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Differential expression of members of the *IGF* system in OPU-derived oocytes from Nelore (*Bos indicus*) and Holstein (*Bos taurus*) cows

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ABSTRACT

The insulin-like growth factor (*IGF*) system is related to quality of oocytes and embryos. The aim of this study was to investigate the mRNA levels of *IGF1* and *IGF2* and their receptors, *IGFR1* and *IGFR2*, as well as *IGFBP2*, *IGFBP4*, and *PAPP-A* in oocytes from Nelore compared to Holstein cows. Pools of oocytes (20 oocytes/pool) from Nelore ($n = 8$ pools) and Holstein ($n = 4$ pools) were obtained via ovum pick-up (OPU, 10 sessions) and *cumulus* cells and zona pellucida were removed. The pools were submitted to total RNA extraction. Expression of members of the *IGF* system was assessed by real time RT-PCR. The mRNA expression of *IGF1* and *IGF2*, *IGFR1* and *IGFR2*, *IGFBP2* and *IGFBP4* was significantly higher ($P < 0.01$) in oocytes from Holstein whereas the expression of *PAPP-A* was significantly higher ($P < 0.05$) in oocytes from Nelore cows. The high *PAPP-A* expression and the low expression of *IGFBP2* and *IGFBP4* are associated with more efficient degradation of *IGFBP*s, which results in greater bioavailability of *IGF* in Nelore oocytes when compared to the Holstein.

1. Introduction

Reproductive performance in the bovine is dependent on many factors amongst them, oocyte quality and the ability of the oocyte to be fertilized, affecting blastocyst yields from in vitro production systems (Rizos et al., 2002; Leroy et al., 2008). Furthermore, there is a difference in reproductive performance between cattle breeds with beef breeds having better fertility than dairy breeds (Oltenacu et al., 1991; Santos et al., 2004).

Insulin-like growth factors (*IGF*s) are present in the follicular fluid (Spicer and Echternkamp, 1995), in uterine secretions (Geisert et al., 1991), in the female reproductive tract (Schmidt et al., 1994; Gabler et al., 1997) and in oocytes and embryos (Yoshida et al., 1998; Lonergan et al., 2000; Yaseen et al., 2001; Lonergan et al., 2003). Both type 1 and 2 *IGF*s and their receptors (*IGFR1* and *2*) are expressed in bovine oocytes (Yoshida et al., 1998) and embryos (Lonergan et al., 2000, 2003). The transport and function of *IGF*s are modulated by at least six binding proteins (*IGFBP*s) present in extracellular fluids in early stages of embryo development (Kaye and Harvey, 1995; Winger et al., 1997) and their expression has been confirmed in bovine embryos (Winger et al., 1997; Sawai et al., 2005). The *IGFBP* degradation occurs by the proteolytyc action of the pregnancy-associated plasma protein A (*PAPP-A*) and the increased expression of *IGFBP* is related with lower bioavailability of *IGF* (Monget et al., 2003).

The *IGF* system acts synergistically to FSH in folliculogenesis to promote follicle growth and estradiol production (Fortune et al., 2004). At the time of deviation, the dominant follicle increases estradiol concentration, reduces FSH

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levels and through the interaction with the *IGF* system, the dominant follicle becomes the only one capable of using low concentrations of FSH to support its final growth (Ginther et al., 2001; Fortune et al., 2004). Moreover, the future dominant follicle has higher *PAPP-A* synthesis and is able to maintain lower concentrations of *IGFBP4* and *5* (Mazerbourg et al., 2001; Rivera and Fortune, 2001; Fortune et al., 2004), which results in higher availability of *IGF1* for follicular growth, enhancing the effects of FSH and estradiol synthesis (Fortune et al., 2004).

The beneficial effects of *IGF1* on embryo development have been described in various species. A greater number of morulae and blastocysts, as well the reduction of apoptosis were observed in several species (Herrler et al., 1998; Lighten et al., 1998; Fabian et al., 2004), particularly in bovine (Block et al., 2008; Bonilla et al., 2011), was observed after adding *IGF1* to the in vitro culture medium. Additionally, several studies have demonstrated that *cumulus*–oocyte complexes treated with *IGF1* showed increased *cumulus* cells expansion and improvement of nuclear maturation (reviewed by Velazquez et al., 2009).

Given the remarkable importance of *IGF* system in oocyte development and adaptive differences between breeds, we aimed to verify the mRNA expression of members of the *IGF* system (*IGF1*, *IGF2*, *IGFR1*, *IGFR2*, *IGFBP2* and *IGFBP4*) and *PAPP-A* in OPU-derived oocytes from Nelore (*Bos indicus*) and Holstein (*Bos taurus*) cows.

2. Materials and methods

The experiment was conducted on private farms in Santa Cruz do Rio Pardo in the central-west region of the state of Sao Paulo, Brazil (latitude 238110 S, longitude 498230 W), during the breeding season, from January to April 2010. Nelore and Hosltein cows (body condition scores from 2.0 to 3.5) were maintained on pasture (*Brachiaria decumbens* or *Brachiaria brizantha*) with ad libitum mineral salt supplementation.

All chemicals were obtained from Sigma–Aldrich Laboratory (St. Louis, MO, USA), Gibco (Langley, OK, USA; Fetal Bovine Serum, Qualified, EU Approved Regions), Vivimed (Ribeirao Preto, SP, Brazil; Choriomon®) and Hertape Calier (Juatuba, MG, Brazil; Pluset®).

Immature *cumulus*–oocyte complexes (COC) from Nelore and Holstein cows were obtained via ultrasound guided transvaginal follicle aspiration (ovum pick up – OPU, 10 sessions) and transported in thermal container at constant temperature (39 °C). Only the COC with homogeneous cytoplasm, surrounded by at least three layers of compact *cumulus* cells were selected for the experiment (Khurana and Niemann, 2000).

Cumulus cells were removed by vortexing in conical tubes containing 1 mL of TCM 199 medium with HEPES for 4 min. The denuded oocytes were washed 4 times in TCM 199 HEPES and were treated with Protease® (5 mg/mL) to eliminate the zona pellucida and possible *cumulus* cells debris and again washed with TCM 199 HEPES medium. Pools with 20 oocytes from Nelore ($n = 8$ pools) and Holstein ($n = 4$ pools) were submitted to total RNA extraction. The mRNA expression of target genes (*IGF1*, *IGF2*, *IGFR1*, *IGFR2*, *IGFBP2* and *IGFBP4* and *PAPP-A*) in oocytes from both breeds was assessed by the reverse transcription (RT), followed by real-time polymerase chain reaction (qPCR) using Power Sybr green PCR Master Mix® – Applied Biosystems.

Real-time RT-PCR analysis was performed with an ABI 7500. Reactions were carried out in 25 μL volumes. Reactions were optimized to provide maximum amplification efficiency for each gene (which were >90%). Each sample was analysed in duplicate, and the specificity of each PCR product was determined by melting curve analysis and amplicon size determination in agarose gels. Negative controls (water replacing cDNA) were run on every plate. The relative expression of each target gene was calculated using the $\Delta\Delta$Ct method with efficiency correction (Pfaffl, 2001) and the housekeeping gene was cyclophilin A (*CYC-A*). Mean efficiency values for each gene were calculated from the amplification profiles of individual samples with LinRegPCR software (Ramakers et al., 2003).

2.1. Statistical analysis

Data for target gene mRNA abundance were transformed to logarithms when not normally distributed. The means were compared using *t*-test. Data are presented as means ± SEM. The analyses were performed using JMP software by SAS (Version 7.0, Stastistical Analysis System v. 9.1.3).

3. Results

3.1. The mRNA expression of the IGF system in oocytes

The relative values (mean ± SEM) for gene expression were significantly higher ($P < 0.01$) in oocytes from Holstein than from Nelore cows for IGF1 (0.96 ± 0.21 vs. 0.48 ± 0.10), IGF2 (0.74 ± 0.27 vs. 0.07 ± 0.02), their receptors IGFR1 (1.08 ± 0.04 vs. 0.04 ± 0.03), IGFR2 (1.19 ± 0.50 vs. 0.06 ± 0.02), IGFBP2 (1.21 ± 0.23 vs. 0.05 ± 0.01), and IGFBP4 (0.53 ± 0.15 vs. 0.03 ± 0.15). However, *PAPP-A* had higher levels of mRNA expression in pools of oocytes from Nelore cows (28.10 ± 18.96) when compared to oocytes from Holstein (1.32 ± 0.17; $P < 0.05$).

4. Discussion

The mRNA expression of *IGF1* and *IGF2*, their receptors *IGFR1* and *IGFR2*, and their binding proteins (*IGFBP2* and *IGFBP4*) was higher in Holstein oocytes when compared to Nelore oocytes. However, *PAPP-A* expression was higher in Nelore oocytes when compared to those from Holsteins. Since *PAPP-A* is responsible for *IGFBP* degradation (Monget et al., 2003), the lower expression of *IGFBP*s found in Nelore, in comparison to Holstein oocytes, indicates greater bioavailability of *IGF* in Nelore oocytes.

Several authors have shown that the *IGF* system plays a crucial role in folliculogenesis, including follicle growth and deviation (Gutierrez-Adan et al., 2000; Louhio et al., 2000; Zhao et al., 2001; Itoh et al., 2002; Fortune et al., 2004) and oocyte maturation (Lorenzo et al., 1994; Sirotkin et al., 2000; Itoh et al., 2002; Walters et al., 2006). *IGF*s are synergistic to FSH to promote follicular growth and estradiol production (Fortune et al., 2004). The dominant follicle