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Systemic and local anti-Mullerian hormone reflects differences in the reproduction potential of Zebu and European type cattle

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ABSTRACT

This study was conducted to evaluate plasma anti-Mullerian hormone (Pl AMH), follicular fluid AMH (FF AMH) and granulosa cell AMH transcript (GC AMH) levels and their relationships with reproductive parameters in two cattle subspecies, *Bos taurus indicus* (Zebu), and *Bos taurus taurus* (European type cattle). Two-dimensional ultrasound examination and serum collection were performed on Zebu, European type and crossbreed cows to determine antral follicle count (AFC), ovary diameter (OD) and Pl AMH concentration. Slaughterhouse ovaries for Zebu and European type cattle were collected to determine FF AMH concentrations, GC AMH RNA levels, AFC, oocyte number, cleavage and blastocyst rate. Additionally GC AMH receptor 2 (AMHR2) RNA level was measured for European type cattle. Relationship between AMH and reproductive parameters was found to be significantly greater in Zebu compared to European cattle. Average Pl AMH mean ± SE for Zebu and European cattle was 0.77 ± 0.09 and 0.33 ± 0.24 ng/ml respectively ($p = 0.01$), whereas average antral FF AMH mean ± SE for Zebu and European cattle was 4934.3 ± 568.5 and 2977.9 ± 214.1 ng/ml respectively ($p < 0.05$). This is the first published report of FF and GC AMH in Zebu cattle. Levels of GC AMHR2 RNA in European cattle were correlated to oocyte number ($p = 0.01$). Crossbred animals were found more similar to their maternal Zebu counterparts with respect to their Pl AMH to AFC and OD relationships. These results demonstrate that AMH reflects differences between reproduction potential of the two cattle subspecies therefore can potentially be used as a reproductive marker. Furthermore these results reinforce the importance of separately considering the genetic backgrounds of animals when collecting or interpreting bovine AMH data for reproductive performance.

1. Introduction

The genetic background of cattle significantly impacts their reproductive performance. It is believed that increased inbreeding to achieve maximal milk yield has

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negatively impacted reproductive efficiency in temperate, *Bos taurus taurus* (European type cattle) animals (Bachelot and Binart, 2007; Leroy et al., 2015). By comparison, the tropical and subtropical subspecies of cattle, *Bos taurus indicus* (Zebu), generally found in India and South America, are known for lower milk yields, but greater reproductive efficiency (Bó et al., 2003). In a direct *in vitro* production (IVP) comparison with European type cattle vs. Zebu cattle reported blastocyst rate was 25.6% and 32.1% respectively, which ultimately gave rise to two thirds more pregnancies (Viana et al., 2010). However, Zebu were found to have lower breeding capacity due to delayed puberty, decreased estrous duration and intensity, longer gestation length and postpartum period, and lower twinning rate compared to European type cattle (Abeygunawardena and Dematawewa, 2004; Bó et al., 2003; Silva-Santos et al., 2011). Taken together, the greater embryo and pregnancy rate but lower breeding capacity make Zebu cattle an ideal candidate for *in vitro* embryo production (IVP). To date, there is an incomplete understanding of the underlying endocrine, molecular and physiological factors necessary to achieve optimal IVP protocols in Zebu and European type cattle.

Anti-Mullerian hormone (AMH) is a 140 kDa glycoprotein dimer member of the TGF-beta protein subfamily secreted by sertoli cells in testes, and by granulosa cells (GC) in the ovary reviewed by (di Clemente et al., 2003). In females AMH is believed to inhibit premature follicular growth and maturation, making it the ovarian reserve "guardian" (Visser et al., 2006). Its minimal intra and inter estrous variability makes it a useful predictor of ovarian reserve (Fanchin, 2003; Ireland et al., 2007). The exact mechanism by which AMH acts in the ovary is not certain, but it is believed to be through decreasing GC sensitivity to luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Visser and Themmen, 2005). Due to its link with ovarian reserve, AMH is used extensively as a quantitative marker of fertility in humans, and is also being increasingly examined as a potential biomarker of reproductive efficiency in other species, including cattle (Ireland et al., 2011; Monniaux et al., 2011; Rico et al., 2011). Various human studies have reported correlation of AMH concentration to a number of reproductive parameters related to the oocyte developmental potential, such as fertilization, implantation and pregnancy rates (Fanchin et al., 2007; Majumder et al., 2010; Takahashi et al., 2008). In addition to several reports of AMH in European type cattle (Ireland et al., 2009; Ireland et al., 2008) there are three published investigations involving Zebu cattle (Guerreiro et al., 2014; Baldrighi et al., 2014; Batista et al., 2014). These studies focused on comparing plasma AMH (Pl AMH) concentration between Zebu and European type cattle and correlating to the number of antral follicles (AFC) as a quantitative reproductive parameter. The findings of these studies provide evidence for plasma AMH (Pl AMH) to AFC correlation with Zebu animals displaying greater AMH values for the same AFC compared to European type animals.

The aim of the current study was to investigate and compare the relationship between both systemic and local AMH and reproductive parameters: AFC, ovary diameter (OD), oocyte number, cleavage and blastocyst rate in both Zebu and European type cattle, as well as a small cohort of crossbred animals. It was hypothesized that irrespective of its source, AMH correlated to reproductive parameters with Zebu having greater AMH value compared to European type cattle.

2. Materials and methods

2.1. Animals and experimental design

2.1.1. Study group 1

B. taurus indicus or Zebu (Nelore breed, $n = 30$), *B. taurus taurus* or European type cattle (Red Angus breed, $n = 10$), and crossbred *B. taurus taurus* × *B. taurus indicus* (Brangus breed, $n = 10$) cows of average reproductive age (mean ages 8.6, 7.8 and 7.1 years respectively) raised on pasture in Paraná state, Brazil were subject to ovarian examination of AFC and OD with the 2-D trans-rectal Mindray adapted with a linear transductor ultrasound, and peripheral blood collection via puncture of the tail vein. All sampling was done at once irrespective of the estrous

Table 1
Real Time PCR primers.

Gene	GenBank accession number	Sequence	Reference	Size (bp)
AMH	NM_173890.1	F-5′-CAGGGAAGAAGTCTTCAGCA-3′ R-5′-AAGGTGGTCAAGTCACTCAG-3′	(Ireland et al. (2009) Scheetz et al. (2012)	270
GAPDH	NM_001034034.2	F-5′-TTCCT GGTACGACAATGAATTTG-3′ R-5′-GGAGATGGGG CAGGACTC-3′	Hamilton et al. (2011)	153
AMHR2	NM_001205328.1	F-5′-GTGCTTCTCCCAGGTCATAC-3′ R-5′-AATGTGGTCATGCTGTAGGC-3′	Monniaux et al. (2011)	163