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Influence of sire and sire breed (Gyr versus Holstein) on establishment of pregnancy and embryonic loss in lactating Holstein cows during summer heat stress

Marcelo F. Pegorer [a], José L.M. Vasconcelos [b], Luzia A. Trinca [c], Peter J. Hansen [d], Ciro M. Barros [a,*]

[a] *Department of Pharmacology, Institute of Bioscience, University of São Paulo State (UNESP), Botucatu, São Paulo, Brazil*
[b] *Department of Animal Production, FMVZ-UNESP, Botucatu, São Paulo, Brazil*
[c] *Department of Biostatistic, Institute of Bioscience, University of São Paulo State (UNESP), Botucatu, São Paulo, Brazil*
[d] *Department of Animal Sciences, University of Florida, Gainesville, FL, USA*

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Abstract

Heat stress has negative effects on pregnancy rates of lactating dairy cattle. There are genetic differences in tolerance to heat stress; *Bos taurus indicus* (*B. t. indicus*) cattle and embryos are more thermotolerant than *Bos taurus taurus* (*B. t. taurus*). In the present study, the effects of sire and sire breed on conception and embryonic/fetal loss rates of lactating Holstein cows during the Brazilian summer were determined. In Experiment 1, cows ($n = 302$) were AI after estrus detection or at a fixed-time with semen from one Gyr (*B. t. indicus*) or one Holstein sire (*B. t. taurus*). Pregnancy was diagnosed 80 days after AI. In Experiment 2, cows ($n = 811$) were AI with semen from three Gyr and two Holstein sires. Pregnancy was diagnosed at 30–40 and at 60–80 days after AI. Cows diagnosed pregnant at the first examination but non-pregnant at the second were considered as having lost their embryo or fetus. Data were analyzed by logistic regression. The model considered the effect of sire within breed, sire breed, days postpartum, period of lactation, and AI type (AI after estrus versus fixed-time). There was no effect of the AI type, days postpartum or milk production on conception or embryonic loss rates. The use of Gyr bulls increased pregnancy rate when compared to Holstein bulls [9.1% (60/657) versus 5.0% (23/456), respectively, $P = 0.008$; data from Experiments 1 and 2 combined]. Additionally, in Experiment 2, cows inseminated using semen from sire #4 (Gyr) had lower embryonic loss (10%) when compared with other *B. t. indicus* (35.3% and 40%) or *B. t. taurus* sires (18.2% and 38.5%, $P = 0.03$). In conclusion, the use of *B. t. indicus* sires may result in higher conception rates in lactating Holstein cows during summer heat stress. Moreover, sire can affect embryonic loss and selection of bulls according to this criterion may result in higher parturition rates in lactating Holstein cows.

Keywords: Heat stress; Artificial insemination; Pregnancy; *Bos indicus*; *Bos taurus*

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1. Introduction

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 [1]. Heat stress is a particularly severe problem in lactating dairy cattle. Over 50% of the bovine population is located in the tropics; it has been estimated that heat stress causes severe economic loss in approximately 60% of the dairy farms around the world [2]. The magnitude of the effect of heat stress on reproduction in dairy cattle is increasing as milk yield

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* Corresponding author. Fax: +55 14 3815 3744.
E-mail address: cmbarros@ibb.unesp.br (C.M. Barros).

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makes cows more susceptible to the deleterious effects of heat stress [3,4].

As compared to European breeds (*Bos t. taurus*), zebu cattle (*Bos t. indicus* [5]) experience less severe reduction in feed intake [6–9], growth rate [10,11], milk yield [12], and reproductive function [13–15] in response to heat stress. Recently, Paula-Lopes et al. [16] reported that cultured embryos (≥9 cells) from a heat-tolerant breed (Brahman) were more likely to develop to the blastocyst stage after exposure to heat shock (41 °C for 6 h) than embryos from heat-sensitive breeds (Holstein and Angus). Similar results have been found by others, comparing zebu (Nelore) with European (Holstein [17]) or crossbred breeds (*indicus* versus *taurus* [18]) and by comparing Brahman and thermotolerant *B. t. taurus*, Romosinuano, as compared to Angus [19].

Given that one action of elevated body temperature is to block embryonic development [20,21], perhaps embryos derived by insemination using *B. t. indicus* semen would be more thermotolerant than embryos using *B. t. taurus* semen. Use of *B. t. indicus* sires might also improve fertility because Holsteins are experiencing inbreeding [22] and there is evidence for heterosis effects on embryonic development [23]. Therefore, the objective of the present work was to determine whether insemination of lactating Holstein cows during the Brazilian summer with semen from Gyr, a *B. t. indicus* breed, would have higher fertility than cows inseminated with Holstein semen.

2. Materials and methods

2.1. Location and experimental animals

Experiments were conducted at a dairy farm located in Inhauma, Minas Gerais, Brazil (latitude 19°29′28″S, longitude 44°23′23″W) during the summer of 2003 and 2004. Lactating Holstein cows (*B. t. taurus*) were maintained in a free-stall barn containing sprinklers and fans that were turned on during the hottest hours of the day (10:00–15:00 h). Cows were inseminated with frozen-thawed semen from a total of seven sires (four Gyr and three Holstein). Semen was processed at Central Bela Vista (Pardinho, São Paulo, Brazil), and each straw of semen had at least 15 million sperm at the time of freezing.

2.2. Experiment 1

Lactating Holstein cows (milk production = 20.3 ± 0.33 kg/day; period of lactation = 191 ± 4.8 days, mean ± S.E.M.) were submitted randomly to three different AI protocols during the summer of 2003. For Protocol 1 (control, $n = 69$), cows were artificially inseminated 12 h after estrus detection, whereas fixed-time AI was used for Protocol 2 (Ovsynch/P4, $n = 116$) and Protocol 3 (Presynch/P4, $n = 117$). Three technicians performed all inseminations, and the cows were inseminated with frozen-thawed semen from either one Gyr sire (*B. t. indicus*, sire number 1, $n = 147$) or one Holstein sire (*B. t. taurus*, sire number 2, $n = 155$). Pregnancy was diagnosed by rectal palpation 80 days after insemination.

Details of synchronization protocols are as follows. For Protocol 2, cows received, at a random stage of the estrous cycle, an intravaginal device containing 1.9 g of progesterone (CIDR®, Pfizer, Cambridge, USA) and GnRH (100 μg of gonadorelin, Fertagyl®, Intervet, Boxmeer, Netherlands, im, Day 0). Seven days later, the CIDR® was removed and animals were treated with a PGF2α analog (150 μg of D-cloprostenol, Prolise®, ARSA, Buenos Aires, Argentina, im, Day 7). At 48 h after PGF2α, cows received a second dose of GnRH (100 μg, Day 9), and AI was performed 16 h afterwards (Day 10). In Protocol 3 (Presynch/P4), cows received a CIDR® device at a random stage of the estrous cycle for 7 days (inserted at Day 10). At CIDR® removal (Day 3), cows were treated with PGF2α (150 μg of D-cloprostenol, im) and 1.0 mg of estradiol cypionate (ECP®, Pfizer, Cambridge, MA, USA). At Day 0, cows received a new CIDR® device, which was removed 7 days later after administration of D-cloprostenol (150 μg, im, Day 7). Cows were treated with GnRH (100 μg of gonadorelin, im) on Day 9, and AI was performed 16 h afterwards (Day 10).

Rectal temperature was determined in a subsample of cows inseminated with Gyr ($n = 44$) and Holstein ($n = 44$) semen, between 10:00 and 15:00 h on Days −1, 0, and 1 relative to AI. Body condition score (0–5 point scale [24]) was ascertained at the start of FTAI protocols.

During the month of January 2003, mean (±S.E.M.) minimal, average and maximal temperatures were 19.5 ± 0.3, 23.2 ± 0.2, 28.4 ± 0.5 °C, respectively, and the level of humidity was 78.8 ± 2.5%; for February 2003, the corresponding data were 18.0 ± 0.5, 23.7 ± 0.4, 30.7 ± 0.7 °C and 67.8 ± 4.7% relative humidity. Temperature and humidity data were recorded at the National Institute of Meteorology (INMET), located in Sete Lagoas (Minas Gerais), approximately 20 km from the farm.

2.3. Experiment 2

Lactating Holstein cows (milk production = 27.7 ± 0.24 kg/day; duration of lactation = 270 ± 9.5 days) were submitted randomly to insemination after estrus detection (control, $n = 364$) or at a fixed-time after insemination using the Ovsynch/P4 protocol as for Experiment 1 ($n = 447$), during the summer of 2004. Three technicians performed all inseminations, and the cows were inseminated with frozen-thawed semen using a total of three Gyr sires (number 3, $n = 172$; number 4, $n = 161$; number 5, $n = 177$) and two Holstein sires (number 6, $n = 169$; number 7, $n = 132$). Pregnancy was diagnosed by ultrasonography 30–40 days after AI, and confirmed 60–80 days after AI (by transrectal palpation). Cows diagnosed pregnant at the first examination but non-pregnant at the second were considered as having lost their embryo or fetus.

Body condition score (0–5 point scale [24]) was ascertained at the beginning of FTAI protocols. Body condition score was not determined for animals inseminated after estrus detection (control group).

Minimal, average and maximal temperatures and humidity were 18.9 ± 0.2, 22.9 ± 0.2, 27.7 ± 0.4 °C and $75.5 \pm 2.1\%$ during January 2004 and were 18.3 ± 0.2, 21.9 ± 0.3, 27.4 ± 0.5 °C and $80.7 \pm 1.8\%$ during February (data from same source as in Experiment 1).

2.4. Statistical analysis

Results were analyzed by logistic regression using the GENMOD procedure of the Statistical Analysis System [25]. The mathematical model considered the effect of sire nested within breed, sire breed, inseminator, milk production 7 days before AI (above average versus below average), period of lactation from parturition until AI (above average versus below average), AI type (Presynch/P4, Ovsynch/P4 and AI after estrus detection), body condition score, and rectal temperature. Body condition score was considered a class variable (2.0 and 3.0), except for the control group, in which body condition score was not measured. Rectal temperature and period of lactation were considered as continuous variables.

3. Results

3.1. Experiment 1

There was no difference in conception rate per AI between cows inseminated after estrus detection (4.3%; $n = 69$) as compared to those inseminated using the Ovsynch/P4 protocol (7.7%; $n = 117$) or those inseminated using the Presynch/P4 protocol (8.6%; $n = 116$; $P = 0.39$, Table 1). Cows inseminated with the Gyr sire ($n = 147$) had a higher pregnancy rate at 80 days after insemination than those artificially inseminated with the Holstein sire ($n = 155$, $P = 0.047$). Milk production, period of lactation, and BCS did not influence conception rate per AI at Day 80.

Rectal temperatures for cows inseminated with the Holstein sire ($n = 44$) were 39.6 ± 0.1, 39.2 ± 0.1 and 39.1 ± 0.1 °C (mean ± S.E.M.) on Days −1, 0 and +1 relative to AI, respectively. For cows inseminated with the Gyr sire ($n = 44$), rectal temperatures were 39.4 ± 0.1, 39.1 ± 0.1 and 38.9 ± 0.1 °C on Days −1, 0 and +1, respectively. The rectal temperature on the day of AI had a negative effect over pregnancy rate/AI, i.e., the cows with highest temperatures had the lowest pregnancy rates ($n = 88$, $P = 0.04$). There was no significant effect for temperatures on Days −1 or +1.

3.2. Experiment 2

Milk production, period of lactation, BCS, breed of sire (Gyr versus Holstein) or AI protocol (Ovsynch/P4 versus AI after estrus) did not significantly influence pregnancy rate/AI at Days 30–40 (Table 2). However, cows inseminated with semen from one sire (number 4) had lower pregnancy loss when compared to others ($P = 0.043$, Table 3).

Table 1
Conception rates, body condition scores (BCS), milk production and calving to conception intervals in Holstein cows submitted to various AI protocols with semen from two sires (Experiment 1)

	AI after estrus	Ovsynch/P4	Presynch/P4	Total
Sire 1 (Gyr)	2/32 (6.2%)	5/56 (8.9%)	9/59 (15.2%)	16/147 (10.9%)[a]
Sire 2 (Holstein)	1/37 (2.7%)	4/61 (6.5%)	1/57 (1.7%)	6/155 (3.9%)
Total conception rate (80 days post AI)	3/69 (4.3%)	9/117 (7.7%)	10/116 (8.6%)	22/302 (7.2%)
Total BCS	–	3.0 ± 0.03	3.1 ± 0.03	3.04 ± 0.02
Milk production[b] (kg/day)	21.2 ± 0.8	20.3 ± 0.6	19.6 ± 0.5	20.2 ± 0.3
Total calving to conception interval (days)	199 ± 9.4	190 ± 8.1	189 ± 7.7	191 ± 4.8

[a] Different from sire 2 ($P = 0.047$).

[b] Average milk production during 7 days before AI.

Table 2
Conception rates (30–40 and 60–80 days after insemination), body condition scores (BCS), milk production and calving to conception intervals in Holstein cows submitted to various AI protocols with semen from Gyr ($n = 3$) or Holstein ($n = 2$) sires (Experiment 2)

	AI after estrus (%)		Ovsynch/P4 (%)		Total[a] (%)	
	30–40 days	60–80 days	30–40 days	60–80 days	30–40 days	60–80 days
Sire 3 (Gyr)	11/84 (13.1)	6/84 (7.1)	14/88 (15.9)	9/88 (10.2)	25/172 (14.5)	15/172 (8.7)
Sire 4 (Gyr)	8/70 (11.4)	4/70 (5.7)	9/91 (9.9)	7/91 (7.7)	17/161 (10.5)	11/161 (6.8)
Sire 5 (Gyr)	10/79 (12.6)	9/79 (11.4)	10/98 (10.2)	9/98 (9.2)	20/177 (11.3)	18/177 (10.2)
Sire 6 (Holstein)	5/77 (6.5)	4/77 (5.2)	6/92 (6.5)	5/92 (5.4)	11/169 (6.5)	9/169 (5.3)
Sire 7 (Holstein)	6/54 (11.1)	3/54 (5.5)	7/78 (8.9)	5/78 (6.4)	13/132 (9.8)	8/132 (6.1)
Total conception rate	40/364 (10.9)	26/364 (7.1)	46/447 (10.3)	35/447 (7.8)	86/811 (10.6)	61/811 (7.5)
Total BCS	3.1 ± 0.02		3.1 ± 0.02		3.1 ± 0.01	
Milk production[b] (kg/day)	27.1 ± 0.5		27.3 ± 0.4		27.2 ± 0.3	
Total calving to conception interval (days)	283 ± 7.5		259 ± 7.0		270 ± 5.1	

[a] No significant difference in conception rates among sires or between protocols.
[b] Average milk production during 7 days before AI.

Pregnancy loss was higher in cows with milk production below the average [<27 kg/day; 45.9% ($n = 17/37$) versus 15.7% ($n = 8/51$), >27 kg/day; $P < 0.05$].

3.3. Analysis of pooled data from Experiments 1 and 2

When data for conception rate at 60–80 days after AI from Experiments 1 and 2 were pooled, conception rate was higher for cows inseminated with Gyr semen as compared to cows inseminated with Holstein semen ($P = 0.008$, Table 3). Additionally, cows inseminated with one sire (number 4, Gyr) had lower embryonic/fetal loss when compared to other *B. t. indicus* or *B. t. taurus* sires.

Table 3
Conception rates (60–80 days after AI) and embryonic/fetal loss between first and second pregnancy diagnosis (30–40 and 60–80 days after AI, respectively) in Holstein cows inseminated with semen from Gyr ($n = 4$) or Holstein ($n = 3$) sires (Experiments 1 and 2 combined)

	Conception rate (%)	Embryonic/fetal loss (%)
Gyr sires		
1	16/147 (10.9)	–
3	15/172 (8.7)	10/25 (40)
4	11/161 (6.8)	06/17 (35.3)
5	18/177 (10.2)	02/20 (10)[a]
Sub-total	60/657 (9.1)[b]	18/62 (29)
Holstein sires		
2	06/155 (3.9)	–
6	09/169 (5.3)	02/11 (18.2)
7	08/132 (6.1)	05/13 (38.4)
Sub-total	23/456 (5.0)	07/24 (29.2)

[a] Different from the other Gyr or Holstein sires ($P = 0.036$).
[b] Conception rates differ between Gyr and Holstein sires ($P = 0.008$).

4. Discussion

Analysis of pooled data from Experiments 1 and 2 demonstrated that insemination of Holstein cows with semen from Gyr bulls increased conception rate compared to insemination with Holstein semen. Additionally, embryos sired by one bull were more likely to proceed through gestation without pregnancy loss than embryos sired by the other bulls. There are two possible explanations for the higher pregnancy rate in cows inseminated with Gyr semen as compared to those sired with Holstein semen. One possibility is that the higher pregnancy rate in crossbred embryos was because these embryos were more resistant to maternal hyperthermia. The other possibility is that heterosis caused by mating Holstein cows to Gyr bulls resulted in an embryo with superior ability for development.

Four studies have demonstrated that *B. t. indicus* embryos are more resistant to exposure to elevated temperature in culture than *B. t. taurus* embryos [16,18,19,26]. In a recent unpublished experiment (Sartorelli and Barros), embryos from Angus or Nelore cows were cultured or not cultured at 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% (6/28) for non-stressed Angus embryos and 7.1% (1/14) for stressed Angus embryos. We inferred that *B. t. indicus* embryos are better able to survive elevated temperature at early stages of development and more capable of originating pregnancies following heat stress than *B. t. taurus* embryos.

There is a discrepancy in the literature, however, as to whether crossbred embryos produced by mating of *B. t. indicus* and *B. t. taurus* have increased resistance to elevated temperature. Embryos produced by insemination of Brahman oocytes with Angus sperm were more thermotolerant than embryos produced by insemination of Holstein oocytes with Angus semen [27]. In contrast, there were no differences in thermotolerance between Holstein × Brahman embryos and Holstein × Angus embryos. These results were interpreted to indicate that the contribution of the oocyte has a more crucial role in determining the genetic ability of an embryo to resist effects of heat shock than the contribution of the spermatozoa. In contrast, Eberhardt et al. [26] observed that embryos produced by insemination of Holstein oocytes with Nelore semen were more resistant to elevated temperature in culture as compared to embryos produced by insemination of Holstein embryos with Angus semen. This result suggests that the breed of the sire does influence the genetic ability for thermotolerance of the embryo.

Another interpretation of the present results is that the higher pregnancy rates observed in Holstein cows inseminated with Gyr as compared to Holstein sires was caused by beneficial effects of heterosis on embryonic development. There was no evidence for heterosis in early embryonic development when comparing different dairy and beef breeds [28]. However, Boediono et al. [23] found heterosis when comparing purebred (Japanese Black) and hybrid (Japanese Black × Holstein) in vitro-derived embryos.

Pregnancy rates were similar comparing cows AI after estrous detection or AI at fixed-time. Additionally, there was no difference in the pregnancy rates when comparing the two fixed-time protocols (Ovsynch/P4 versus Presynch/P4). These results were similar [29,30,32,33], i.e., pregnancy rates did not differ when comparing AI after estrus versus AI at fixed time protocols, or different [31,32,34,35] from those obtained by others, indicating that the use of protocols for fixed-time AI to facilitate management of cows, can be advantageous, depending on many things, including body condition score, climate, and reproductive management of the animals [33–37].

Embryonic loss varied from 10% to 40% (Experiment 2). These results seemed higher than those reported by others (10–21% [31,38–40], although there are reports of elevated embryonic lost in lactating dairy cows (60% [37]). Additionally, the cows with a milk production below the average ($<27.7 \pm 0.24$ kg/day) had the highest embryonic lost. According to Chebel et al. [38], dairy cows with low milk production are those that could have acquired infection of the mammary gland. The presence of clinical or subclinical mastitis from AI until pregnancy diagnosis was associated with an increased risk of embryonic loss [31,41–43]. One interesting finding in the present study was that cows inseminated using semen from one sire had lower embryonic loss when compared with other *B. t. indicus* or *B. t. taurus* sires (Experiment 2). Therefore, the sire can affect embryonic loss and selection of bulls according to this criterion may result in higher parturition rates in lactating Holstein cows.

In conclusion, the use of *B. t. indicus* sires may increase conception rates in lactating Holstein cows during summer heat stress. It remains to be seen whether this advantage would also occur in the cool weather, i.e., be the result of heterosis, or is limited to periods of heat stress, i.e., is related to embryonic resistance to elevated temperature.

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