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Resistance of embryos from *Bos indicus* cattle during early stages of in vitro development to heat shock compared to embryos crossbred from crossbred cattle

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Abstract

There is evidence that the detrimental effects of heat stress on fertility are less pronounced in heat-tolerant breeds, due primarily to differences in thermoregulation. The current objective was to test the hypothesis that Nelore embryos (*Bos indicus*, I) at early stages of in vitro development are more resistant to heat shock than those from *Bos taurus* (T) oocytes fertilized with Nelore spermatozoa (crossbred *taurus* embryos, IT) or oocytes from crossbred animals (Nelore x *taurus*) fertilized with Nelore spermatozoa (crossbred *indicus* embryos, ITI). Embryos carrying different genotypes were exposed to a control temperature of 39°C continuously or heat shock of 41°C for 9 or 12 h at 12, 48, or 80 hours post-insemination (hpi) and 39°C thereafter. Overall exposure to 9 or 12 h heat shock at 12, 48 or 80 hpi did not affect cleavage rate in Nelore (I), Nelore vs crossbred (ITI), and Nelore vs *Bos taurus* (IT). For embryos exposed to 9 or 12 h heat shock at 12 hpi, the number of oocytes that developed to the blastocyst stage (blastocyst/oocyte) at Day 8 was: I (45/94, 47.9% at 39°C; 47/117, 40.2% at 41°C/9h; 43/115, 37.4% at 41°C/12h; $P > 0.05$) vs. ITI (69/150, 46%; 85/172, 49.4%; 48/164, 29.3%, respectively; $P < 0.05$); I (48/118, 40.7% at 39°C; 33/80, 41.2% at 41°C/12h; $P > 0.05$) vs. IT (34/68, 50%; 17/72, 23.6%, respectively; $P < 0.05$). The detrimental effect of heat shock on the Nelore breed was noted only when Nelore data at 12 hpi were pooled in a single dataset (140/269, 56.7% at 39°C; 89/209, 42.6% at 41°C/9h; 115/262, 43.9% at 41°C/12h; $P < 0.05$). For Nelore (I), crossbred (ITI), or *Bos taurus* (T) oocytes submitted to heat shock 48 or 80 hpi during 12h, there was no significant decrease in cleavage or blastocyst rates. However, oocytes from *Bos taurus* cows produced fewer blastocysts than those from *Bos indicus* cows ($P < 0.05$).

In conclusion, the results of this study indicate that Nelore embryos at an early stage of *in vitro* development are more resistant to heat shock than those from *Bos taurus* oocytes fertilized with Nelore spermatozoa. However, the resistance of Nelore embryos to heat shock was similar to those embryos from crossbred oocytes fertilized with Nelore spermatozoa.

Keywords: *Bos indicus*, pre-implantation embryo, heat shock, crossbred, genotype.

Introduction

Over 50% of the bovine population is located in the tropics and are subjected to a higher degree of stress than animals from temperate climates due to a combination of high temperatures and humidity, parasites, and inadequate nutrition. *Bos indicus* cattle are predominant in many countries in the Southern hemisphere, and Nelore and crossbred cattle are the most common beef breeds in Brazil (over 120 million) and are therefore important for future genetic improvement (ANUALPEC, 2006).

Reproductive processes in male and female mammals are very sensitive to disruption by hyperthermia with the most pronounced consequences being reduced quantity and quality of sperm production in males and decreased fertility in females (Hansen *et al*., 2001). It is well documented that exposure of female mammals to heat shock (HS) increases embryo mortality (Thatcher and Hansen, 1993). The major cause for reduced embryo survival induced by HS may be elicited by the deleterious effects of elevated temperatures on the development of both zygotes and embryos (Ealy *et al*., 1993).

The preimplantation period represents an extremely dynamic period of embryogenesis in which the embryo develops from a single quiescent cell under maternal control of genetic trancription to a highly metabolically-active group of cells under its own control. During this period, the embryo must go through several cell-cycle divisions, activate its own genome, undergo compactation, form a transport epithelium, differentiate into two distinct cellular types, and form a blastocoele cavity. These functions require precise regulation of several cellular functions such as homeostasis, metabolism, and gene expression (Lane, 2001).

Recently, Paula-Lopes *et al*. (2003) reported

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that embryos (≥ 9 cell stage) from a heat tolerant breed (Brahman) exposed to heat shock (41°C for 6 h) had better development to the blastocyst stage than embryos from heat-sensitive breeds (Holstein and Angus). Similar results have been found by others (Hernandez-Ceron *et al*., 2004) comparing Brahman and Romosinuano (a thermotolerant *Bos taurus* breed) with Angus.

The aim of the present work was to test the hypothesis that Nelore embryos (*Bos indicus*,) at early stages of *in vitro* development are more resistant to HS than those from *Bos taurus* oocytes fertilized with Nelore spermatozoa (crossbred *taurus* embryos) or oocytes from crossbred animals (Nelore x *taurus*) fertilized with Nelore spermatozoa (crossbred *indicus* embryos).

Materials and Methods

Oocyte recovery and selection

Unless otherwise stated, all reagents were from Sigma-Aldrich (St. Louis, MO, USA). Bovine ovaries were transported from the slaughterhouse to the laboratory in a 0.9% (w/v) NaCl solution at 30-37ºC in a thermocontainer. Imature cumulus-oocyte complexes (COCs) were aspirated from 2- to 6-mm follicles using an 18-gauge needle attached to a 10-ml syringe. Only oocytes with a homogeneous cytoplasm and surrounded by at least three layers of cumulus cells or just partially denuded were used (Ali *et al*., 2003). These COCs were then washed two times in Hepes-buffered Tissue Culture Medium-199 (TCM-199), supplemented with 0.25 mM of sodium pyruvate, 75 µg/ml gentamicin, and 10% (v/v) fetal calf serum (FCS, Gibco, BRL, Burlington, Ont., Canada).

In vitro maturation

Groups of 20-25 COCs were incubated in 90-µl droplets of maturation medium that consisted of TCM-199 medium Earle's salt TCM-199 supplemented with 0.25 mM of sodium pyruvate, 75 µg/ml gentamicin, 20 µl/ml FSH (Pluset®, Serovet, Roma, Italy), 2 IU/ml hCG (Profasi®, Serono Pharma), 1 µl/ml estradiol 17β, and covered with mineral oil for 22-24 h at 39ºC in an atmosphere of 5% (v/v) CO_2 in humidified air.

In vitro fertilization

In vitro fertilization (IVF) took place in 90-µl droplets of modified Tyrode's medium (IVF-TALP) supplemented with 0.6% (w/v) fatty-acid-free bovine serum albumin (BSA), 0.25 mM of sodium pyruvate, 75 µg/ml gentamicin, 11 µg/ml heparin, and 44 µg/ml PHE (2 mM penicillamine, 1 mM hypotaurine, 250 mM epinephrine). All experiments were carried out using frozen spermatozoa from the same Nelore bull (*Bos indicus*). Spermatozoa were thawed in a water bath at 37ºC for 30 s and purified in a discontinuous Percoll gradient. For the Percoll gradient, 2 ml of 90% (v/v) Percoll was layed under 2 ml of 45% (v/v) Percoll (using modified Tyrode's medium, SP-TALP) in a 15-ml centrifuge tube.The semen samples were added on top of the Percoll gradient and centrifuged at 900 X g for 25 min. After removal of the supernatant, spermatozoa were resuspended in IVF-TALP and added in 8-µl aliquots to each droplet containing oocytes (20-25 oocytes/droplet) for a concentration of 1 X 10^6 cells/ml. Gametes were co-incubated at 39ºC in 5% (v/v) CO_2 in air with saturated humidity for 12 to 14 h.

In vitro culture

In all experiments, embryo culture took place in droplets (90 µl) composed of TCM-199 medium (Earle's salt) co-cultured with granulosa cells, supplemented with 0.25 mM of sodium pyruvate, 75 µg/ml gentamicin and 10% (v/v) FCS (Gibco) under mineral oil at 39ºC in an atmosphere of 5% (v/v) CO_2 in humidified air. Between 12-14 h post-insemination, presumptive zygotes (20-25 per droplet) were denuded of surrounding cumulus cells by repeated pipetting and washed three times in Hepes-buffered TCM-199 medium, supplemented with 75 µg/ml gentamicin. Cleavage rate was assessed after 24 h of culture, and the number of embryos developing to the morula and blastocyst stage was assessed on Day 8. Culture medium was replaced after 48 and 120 h of culture.

Experimental groups

Experiment 1 – embryos exposed to heat shock at 12 hpi. In the first part of this experiment, oocytes from Nelore (I) and crossbred (IT) cows were randomly distributed across three treatment groups: Control group - oocytes were matured, fertilized with Nelore spermatozoa, and cultured at 39ºC and groups 9HS and 12HS - 12 h after insemination with Nelore spermatozoa, the presumptive zygotes were subjected to a 41°C heat shock (HS) for 9 or 12 h, respectively. After the HS period, embryos were maintained at 39°C until the end of the experiment. In the second part of this experiment, Nelore (*Bos indicus,* I) oocytes were compared to Holstein (*Bos taurus,* T) oocytes, except that the embryos were submitted to 12HS only, i.e., there was no group with 9HS, due to the difficulty in obtaining sufficient Holstein oocytes for both groups. Presumptive zygotes exposed to heat shock at 41ºC had the percentage of incubator CO_2 adjusted to 7% (v/v) to avoid pH changes due to lower solubility of CO_2 at high temperatures (Rivera and Hansen, 2001). Cleavage and blastocyst rates were calculated considering the total number of oocytes used for fertilization from Nelore x Crossbred cows (5 replicates), Nelore x *Taurus* cows (3 replicates), and Nelore females (8 replicates).

Experiment 2 – embryos exposed to heat shock

at 48 or 80 hpi. In the first part of this experiment, oocytes from Nelore (I) and crossbred (IT) cows were randomly distributed across four treatment groups. In the Control group, the oocytes were matured, fertilized with Nelore spermatozoa, and cultured at 39°C. In groups 9HS and 12HS, 48 h after fertilization with Nelore spermatozoa, the embryos were subjected to a 41°C HS for 9 or 12 h, respectively. In group 12HS80 (80 hpi), the embryos were subjected to 41°C for 12 h. After the HS period, embryos were maintained at 39°C until the end of the experiment. In the second part this experiment, Nelore (*Bos indicus,* I) oocytes were compared to Holstein (*Bos taurus,* T) oocytes, except that the embryos were submitted to 12HS only. Cleavage and blastocyst rates were calculated considering the total number of oocytes used for fertilization from Nelore x Crossbred (5 replicates), Nelore x *taurus* (4 replicates), and Nelore females (9 replicates).

Statistical analysis

Cleavage and blastocyst rates were analyzed using Proc GENMOD of SAS (SAS, 1999). Logistic regression analysis was used to study the breed effects and the experimental groups within each breed. Each treatment group was compared to the control group within each breed using orthogonal contrasts. For the experiments with 2 groups, the comparison of interest was: control vs. 9HS. For the experiments with 3 groups, the comparisons were: control vs. 9HS + 12HS and 9HS vs. 12HS. For the assays with 4 groups, the comparisons were: control vs. 9HS + 12HS + 12HS80, 9HS vs. 12HS + 12HS80, and 12HS vs. 12HS80.

Results

Experiment 1 – embryos exposed to heat shock at 12 hpi

Nelore (I) x Crossbred indicus (ITI). In I, adjustment of the logistic model showed a significant effect on cleavage rate when comparing Control vs. 12HS ($P < 0.03$). Orthogonal contrasts showed a significant difference when comparing Control vs. 9 HS and 12HS ($P < 0.02$) but not 9HS vs. 12HS ($P = 0.7$). There was no significant effect on blastocyst rate among groups (Table 1). In ITI, there was no significant difference in cleavage rate among groups ($P > 0.05$). For blastocyst yield, adjustment of the logistic model showed a significant effect when comparing Control vs. 12HS ($P < 0.002$). Analysis of the orthogonal contrasts showed that the blastocyst yield was not different when comparing Control vs. 9HS and 12HS ($P = 0.14$). Nevertheless, there was a significant difference among 9HS vs. 12HS ($P < 0.0001$; Table 1). There was a significant difference when comparing the cleavage rate among I vs. ITI within 9HS ($P < 0.004$) and within 12HS ($P < 0.03$). On the other hand, there was no difference ($P > 0.05$) in blastocysts rate among I vs. ITI, in the same experimental group (Table 1).

Table 1. Number of oocytes and cleavage and blastocyst rates from Nelore (I) and crossbred *indicus* (ITI) females cultured at 39°C (control) or submitted to HS (41°C) for 9h (9HS) or 12h (12HS) 12 hpi.

Group	Control (c)		9HS		12HS	
Breed	I	ITI	I	ITI	I	ITI
Oocytes	94	150	117	172	115	164
Cleavage	79 (84.0)	126 (84.0)	86 (73.5)	150 (87.2)	82 (71.3)	135 (82.3)
Blastocyst	45 (47.9)	69 (46.0)	47 (40.2)	85 (49.4)	43 (37.4)	48 (29.3)

HS = heat shock; hpi = hours post-insemination.
I: Cleavage, C > 12HS ($P < 0.03$); C > 9HS+12HS ($P < 0.02$) and 9HS=12HS ($P = 0.71$). Blastocyst, no differences among groups.
ITI: Cleavage, no difference among groups. Blastocyst, C > 12HS ($P < 0.02$); 9HS > 12HS ($P < 0.001$).
I vs. ITI: Cleavage, I < ITI, within 9HS ($P < 0.004$) and 12HS ($P < 0.03$). Blastocyst; no difference within groups.

Nelore (I) x Crossbred taurus (IT). In I, there was no significant difference in cleavage or blastocyst rates among groups ($P > 0.10$; Table 2). In IT, adjustment of the logistic model showed a significant effect when comparing Control vs. 12HS for both cleavage ($P < 0.01$) and blastocyst rates ($P < 0.001$). There was no difference ($P > 0.10$) in cleavage rate when comparing I vs. IT within the same experimental group. However, there was a significant difference in the blastocyst rate with I vs. IT within 12HS ($P < 0.02$).

Nelore (I). The results of all experiments performed with I embryos subjected to HS 12 hpi are summarized in Table 3. Adjustment of the logistic model showed that there was no significant difference in cleavage rates for the groups exposed to HS vs. Control. There was a significant effect difference in blastocyst rate in 9HS ($P < 0.04$) compared to the Control group, and there was a tendency in 12HS ($P < 0.06$) when compared to the Control group. Analysis of the orthogonal contrasts showed that there was a

significant difference for blastocyst rate among Control vs. 9HS and 12HS ($P < 0.02$). However, there was no difference among 9HS and 12HS ($P = 0.78$; Table 3).

Table 2. Number of oocytes and cleavage and blastocyst rates from Nelore (I) and crossbred *taurus* (IT) cultured at 39°C (control) or submitted to HS (41°C) for 12h (12HS) 12 hpi.

Group	Control (C)		12HS	
Breed	I	IT	I	IT
Oocytes	118	68	80	72
Cleavage	86	56	53	46
	(72.9)	(82.4)	(66.2)	(63.9)
Blastocyst	48	34	33	17
	(40.7)	(50.0)	(41.2)	(23.6)

HS = heat shock; hpi = hours post-insemination.
I: Cleavage and blastocyst rates, no significant difference among groups.
IT: Cleavage, C > 12HS ($P < 0.01$). Blastocyst, C > 12HS ($P < 0.001$).
I vs. IT: Cleavage, no significant difference within groups. Blastocyst, I > IT within 12HS ($P < 0.002$).

Table 3. Number of oocytes and cleavage and blastocyst rates from Nelore females cultured at 39°C (control) or submitted to HS (41°C) for 9h (9HS) or 12h (12HS) 12 hpi.

Groups	Control (C)	9HS	12HS
Oocytes	269	209	262
Cleavage	210	157	196
	(78.1)	(75.1)	(74.8)
Blastocyst	140	89	115
	(52.0)	(42.6)	(43.9)

HS = heat shock; hpi = hours post-insemination.
No significant difference for cleavage rate. Blastocysts: C > 9HS ($P < 0.04$), C=12HS ($P < 0.06$); C > 9HS+12HS ($P < 0.02$) and 9HS=12HS.

Experiment 2 – Embryos exposed to heat shock at 48 or 80 hpi

Nelore (I) x Crossbred indicus (ITI). In I, there was no effect of heat shock on cleavage or blastocyst rates. In ITI, there was no significant difference among groups for cleavage rate; however, interestingly, there was a significant increase in blastocyst rate in embryos submitted to 9HS when compared to the Control group. Analysis of the orthogonal contrasts showed a significant difference in 9HS vs. 12HS and 12HS80 ($P < 0.002$, Table 4). There was no significant difference ($P > 0.05$) in cleavage and blastocyst rate when comparing I vs. ITI within the same experimental group (Table 4).

Nelore (I) x Crossbred taurus (IT). In I, there was no effect of heat shock on cleavage or blastocyst rates. In IT, there was a significant effect on cleavage ($P < 0.05$) and blastocyst ($P < 0.05$) rate when comparing Control vs. 12HS (Table 5). There was a significant difference in cleavage rate when comparing I vs. IT within the Control group ($P < 0.02$). However, there was no difference in blatocyst rate among I vs. IT, in the same group (Table 5).

Nelore (I). The results of all experiments performed with I embryos subjected to HS 48 or 80 hpi are sumarized on Table 6. There was no significant difference in cleavage or blastocyst rate among groups.

Table 4. Number of oocytes and cleavage and blastocyst rates from Nelore (I) and Crossbred *indicus* (ITI) females cultured at 39°C (control), HS (41°C) for 9h (9HS) or 12h (12HS) 48 hpi and for 12h 80 hpi (12HS80).

Group	Control (C)		9HS		12HS		12HS80	
Breed	I	ITI	I	ITI	I	ITI	I	ITI
Oocytes	202	170	177	137	190	155	145	135
Cleavage	131	112	126	100	113	103	98	101
	(64.8)	(65.9)	(71.2)	(73.0)	(59.5)	(66.4)	(67.6)	(74.8)
Blastocyst	94	68	76	72	77	51	59	55
	(46.5)	(40.0)	(42.9)	(52.6)	(40.5)	(32.9)	(40.7)	(40.7)

HS = heat shock; hpi = hours post-insemination.
I: Cleavage and Blastocyst, no significant difference among groups.
ITI: Cleavage, no significant difference among groups. Blastocyst, C < 9HS ($P < 0.02$); 9HS > 12HS+12HS80 ($P < 0.002$).
I vs ITI: Cleavage and Blastocyst no signficant difference within groups.

Table 5. Number of oocytes and cleavage and blastocyst rates from Nelore (I) and Crossbred *taurus* (IT) females cultured at 39°C (control), HS (41°C) for 12h (12HS) 48 hpi and 80 hpi (12HS80).

Group	Control (C)		12HS		12HS80	
Breed	I	IT	I	IT	I	IT
Oocytes	195	89	151	111	159	75
Cleavage	130 (66.7)	72 (80.0)	109 (72.9)	76 (68.7)	118 (74.1)	56 (74.7)
Blastocyst	66 (33.5)	39 (43.2)	50 (33.1)	34 (30.3)	59 (37.1)	25 (33.3)

HS = heat shock; hpi = hours post-insemination.
I: Cleavage and Blastocyst, no significant difference among groups.
IT: Cleavage, C > 12HS ($P < 0.048$). Blastocys, C > 12HS ($P < 0.05$).
I vs IT: Cleavage, IT > I in C ($P < 0.01$). Blastocyst, no significant difference among groups.

Table 6. Number of oocytes and cleavage and blastocyst rates from Nelore females cultured at 39°C (control), HS (41°C) for 9h (9HS) or 12h (12HS) 48 hpi and for 12h 80 hpi (12HS80).

Groups	Control (C)	9HS	12HS	12HS80
Oocytes	493	471	448	498
Cleavage	305 (61.9)	290 (61.6)	274 (61.2)	314 (63.01)
Blastocyst	192 (39.0)	154 (32.7)	172 (38.4)	184 (37.0)

HS = heat shock; hpi = hours post-insemination.
Cleavage and Blastocyst, no significant difference among groups.

Discussion

In the present work, there was reduced development to the blastocyst stage in *Bos indicus* (I), crossbred (*Bos indicus* vs. *Bos taurus*, IT), or *Bos taurus* (T) oocytes fertilized with Nelore (*Bos indicus*) spermatozoa submitted to heat shock (41°C) 12 h post insemination. However, in Nelore embryos the decline in blastocyst rate was observed only when all 12 hpi data were combined. The results of HS at 48 and 80 hpi indicated that as Nelore (I) and crossbred indicus (ITI) embryos advanced in development they become more resistent to HS. Neverthless, there was a reduction in both cleavage and blastocyst rates in crossbred *bos taurus* (IT) embryos submitted to HS 48 and 80 hpi, indicating that IT embryos are less resistant to HS than I or ITI. These results are in accordance with those reported by Edwards and Hansen (1996), who observed in Holstein heifers that exposure of oocytes to 41°C for 12 h reduced subsequent development of oocytes to the blastocyst stage (30% vs. 10% for oocytes cultured at 39 and 41°C, respectively). Similar findings were noted in another experiment done by Edwards and Hansen (1997, 35% and 18% for oocytes cultured at 39°C and 41°C, respectively). Additionally, Riviera and Hansen (2001) found that HS of 41°C applied during fertilization and at one- and two-cell stages disrupt embryonic development.

In vitro experiments in which bovine embryos were exposed to temperatures similar to those experienced by heat-stressed, lactating dairy heifers in Florida (USA; temperature ranging from 38.5 to 40.5°C, throughout 8 days of culture in vitro; Riviera and Hansen, 2001) or in southeast Queensland (Australia, temperature ranging from 39.5 to 41.0°C, during the first 48 h of *in vitro* culture; Sugiyama *et al*., 2003) have shown reduced development to the blastocyst stage.

In most mammals, the effects of HS are more evident from estrus until artificial insemination (Cavestany *et al*., 1985; Putney *et al*., 1989; Al-Katanani *et al*., 2001) and during the first embryo cleavages (Dutt, 1963; Tompkins *et al*., 1967; Putney *et al*., 1988; Ealy *et al*., 1993). The effects of HS on embryonic mortality decrease as pregnancy progresses, and they are minimal between Days 3 and 5 of pregnancy in ewes (Dutt, 1963) and Day 3 in cows (Ealy *et al*., 1993) and sows (Tompkins *et al*., 1967).
There are at least two possibilities to explain why embryos become more thermotolerant as development proceeds. One possibility is that the increased cell number allows the embryo to survive the loss of a fraction of its cells. If one assumes, hypothetically, that the effect of HS is to alter the function of 50% of the blastomeres, a two-cell embryo would be left with only one blastomere to form a viable embryo, whereas a morula or blastocyst would have 30-50 viable blastomeres to continue development. A second possibility is that embryos acquire mechanisms of thermo-protection during development (Edwards and Hansen, 1997).

In most cellular types studied until now, biochemical mechanisms related to thermo-protection comprise some members of the family of heat shock proteins (HSP, for example HSP-70) that are produced in response to increased temperatures, and some antioxidants such as glutathione. HSP-70 seems to protect cells from HS by means of rearranging damaged proteins and protecting the rRNA (Duncan and Hershey, 1989; Nover and Scharf, 1991), while glutathione diminish the effects of free radicals (Loven, 1988). These molecules may be involved in the embryo acquirement of thermo-resistance, since the oocytes become more resistant to heat shock when they are injected with HSP-70 mRNA (Loven, 1988). Furthermore, addition of glutathione to culture medium partially inhibits the HS effects on mice (Aréchiga *et al*., 1995) and bovine embryos (Ealy *et al*., 1992).

Paula-Lopes *et al*. (2001) compared the resistance to HS among *Bos indicus* (Brahman) and *Bos taurus* (Holstein and Angus) breeds. The percentage of embryos submitted to HS (41°C, 96 hpi for 6 h) that developed to blastocyst was significantly higher in Brahman (15.3 ± 4.3) than in Holstein (0.0 ± 5.1) and Angus (2.6 ± 5.7) embryos. In a more recent study, the same group (Paula-Lopes *et al*., 2003) confirmed that exposing embryos at more than the eight-cell stage to HS of 41°C for 6 h reduced development to the blatocyst stage. However, the negative effect of HS on development of embryos to the blastocyst stage was less pronounced for embryos from Brahman cows than for embryos from Holstein or Angus cows.

In the present work, the deleterious effects of HS were only detected in Nelore and crossbred *indicus*, when the oocytes were exposed to high temperatures (41°C for 12 h) 12 hpi. For the *Bos taurus* oocytes fertilized with Nelore spermatozoa, the blastocyst rate declined when the HS was applied at 12 ($P < 0.05$), 48 ($P < 0.05$), and 80 hpi ($P < 0.06$). Therefore, there were breed differences in response to HS for embryos at the 2-8 cell stage (48 hpi) or later stages (80 hpi). This indicated that Nelore or crossbred oocytes fertilized with Nelore spermatozoa are more resistant to HS than Holstein oocytes, since the decline in blastocyst rate was observed only when HS was applied at 12 hpi whereas the deleterious effects of HS on embryos from Hostein oocytes were observed at 12 hpi and even at later stages of development (i.e, 48 and 80 hpi) when the embryos should have acquired mechanisms of thermo-protection (Edwards and Hansen, 1997).

In a recent experiment, embryos from Angus or Nelore cows, produced using oocytes obtained by oocyte pick up procedures, were exposed to a culture temperature of 41°C for 12 h beginning 96 h after fertilization. Thereafter, embryos were transferred at the blastocyst stage to crossbred recipient heifers. The pregnancy rates after transfer were: 29.4% (15/51) for non-stressed Nelore embryos, 29.0% (11/38) for stressed Nelore embryos, 21.4% 196 (6/28) for non-stressed Angus embryos, and 7.1% (1/14) for stressed Angus embryos (Sartorelli *et al*., 2006). These results and a previous report (Eberhardt *et al*., 2005) clearly indicate that Nelore embryos are better able to survive HS at early stages of development and more capable of originating pregnancies following HS than Angus embryos (*Bos taurus*).

The fact that embryo genotype determines resistance to HS leads to the question as to whether embryos sired by thermotolerant breeds exhibit the same resistance to HS. In the present work, fertilization of Holstein oocytes with Nelore spermatozoa did not prevent the decrease in blastocyst production. Since the effects of HS were not tested in oocytes fertilized with Hostein spermatozoa, it is not possible to tell if the use of Nelore spermatozoa in Holtein oocytes may have attenuated the deleterious effect of HS on blastocyst rate. However, in an experiment designed to test whether the ability of Brahman embryos (*Bos indicus*) to resist the deleterious effects of HS was a result of the genetic and cellular contributions from the oocyte, spermatozoa, or a combination of both, Block *et al*. (2002) found that the contribution of the oocyte plays a more crucial role in the ability of Brahman embryos to resist effects of HS than the contribution of the spermatozoa. Embryos were more affected by HS if produced using Holstein oocytes than if using Brahman oocytes. In contrast, breed of sire had no effect on the thermal resistance of embryos produced using Angus and Brahman spermatozoa to fertilize Holstein oocytes. Similar results regarding the contribution of oocyte were obtained by Eberhardt *et al*. (2005) using Nelore or Holstein oocytes fertilized with Nelore or Angus semen, i.e., the deleterious effect of HS (decline in blastocyst rate) was less pronounced in Nelore when compared to Holstein or crossbred embryos (*indicus* vs *taurus*). However, in contrast to the previous report, oocytes from Holstein cows were more resistant to HS when fertilized with Nelore as compared to Angus semen, suggesting that the breed of the sire (*indicus*) influenced the tolerance of the embryo to HS (Eberhardt *et al*., 2005). Such a result suggests that it should be possible to increase pregnancy rates in heat-stressed cows by insemination with semen from zebu bulls. In fact, Pegorer *et al*. (2007) evaluated the effect of sire and sire breed on conception rates of Holstein cows during the summer in Brazil and found that the use of Gyr bulls (*Bos indicus*) increased pregnancy rates when compared to Holstein bulls.

In conclusion, the results of the present study indicate that Nelore embryos (*Bos indicus*) at early stage of *in vitro* development, are more resistant to HS than those from *Bos taurus* oocytes fertilized with Nelore spermatozoa. However, the resistance of Nelore embryos to HS was similar to those embryos from crossbred oocytes (*Bos indicus* vs *Bos taurus*) fertilized with Nelore spermatozoa.

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