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Fetal sex alters maternal anti-Mullerian hormone during pregnancy in cattle

Anja Stojsin-Carter[a], Nathalia N. Costa[b], Rodrigo De Morais[b], Tiago H. De Bem[c], Mayra P. Costa[b], Timothy F. Carter[a], Daniel J. Gillis[d], Michael S. Neal[e], Otavio M. Ohashi[b], Moyses S. Miranda[b], Flavio V. Meirelles[c], Laura A. Favetta[a], W. Allan King[a,*]

[a] Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, 50 Stone Road, Guelph, ON, N1G 2W1, Canada
[b] Instituto de Ciências Biológicas, Universidade Federal do Pará, Rua Augusto Corrêa 1, Belém, PA, 66075-110, Brazil
[c] Departamento de Medicina Veterinaria, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de Sao Paulo, Avenida Duque Caxias Norte 225 – Jardim Elite, Pirassununga, SP, 13635-000, Brazil
[d] School of Computer Science, University of Guelph, 50 Stone Road, Guelph, ON, N1G 2W1, Canada
[e] ONE Fertility, 3210 Harvester Rd, Burlington, ON, L7N 3T1, Canada

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ABSTRACT

Anti-Mullerian hormone (AMH) is expressed by both male and female fetuses during mammalian development, with males expressing AMH earlier and at significantly higher concentration. The aim of the current study was to explore the potential impact of pregnancy and fetal sex on maternal AMH and to determine if plasma (Pl) AMH or placenta intercotyledonary membrane and cotyledonary AMH receptor 2 (AMHR2) mRNA expression differ in pregnant cows carrying male vs. female fetuses. AMH levels in blood were measured using a bovine optimized ELISA kit. Cows pregnant with a male fetus were observed to have a significantly greater difference in Pl AMH between day 35 and 135 of gestation. Average fetal AMH level between 54 and 220 days of gestation was also observed to be significantly higher in male vs. female fetuses. Intercotyledonary membranes and cotyledons were found to express AMHR2 between days 38 and 80 of gestation at similar levels in both fetal sexes. These findings support the hypothesis that fetal sex alters maternal Pl AMH during pregnancy in cattle.

1. Introduction

The powerful effects of exposure to Anti-Mullerian hormone (AMH) during development are best displayed by the freemartin phenotype, which occurs in 90% of opposite sex twin bovine and ovine pregnancies. The condition develops when the female twin is exposed to male twin fetal AMH via direct anastomosis of their placental vasculatures and leads to masculinization and sterility of the female twin (Vigier et al., 1981). Effects of fetal sex on maternal AMH level and physiology have not been investigated in cattle, however there are a few studies in human. Beyond lactation and the more obvious maternal alterations associated with pregnancy, additional subtle changes such as maternal autoimmune disease prevalence and altered cancer risk have also been identified in women and observed to continue long into the postpartum period (reviewed by Boddy et al., 2015). Although many of the mechanisms are not fully understood, these effects can be traced back to fetal factors that escape the placental-endometrial barrier and

* Corresponding author.
E-mail address: waking@uoguelph.ca (W.A. King).

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are shared across the fetal-maternal link in both human and cattle (Malek et al., 1998; Palmeira et al., 2012; Pereira et al., 2013; Turin et al., 2007). In most extreme cases, fetal cells entering maternal circulation give rise to stable populations (fetal cell microchimerism) persisting for decades beyond pregnancy (Herzenberg et al., 1979). This is found to decrease rate of aging and tumor risk (Cirello and Fugazzola, 2016; Fugazzola et al., 2012, 2011; O'Donoghue, 2008) or is further implicated in the maternal response to treatment for chronic conditions on the basis of the sex of the founding fetus (Firoozi et al., 2009; Hocher et al., 2009; Walsh et al., 2015). Therefore, the mammalian fetal-maternal interface is not a simple barrier restricted to nutrient exchange, but a broader link between fetus and mother, with remnants and effects exceeding the pregnancy itself.

Often used as a marker for ovarian reserve and fertility in females in both human and cattle (Durlinger et al., 2001; Ireland et al., 2011; La Marca and Volpe, 2006; Monniaux et al., 2011; Rico et al., 2009) or for testicular function in male infants (Lee et al., 1996), AMH has recently gained attention as a potential early pregnancy sex-related marker in women (Empey et al., 2012). Increase in AMH expression by male fetuses starts from as early as the 7th and 8th week of gestation in cows and humans respectively, allowing it to potentially become one of the earliest molecular markers of fetal sex (Lee et al., 1996; Vigier et al., 1984a). Despite having clear roles in both fetal development and adult fertility, there are few reports on maternal AMH levels during gestation in humans (Empey et al., 2012; Koninger et al., 2013; La Marca, 2005; Nelson et al., 2010; Pankhurst et al., 2016). These initial studies have reported a significant decline in maternal AMH levels during pregnancy, presumably due to reduced follicle growth (Kuijper et al., 2013).

We hypothesized that i) the changes in levels of plasma (Pl) AMH during pregnancy would differ between cows carrying a male fetus and cows carrying a female fetus with cows carrying a male fetus being greater ii) the levels of Pl AMH in male fetuses will be higher than in the female fetuses, iii) the levels of Pl AMH in male fetuses will be higher than in the cows and female fetuses, and iv) the placenta would express AMHR2 at a comparable level in pregnancies of both sexes.

2. Materials and methods

2.1. Animals and experimental design

The study was designed to measure Pl AMH in pregnant cows at multiple and single time points and in their respective female and male fetuses and to determine if the sex of the fetus has an influence on the systemic levels of the maternal AMH. This was performed using three experimental groups with the rationale that the male fetal AMH could have an effect on the difference in levels of the maternal AMH. In the first experimental group, it was possible to sample single animals at the three different time points throughout pregnancy. In a second experimental group, single time point measurements were taken from pregnant cows and their corresponding fetuses. A third experimental group was used to study the expression of AMH receptor 2 (AMHR2) in the intercotyledonary membrane and cotyledon of male and female fetus pregnancies.

2.2. Experimental group I

The first experiment was conducted in accordance with the Ethics Committee on the Use of Animals (CEUA) of Faculty of Animal Science and Food Engineering, University of Sao Paulo (FZEA/USP). *Bos taurus indicus* or Zebu (n = 13) cows of average reproductive age and raised on the pasture in São Paulo state, Brazil were subjected to artificial insemination (AI) and peripheral blood collection via puncture of the tail vein at day 35, 135 and 275 of gestation (Fig. 1). Out of 13 animals, 4 animals aborted, 1 animal sample was compromised during processing, and in 8 animals (n = 8) pregnancy resulted in the birth of a healthy singleton calf with all samples successfully retrieved. Fetal sex determination was assessed using 2-D trans-rectal Mindray® ultrasound adapted with a linear probe during the 2nd trimester and then was confirmed at birth. Out of 8 animals, 5 carried a male and 3 carried a female fetus. All blood sampling was done by the same person and blood was collected in EDTA tubes (BD Vacutaner®, BD Franklin Lakes NJ, USA). Plasma

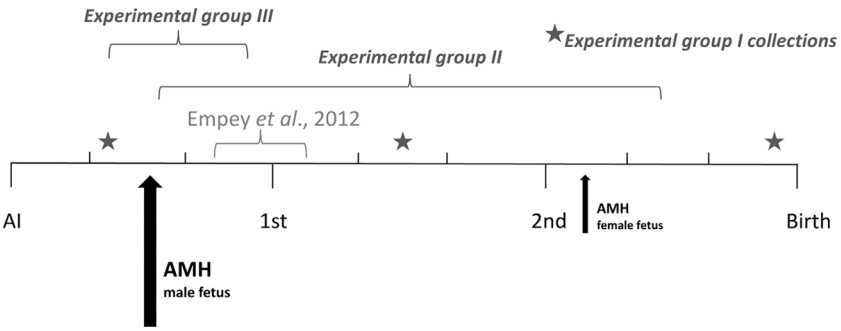

Fig. 1. A visual representation of the three experimental groups. The horizontal line represents the time line of gestation; it is divided into the three trimesters and each trimester into three months, starting with artificial insemination (AI) of the cow and ending at birth. Fetal expression of AMH for male at 60 days and for female at 220 days, with large and small arrows corresponding to the relative level of expression. In dark gray: Experimental group I (marked by the star symbol), collections at day 35, 135 and 275 and Experimental group II collections from 54 to 220 days of gestation and Experimental group III collections from 38 to 80 days of gestation. In light gray: (Empey et al., 2012) detected significant difference in maternal blood AMH levels between male and female fetuses in humans in between 11th and 15th week of pregnancy, approximately corresponding to 77 and 105 days of gestation.