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Effects of sexed semen and interactive effects on commercial *in vitro* embryo production when oocytes are collected from cows of *Bos indicus*, and *Bos taurus* breeding and crossbred cows of these subspecies

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ABSTRACT

A large scale commercial *in vitro* embryo production and *in vitro* fertilization record is reported when there was use of oocytes from *Bos taurus* (BT), *Bos indicus* (BI), and *Bos Taurus*/*indicus* (BT/BI) crossbred subspecies fertilized with sexed (SS) and conventional (CS) semen. The aim of the study was to analyze the impacts of use SS in a commercial embryo production center in Colombia.

Non-pregnant ($n = 800$), healthy, and estrous cycling BT, BI, and BI/BT crossbred cows were selected to be used as oocyte donors. Viable oocytes from 733 ovum pick up sessions in cows of the BT (4663) and BI (7305), BT/BI (3605) groups were matured and fertilized with frozen thawed semen from commercial sires. Of all cultured oocytes ($n = 15{,}573$), 52.3% of the embryos ($n = 8607$) underwent cleavage while 3062 (19.7%) developed to the blastocyst stage.

For cows of the BT, SS and CS groups, there were similar cleavage rates; however, use of SS decreased blastocyst formation (15.6% *versus* 18.9%). In the BI group, use of SS increased cleavage rates (59.1% *versus* 53%, respectively) although there was no effect on blastocyst rate development (22.9% *versus* 21.9). In the BI/BT groups, use of SS decreased cleavage (44.4% *versus* 57.1%, respectively) and blastocyst rate development compared with the CS group (15.3% *versus* 20.1%, respectively).

Data from this study indicate embryos from cows of the BT purebred or BI/BT crossbred had less *in vitro* developmental capacity compared to embryos derived from oocytes of cows that were purebred BI in tropical–subtropical regions.

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1. Introduction

Sex-sorted sperm for artificial insemination and embryo transfer has become an important reproductive tool that can change the production systems for dairy and beef cattle operations (De Vries et al., 2008). Globally, the use of *in vitro* embryo production technologies during the last 5 years has increased the application of sexed semen (SS) to produce animals for replacement females or males for breeding purposes (Stroud and Callesen, 2012).

Bos indicus (BI), *Bos taurus* (BT) and *Bos indicus*/*taurus* (BI/BT) crossbred cattle are important genetic resources for beef production in tropical–subtropical areas. The interest of producing hybrid cows from such different breeds increases the importance of *in vitro* embryo production as a strategy to, with greater capacity, produce embryos and decrease economic expense (Seidel, 2003). Many factors have been reported to negatively affect fertility when sexed semen (SS) is used inartificial insemination or *in-vitro* fertilization (Campanile et al., 2011). The mitochondria organelles and alteration of sperm DNA structure were thought to be responsible, at least in part, for the decreased fertility when SS was used (Rath et al., 2013). Limited knowledge is available regarding subspecies *in-vitro* embryo production using SS (Morotti et al., 2014; Pontes et al., 2010). A recent study reported greater rates of oocyte recovery and *in vitro* embryo production with use of oocytes from cows with BI compared with BT breeding (Morotti et al., 2014). Previous reports on *in vitro* embryo production using SS have been inconsistent in relation to cleavage occurrence and blastocyst rate development (Seidel, 2003; Zhang et al., 2003; Carvalho et al., 2010; Blondin et al., 2009; Lu and Seidel, 2004). The present study aimed to determine the effects of subspecies of BI, BT and BI/BT crossbreeding as potential oocyte donors for *in vitro* embryo production using SS and conventional semen (CS). All data were generated from a commercial embryo production center (Genes de Colombia S.A. located near Santander, in Bucaramanga, Colombia).

2. Materials and methods

To determine whether or not there were significant differences between subspecies and reproductive methods, several chi-square tests were conducted. Data were drawn from a database containing the records of insemination date, bull identification, type of sub-species, breed, and additionally viability classification, cultured and cleaved embryos comparing embryo data for groups derived with CS compared with SS. Data were collected over a period of 7 months between March and November 2013 in a spreadsheet (Microsoft Excel, Microsoft Corporation, 2010).

Non pregnant ($n = 800$), healthy, and estrous cycling *Bos taurus* (Holstein, Simmental, Jersey, Brown Swiss), *B. indicus* (Gir, Brahman, Guzera), and *B. indicus/taurus* crossbred (Girolando, Beefmaster, Simmental/Brahman) cows were selected based on genetic merit to be used as oocyte donors. These cows had typical ovarian activity which was examined by trans-rectal palpation and ultrasonography.

Cow donors were from several Colombian dairy and beef farms. Similar to the methodologies used by Morotti et al. (2014), in the present study cows were randomly used for ovum pick-up (OPU) without a specifically scheduled or predetermined sequence and without hormonal stimulation, but with a minimum interval of 15 days between OPU sessions. Embryo production was conducted in a commercial embryo production center (Genes de Colombia S.A.) located in a region that is in close proximity to Santander, Colombia.

2.1. Collection of oocytes and in vitro maturation

Preceding OPU the cows were restrained and after manual evacuation of the rectum contents was performed the animals received epidural anesthesia using lidocaine (2%). After anesthesia administration the animal's perineal region was washed and disinfected using ethyl alcohol at a 70% concentration. Ultrasonic-guided follicular aspirations were performed by visualizing the follicles using the Mindray DP-2200 ultrasonic device (Mindray, Shenzhen, China) with a 5 MHz transductor. Follicular fluids from all visible follicles were aspirated using 16 (0.9 mm × 50 mm) gauge needles (Terumo Medical Co., USA) and a vacuum pump (Cook Veterinary Products, New Buffalo, MI, USA) with 120 a 150 mm Hg pressure. The follicular fluid was collected in a 50 ml Falcon tube through a cannula. Oocytes were immediately washed through a filter with Euroflush and were collected under a stereomicroscope. After several washes in the same medium, oocytes were transferred to 400 μl of tissue culture medium supplemented with follicular stimulating hormone (0.5 μg/ml), luteinizing hormone (0.5 μg/ml), IGF1 (100 ng/ml), ITS (insulin 5 μg/ml, transferrin 5 μg/ml and selenium 5 ng/ml), estradiol (1 μg/ml) and gentamycine (25 μg/ml) in a Falcon tube (code 352054) covered by mineral oil (Sigma). The tubes were gassed with 5% CO_2, 5% O_2 and 90% N_2 mixture and a silicone rubber device was inserted to cap the tube followed by placement of the tube in a portable incubator (WTA, Brazil) at 38.5 °C to be transported to the laboratory. At the laboratory the tubes were opened and placed in a humidified incubator with 5% CO_2 in the environment and cultured for 18–20 h.

2.2. In vitro fertilization and embryo culture

Equal amounts of commercial CS and SS were prepared for IVF as follows. Sperm straws were thawed at 37 °C for 30 s and washed in a 15 Falcon tube using percoll gradients of 45% and 90% prepared with Talp (Bavister et al., 1983) solution (2 ml of 45% percoll in a top and 2 ml of 90% percoll in the bottom of the tube) by centrifugation at 9000 rpm for 5 min, then the pellet was diluted with 3 ml Talp solution and centrifuged a second time at 4000 rpm for 3 min. Sperm concentration was calculated after sperm counting using a hemocytometer and adjusted to 0.5 million per ml for conventional semen or 1million per ml for sexed semen. Droplets of 50 (sexed sperm) or 100 μl (conventional semen) of synthetic oviductal fluid medium (SOF; Takahashi et al., 1996) containing 10 μg/ml heparin were prepared in a 35 mm culture dish covered with mineral oil and kept in the incubator with 90% humidified environments and 5% CO_2, 5% O_2 and 90% N_2 gas mixture for co-culture of sperm and oocytes for 18–20 h.