่คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น ในวันที่ 3 หลังการปฏิสนธินำเอ็มบริโอที่ระยะ 4-8 เซลล์ แบ่งเป็น 3 กลุ่มได้แก่ กลุ่มที่ 1 เพาะเลี้ยงที่อุณหภูมิ 38.5°C (กลุ่มควบคุม) กลุ่มที่ 2 เพาะเลี้ยงที่ 41°C นาน 8 ชั่วโมงครั้งเดียว (heat 8 h) และกลุ่มที่ 3 เพาะเลี้ยงที่ 41°C นาน 8 ชั่วโมงทุกวันจนกระทั่งวันที่ 7 หลังการปฏิสนธิ (heat 8 h/d) จดบันทึกการพัฒนาการของเอ็มบริโอที่พัฒนาถึงระยะบลาสโตซีสต์ในวันที่ 8 หลังการปฏิสนธิ และทำการนับจำนวนเซลล์โดยวิธี Differential staining

ผลการศึกษาพบว่า การให้ความเครียดเนื่องจากความร้อนทุกวัน (heat 8 h/d) ลดการพัฒนาของเอ็มบริโอระยะ 4-8 เซลล์ถึงระยะบลาสโตซีสต์ได้เมื่อทำการเปรียบเทียบกับกลุ่มควบคุม ($P<0.05$) เมื่อนำเปอร์เซ็นต์การพัฒนาของเอ็มบริโอถึงระยะบลาสโตซีสต์ของกลุ่มที่เหนี่ยวนำให้อยู่ในสภาวะความเครียดเนื่องจากความร้อนมาทำการ normalize กับกลุ่มควบคุม (control = 100%) พบว่าจำนวนการพัฒนาถึงระยะบลาสโตซีสต์รวมทั้งหมดในกลุ่ม heat 8 h/d จากพ่อพันธุ์ตัวที่ 3 (62.9%) ต่ำกว่ากลุ่มควบคุม ($P<0.05$) อย่างไรก็ตาม จำนวนการพัฒนาถึงระยะ expanded blastocyst ในกลุ่ม heat 8 h/dจากพ่อพันธุ์ทุกตัวลดลงอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) จำนวนเซลล์รวม และจำนวนเซลล์ trophectoderm ของเอ็มบริโอระยะ
บลาสโตซีสต์ ในกลุ่มheat 8 h/d มีจำนวนต่ำกว่ากลุ่ม heat 8 h และกลุ่มควบคุม จำนวนเซลล์ inner cell mass ไม่มีความแตกต่างระหว่างกลุ่มการทดลอง

สรุปผลการศึกษาครั้งนี้ยังไม่สามารถยืนยันได้แน่ชัดถึงอิทธิพลของพ่อพันธุ์ ที่มาจากสายพันธุ์เดียวกัน มีผลต่อการพัฒนาของเอ็มบริโอถึงระยะบลาสโตซีสต์เมื่ออยู่ในสภาวะความเครียดเนื่องจากความร้อน ผลของความเครียดเนื่องจากความร้อนสามารถลดจำนวนเซลล์รวมของเอ็มบริโอในระยะบลาสโตซีสต์รวมทั้งจำนวนเซลล์ trophectoderm

คำสำคัญ: ความเครียดเนื่องจากความร้อน เอ็มบริโอโค และ จำนวนเซลล์

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Introduction

Elevated temperature has negative effect on embryonic development causing embryonic mortality and loss of pregnancy (Hansen, 2007). Exposure to elevated temperature during oocyte maturation at 41°C has also been reported to reduce fertilization rate by reducing the proportions of oocytes that can complete nuclear maturation (Payton *et al*., 2004; Roth and Hansen, 2004) and increasing the proportions of abnormal spindle formation.*In vitro* fertilization under heat stress was resulted in lower the embryonic development to blastocyst stage (Rivera and Hansen, 2001). Heat stress during cleavage stage reduced the proportions of embryonic development to blastocyst stage (Block *et al*., 2002). In bovine embryos, heat stress was more detrimental to development when applied to early stages (zygote to 4-8 cells stage) than when applied to later stages of development (Edward and Hansen, 1997; Sakatani *et al*., 2004; Bonilla *et al*., 2011).

Various models in studying effect of heat stress on bovine embryos have been reported. However, to our knowledge, all studies exposed oocytes, zygotes, or embryos to an only one session of heat stress. Heat stress at 40-41°C for 8-24 hours was given during oocyte maturation (IVM) (Rivera and Hansen, 2004; Roth and Hansen, 2004; Sakatani *et al*., 2012, 2013; Alves *et al*., 2013). Heat stress at 41°C for 3-12 hours was given to presumptive zygotes and 2-4 cells stage embryos (Rivera and Hansen, 2001; Rivera *et al*., 2004; Sakatani *et al*., 2004; Eberhardt *et al*., 2009; Gendelman *et al*., 2010; Alves *et al*., 2013). Heat stress at 41-42°C for 6-18 hours was given to 4-8 cells stage embryos, 8-16 cells stage embryos, or morulae (Paula-Lopez and Hansen, 2002; Sakatani *et al*., 2004; Satrapa *et al*., 2011; Silva *et al*., 2013). In this study, we used a daily exposure to 8 hours of heat stress at 41°C initiated at 4-8 cells stage embryos to study effect of heat stress on development.

The heat tolerance ability of embryos is profoundly affected by various factors, especially the genetic variations of animal and stages of development. In general, *Bos indicus* breeds embryos can tolerate to heat stress better than *Bos taurus* breeds embryos (Eberhardt *et al*., 2009; Gendelman *et al*., 2010; Satrapa *et al*., 2011; Paula-Lopez *et al*., 2013).In bovine embryos, the major embryonic genome activation (EGA) is established at the 4-8 cells stage (Graf *et al*., 2014). Before EGA, the genotype of oocytes plays a vital role in the ability of embryos to endure effects of heat stress. Embryos produced from *Bos indicus* oocytes and fertilized with *Bos taurus* spermatozoa were able to endure heat stress better than embryos produced from *Bos taurus* oocytes and fertilized with *Bos indicus* spermatozoa (Block *et al*., 2002; Eberhardt *et al*., 2009). After EGA, however, the genotypes of spermatozoa and oocytes should contribute equally to embryonic heat tolerance. Therefore, the influence of spermatozoa on embryonic heat tolerance can be evaluated in embryos at later than 4-8 cells stage.In previous studies, effects of sires on embryonic heat tolerance were studied as an effect of breed whether they are *Bos taurus* or *Bos indicus* (Eberhardt *et al*., 2009; Satrapa *et al*., 2011; Paula-Lopez *et al*., 2013) In this study, we were interested in examine the paternal effect from different sires in the same breed on embryonic development under heat stress conditions.

The objectives of this study were to determine the effect of heat stress initiating at the 4-8 cells stage on bovine embryonic development and effect of sires on development under heat stress conditions.

Materials and Methods

Materials

All chemicals were purchased from Sigma Chemical Company (St. Louis, MO) unless otherwise noted. Fetal bovine serum was purchased from Gibco™ (Gibco, USA.).

Oocyte recovery and in vitro maturation (IVM)

In vitro production of embryos was performed using oocytes from slaughter houses in Khon Kaen Province. Briefly, cumulus-oocyte complexes (COCs) with at least three layers of cumulus surrounding and homogeneous cytoplasm aspirated from antral follicles were selected and washed in TCM-199. Selected COCs were cultured in TCM-199 supplemented with 5% fetal bovine serum (Gibco, USA.) and follicle stimulating hormone (Sigma Aldrich, USA.) for 23 hours at 38.5^{O}C, 5%CO_2 and humidified air.

In vitro fertilization (IVF)

This study used frozen semen from 4 sires. After 23 h of IVM, fertilization was performed with swim-up prepared frozen-thawed spermatozoa. Briefly, two straws of frozen semen were thawed at 37^{O}C 30 sec and deposited in 6 tubes containing 1,500 µL of Tyrode's albumin-lactate-pyruvate (TALP-HEPES) and incubated at 38.5^{O}C for 45 minutes. After incubation, sperm were centrifuged at 170 G for 10 minutes and supernatant was removed. The sperm pellet was re suspended and counted for sperm concentration. Mature oocytes were inseminated with 2×10^{6} spermatozoa/mL for 18-20 h in 100 µL/drop of TALP-IVF and incubated at 38.5^{O}C, 5%CO_2 and humidified air.

In vitro culture (IVC)

At 18-20 hours post insemination (hpi), sperm were washed out and presumptive zygotes were transferred to synthetic oviductal fluid (SOF) supplemented with bovine serum albumin (BSA) and incubated at 38.5°C, 5%O_2 and 5%CO_2. At 72 hpi, only embryo shaving at least 4 cells were collected and randomly distributed into 3 groups.

Group 1: culture at 38.5°C (control)

Group 2: culture at 41°C for 8 h once (heat 8h)

Group 3: culture at 41°C for 8 h every day until day 7 after fertilization (heat 8h/d)

Blastocyst development was determined on day 8 after fertilization. To assess the proper speed of development, embryos reaching blastocyst by day 8 after fertilization were classified into 4 stages of blastocyst; early blastocyst, full blastocyst, expanded blastocyst, and hatched blastocyst.

The data were presented as the% of blastocyst development from 4-8 cells stage embryos. The% of blastocyst development in heat stress groups were also normalized to the control group (control =100%). Some of expanded blastocysts were used for determination of cell number with differential staining.

Differential staining

The cell number of blastocyst stage embryos was counted using differential staining technique developed by Thouas *et al*. (2001). Briefly, embryos were placed in 1% Triton X-100

(Sigma, USA) in phosphate buffer saline (PBS) for 10-15 sec. Embryos were washed in TCM-199 before stained with 20 mg/mL propidium iodide (PI) and 25 µg/mL bisbenzimide (Hoechst 33258) in TCM-199 for 30 min (38.5°C). After staining, embryos were washed with TCM-199 and fixed in 2% glutaraldehyde for 20 sec then washed again with TCM-199. Stained embryos were placed in one drop of anti-fading on glass slide and covered with cover-glass. The stained embryos were examined immediately under fluorescence microscope (Carl Zeise Jena, SH 250). The numbers of inner cell mass (stained in blue color) and trophectoderm (stained in red color) were recorded.

Statistical analysis

The experimental design was completely randomized design (CRD). Data were analyzed with one-way ANOVA using the SPSS program. Differences between treatments were subjected to student Duncan Multiple Range Test (DMRT). Differences of $P<0.05$ were considered significant.

Results and Discussion

Results

The results of this study were showed that daily exposure to heat stress (heat 8h/d) reduce the% of blastocyst development from 4-8 cell stage embryos compared to control ($P<0.05$). No different in blastocyst development between embryos in heat 8h and heat 8h/d groups was found (Fig. 1). The daily exposure to heat stress (heat 8h/d) also reduced the% of development to expanded blastocyst stage (Fig.2) compared to control ($P<0.05$).

When the% of embryonic development to blastocyst stage in the heat stress groups were normalized to control (control = 100%) in each sire (Fig.3). The total blastocyst development of embryos in heat 8h/d groups from sire 3 (62.9%) was lower than that of control group ($P<0.05$). No different within groups in sire 1, sire 2, and sire 4 were found. Additionally, the development to expanded blastocyst stage (Fig.4) of embryos in heat 8h/d groups from sire 2, 3 and 4 were lower than that of control group ($P<0.05$).

The total cell number and numbers of trophectoderm of blastocyst in heat 8h/d group were lower than those of control and heat 8 h groups ($P<0.05$). The numbers of inner cell mass among groups were not different; data were shown in Table 1.

Discussion

In this study we placed embryos into two models of heat stress conditions; group 1, embryos were induced to heat stress once, represent an acute heat stress; and group 2, embryos were induced to heat stress daily, represent a chronic heat stress condition. Our results showed that single exposure to heat stress (heat 8h group) produced no effect on development suggesting that these embryos were genetically tolerant to an elevated temperature.The 4-8 cells stage embryos in our study were able to develop to the blastocyst stage at a comparable rate to that of normal temperature. This was in contrast to other studies (Rivera and Hansen, 2001; Roth and Hansen, 2004; Sakatani *et al*., 2012; Sakatani *et al*., 2015). This discrepancy might be explained by the fact that oocytes used in our study came from Thai native cross bred (*Bos indicus*) cows while other studies

used oocytes collected from *Bos taurus* breeds, mainly Holstein.However, repeated exposure to heat stress (heat 8h/d) significantly reduced the% of embryos reaching any stage of blastocyst (total blastocyst) and reaching expanded blastocyst or more. This suggested that our model is suitable to study heat stress in genetically-adapted to subtropical climate embryos.

The influence from breed is one of several factors contributed to heat-tolerance. Block *et al*. (2002) reported that *Bos indicus* embryos had a better heat tolerance than *Bos taurus* embryos.The embryos produced from *Bos indicus* oocytes were more resistant to heat stress than embryos produced from *Bos taurus* oocytes (Eberhardt *et al*., 2009; Gendelman *et al*., 2010; Satrapa *et al*., 2011; Paula-Lopez *et al*., 2013).

The developmental ability to blastocyst stage under heat stress conditions could be affected by individual characteristic or genetic of sire (Ward *et al*., 2001). In this study, oocytes from Thai native crossbred cows were fertilized by four Australia Charloias' sire (*Bos taurus*) imported to Thailand by Department of Livestock Development. It was found that embryos produced from sire 3 under daily exposure to heat stress had lower proportions of total blastocyst compared to control and lowest among four sires in our knowledge, the influence of different bulls in the same breed on heat stress tolerant ability of embryos has not been reported. However, when the speed of blastocyst development was taken into considered the % of expanded blastocysts were also reduced in every sire under daily exposure of heat stress. This indicated that there was no sire that gave rise to embryos with highly resistant to heat stress. Interestingly, embryos in our study were profoundly affected by chronic heat stress despite the good heat tolerance from both maternal and paternal genome.

The number of cells of blastocysts reflects the quality of embryos.The numbers of blastocyst total cells in our study were comparable with other studies (Paula-Lopez *et al*., 2003; Sakatani *et al*., 2004; Jousan and Hansen, 2004; Sakatani *et al*., 2012). It was found that the numbers of trophectoderm of blastocysts in heat 8h/d group were lower than those of control and heat 8h groups whereas the numbers of the inner cell mass were not different among groups. In agreement to other studies, the numbers of trophectoderm and total cells were decreased in heat stress group (Paula-Lopez *et al*., 2003; Sakatani *et al*., 2004; Jousan and Hansen, 2004; Sakatani *et al*., 2012). Although the numbers of inner cell mass were not affected by heat stress the pregnancy rate from heat stress could be compromised. The trophectoderm plays a crucial role in the pregnancy recognition process as the source to secrete interferon-tau (IFN- **τ**), the pregnancy recognition signal in ruminants (Bazer *et al*., 1997; El-Sayed *et al*., 2006; Bazer. 2013).

Table 1 Numbers (means ± SE) of inner cell mass (ICM) and trophectoderm (TE) in expanded blastocyst under heat stress conditions

Treatment	n	ICM	TE	Total
Control	16	24.6±2.1	96.6±3.8[a]	123.1±5.1[a]
Heat 8h	16	24.9±2.0	97.9±3.2[a]	122.9±4.5[a]
Heat 8h/D	12	20.6±1.4	88.3±2.7[b]	108.8±3.3[b]

[a,b] difference superscripts were significant (P<0.05)

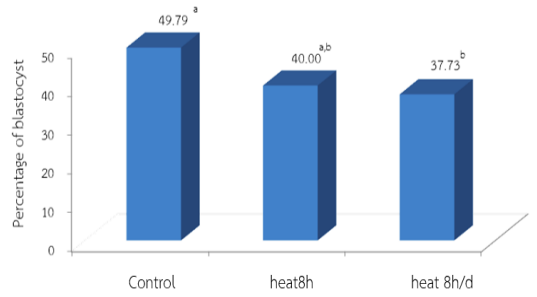

Fig. 1 The% of 4-8 cell stage embryos development to total blastocyst under heat stress conditions.

a,b different superscripts are significant (P<0.05)

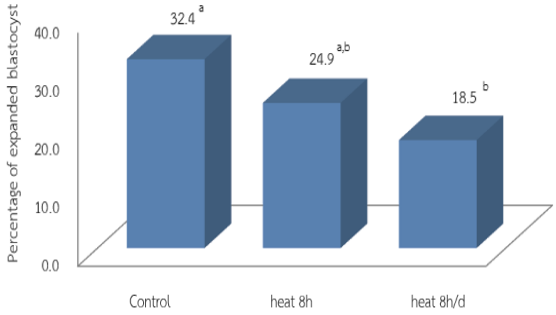

Fig. 2 The% of 4-8 cell stage embryos development to expanded blastocyst stage under heat stress conditions.

a,b different superscripts are significant (P<0.05)

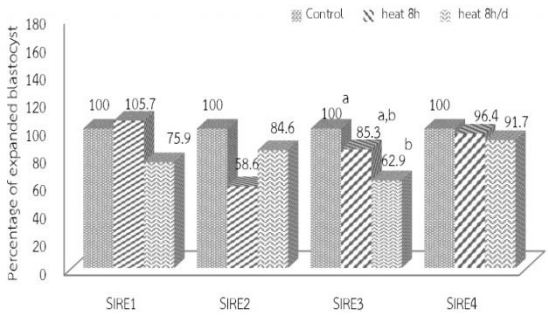

Fig. 3 The% of 4-8 cell stage embryos development to blastocyst stage normalized to control group (control=100%) under heat stress conditions.

a,b different superscripts are significant (P<0.05)

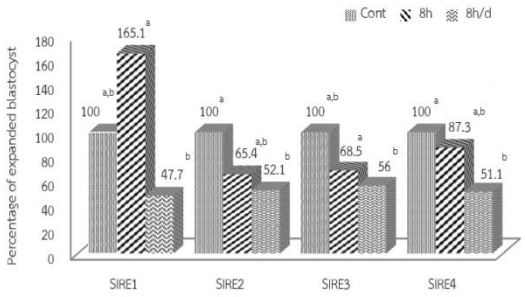

Fig. 4 The% of 4-8 cell stage embryos development to expanded blastocyst stage normalized to control group (control=100%) under heat stress conditions.

a,b different superscripts are significant (P<0.05)

Conclusion

In conclusion, this study was suggested that there was no distinct sire affection development to blastocyst stage under heat stress condition. Heat stress reduced the number of total cell in blastocyst stage through the reduction in numbers of trophectoderm.It is interesting to study the effect of heat stress on expression of heat stress related genes.

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References

Alves, M.F.,Gonçalves, R.F., Pavao, D.L., Palazzi, E.G., Souza, F., de Queiróz, R.K.R., D'Angelo, M. and Antônio de Achilles, M. 2013. Effect of heat stress on the maturation, fertilization and development rates of *in vitro* produced bovine embryos. J.Anim. Sci. 3: 174-178.

Bazer, F.W., Spencer, T.E. and Ott, T.L. 1997. Interferon Tau: A novel pregnancy recognition signal. AJRI. 37: 412-420.

Bazer, F.W. 2013. Pregnancy recognition signaling mechanisms in ruminants and pigs. J. Anim. Sci. Biotechnol. 4(23): 1-10.

Block, J., Chase, C.C. Jr. and Hansen, P.J. 2002. Inheritance of resistance of bovine pre implantation embryos to heat shock: relative importance of the maternal versus paternal contribution. Mol. Reprod. Dev. 63: 32-37.

Bonilla, A.Q.S., Oliveira, L.J., Ozawa, M., Newsom, E.M., Lucy, M.C. and Hansen, P.J. 2011. Developmental changes in thermo protective actions of insulin-like growth factor-1 on the pre implantation bovine embryo. Mol. Cell. Endocrinol. 332: 170-179.

Eberhardt, B.G., Satrapa, R.A., Capinzaiki, C.R.L., Trinca, L.A. and Barros, C.M. 2009. Influence of the breed of bull (*Bos taurus indicus* vs. *Bos taurus taurus*) and the breed of cow (*Bos taurus indicus*, *Bos taurus taurus* and crossbred) on the resistance of bovine embryos to heat. Anim.Reprod. Sci.114: 54-61.

Edwards, J.L. and Hansen, P.J. 1997. Differential responses of bovine oocytes and pre implantation embryos to heat shock. Mol. Reprod. Dev. 46: 138-145.

El-Sayed, A., Hoelker, M., Rings, F., Salilew, D., Jennen, D., Tholen, E., Sirard, M., Schellander, K. and Tesfaye, D. 2006. Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. Physiol. Genomics. 28: 84–96.

Gendelman, M., Aroyo, A., Yavin, S. and Roth, Z. 2010. Seasonal effect on gene expression, cleavage timing, and developmental competence of bovine pre implantation embryos. Reprod. Ferti. 140: 73-82.

Graf, A., Krebs, S., Heininen-Brown, M., Zakhartchenko, V., Schwalb, B., Bluma, H. and Wolf, E. 2014. Fine mapping of genome activation in bovine embryos by RNA sequencing. PNAS. 111(11): 4139–4144.

Hansen, P.J. 2007. To be or not to be-determinants of embryonic survival following heat shock. Theriogenology. 68 Suppl 1: S40-8.

Jousan, F.D. and Hansen, P.J. 2004. Insulin-link growth factor-I as a survival factor for the bovine pre implantation embryo exposed to heat shock. Biol. Reprod. 71: 1665-1670.

Payton, R.R., Romar. R., Coy, P., Saxton, A.M, Lawrence, J.L. and Edwards, J.L. 2004. Susceptibility of bovine germinal vesicle-stage oocytes from antral follicles to direct effects of heat stress *in vitro*. Biol. Reprod. 71:1303–8.

Paula-Lopez, F.F. and Hansen, P.J. 2002. Heat-shock induced apoptosis in pre implantation bovine embryos is a developmentally-regulated phenomenon. Biol. Reprod. 66:1169-1177.

Paula-Lopez, F.F.,ChaseJr, C.C., Al-Katanani, Y.M., Krininger,C.E., Rivera, R.M., Tekin, S., Majewski, A.C., Ocon,

O.M., Olson, T.A. Hansen, P.J. 2003. Genetic divergence in cellular resistance to heat shock in cattle: differences between breeds developed in temperate versus hot climates in responses of pre implantation embryos, reproductive tract tissues and lymphocytes to increased culture temperatures. Reprod. 125: 285–294.

Paula-Lopez, F.F., Lima, R.S., Satrapa, R.A. and Barros, C.M. 2013. Physiology and endocrinology symposium: Influence of cattle genotype (*Bos indicus* vs. *Bos taurus*) on oocyte and pre implantation embryo resistance to increased temperature. J Anim Sci. 11: 43-1153.

Rivera, R.M. and Hansen, P.J. 2001. Development of cultured bovine embryos after exposure to high temperatures in the physiological range. Reprod. 121 (1): 107-15.

Rivera, R.M., Dahlgren, G.M., De Castro, E., Kennedy, R.T. and Hansen, P.J. 2004. Actions of thermal stress in two-cell bovine embryos: oxygen metabolism, glutathione and ATP content, and the time-course of development. Reprod. 128(1): 33-42.

Roth, Z. and Hansen, P.J. 2004. Involvement of apoptosis in disruption of developmental competence of bovine oocytes by heat shock during maturation. Biol. Reprod. 71: 1898–1906.

Sakatani, M., Yamanaka, K., Balboula, A.Z., Takenouchi, N. and Takahashi, M. 2015. Heat stress during *in vitro* fertilization decreases fertilization success by disrupting anti-polyspermy systems of the oocytes. MolReprod Dev. 82(1): 36-47.

Sakatani, M., Alvarez, N.V., Takahashi, M. and Hansen, P.J. 2012. Consequences of physiological heat shock beginning at the zygote stage on embryonic development and expression of stress response genes in cattle. J. Dairy. Sci. 95:3080-3091.

Sakatani, M., Kobayashi, S. and Takahashi, M. 2004. Effects of heat shock on *in vitro* development and intracellular oxidative state of bovine pre implantation embryos. Mol. Reprod. Dev. 67: 77– 82.

Satrapa, R.A., Nabhan, T., Silva, C.F., Simões, R.A.L., Razza, E.M., Puelker, R.Z., Trinca, L.A. and Barros, C.M. 2011. Influence of sire breed (*Bos indicus* versus *Bos taurus*) and interval from slaughter to oocyte aspiration on heat stress tolerance of *in vitro*-produced bovine embryos. Theriogenology. 76: 1162-1167.

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. and Barros, C.M. 2013. Effects of heat stress on development, quality and survival of *Bos indicus* and *Bos taurus* embryos produce d *in vitro*. Theriogenelogy. 79:351-357.

Thouas, G.A., Korfiatis, N.A., French, A.J., Jones, G.M. and Trounson, A.O. 2001. Simplified technique for differential staining of inner cell mass and trophectoderm cells of mouse and bovine blastocysts. Reprod. Biomed. Online. 3(1): 25-29.

Ward, F., Rizos, D., Corridan, D., Quinn, K., Boland, M. and Lonergan, P. 2001. Paternal influence on the time of first embryonic cleavage post insemination and the implications for subsequent bovine embryo development *in vitro* and fertility in vivo. Mol. Reprod. Develop. 60: 47–55.