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Use of sexed sorted semen for fixed-time artificial insemination or fixed-time embryo transfer of *in vitro*–produced embryos in cattle

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ABSTRACT

Artificial insemination and *in vitro* embryo production are powerful tools for disseminating superior genetic qualities and improving the reproductive performance of dairy and beef cattle. In conjunction with these biotechnologies, sexed-sorted semen has been used to obtain offspring of a predetermined sex. This study compared the pregnancy rates obtained using *in vitro* fertilization/timed embryo transfer (IVF/TET) and timed artificial insemination (TAI), both performed using sexed-sorted (Y-chromosome-bearing) semen obtained from the same bull. For the *in vitro* embryo production, the ovaries of 250 Nelore cows with known histories were collected in the slaughterhouse and used for IVF. After evaluation of the recipients (IVF/TET group; n = 974), the resultant embryos were transferred to the females with corpus luteum (n = 822). The pregnancy-related data for this group were compared with those for the TAI group (n = 974). Ultrasonography was performed at 60 days to determine the pregnancy status and confirm the sex of the fetus. A total of 2008 oocytes produced 1050 embryos, with 52% of them reaching the blastocyst stage. The pregnancy rate and the accuracy in determining the fetal sex were 35.4% (345/974) and 95.07% (328/345), respectively, for the IVF/TET group and 30% (293/974; $P < 0.05$) and 94.88% (278/293), respectively, for the TAI group. In the present study, we concluded that male calves could be better obtained using IVF/TET rather than TAI; therefore, this strategy can be considered to increase the pregnancy rate of beef cattle.

1. Introduction

Considering the worldwide aspiration to increase meat consumption as well as the need for sustainable livestock, beef cattle producers must adapt more efficient and economical management practice methods. Using reproductive biotechnologies together with genetic selection is the best strategy to meet this goal because reproducing genetically superior animals could rapidly increase the productivity of the livestock [1,2].

Artificial insemination and embryo production are powerful tools for disseminating superior genetic qualities and improving the genetics and reproductive performance of dairy and beef cattle. The biotechnology of embryo production through *in vitro* fertilization (IVF) has been constantly improved over the last decade [3], and its efficacy has been reported in large-scale studies of cattle [4,5], resulting in its application on a commercial

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scale in many countries. Currently, combining IVF with the use of sexed- [6] or reverse-sorted sperm [5] is the advisable biotechnological strategy for obtaining offspring of a predetermined sex [7,8] to improve the productivity of farms and promote the performance features related to a particular sex, such as encouraging the birth of male beef cattle, which may have greater commercial value than do female beef cattle due their performance characteristics.

Currently, the vast majority of IVF programs use *in vivo* ovum pick-up (OPU) in an oocyte donor of high genetic value, and this step of the process accounts for a significant fraction of the final cost of using this biotechnology. Furthermore, many cows of considerable zootechnical value (products of genetic improvement) are slaughtered owing to the constant needs of the consumer market. Collecting the ovaries of these females for *in vitro* OPU is an interesting strategy for large-scale IVF programs because this procedure is easier than *in vivo* OPU using the follicular puncture method and the oocytes can be obtained at a low cost. In addition, performing IVF using sexed-sorted sperm favors the birth of calves with the performance features of the chosen sex [8].

Performing artificial insemination using sexed-sorted semen is another option for obtaining offspring of the desired sex [9,10]. Studies have shown that when fixed-time artificial insemination (TAI) was performed 60 hours after the removal of the progesterone source [11,12], the presence of a dominant follicle (≥9 mm) on the day of the TAI and the female being in estrus [13] increased the conception rate obtained using sexed-sorted sperm. However, despite significant advances in modifications of this biotechnology (in the sex-selection process and in the hormonal protocols for synchronizing estrous or ovulation), the use of sexed-sorted sperm for TAI is still largely limited to heifers owing to their more desirable rates of pregnancy [14,15]. Moreover, transferring embryos produced *in vivo* [16] or *in vitro* [4], which is performed mainly using dairy cattle, is a strategy that has several advantages, including an increased pregnancy rate.

To our knowledge, no studies have evaluated the large-scale use of these two reproductive biotechnologies with a strong potential for obtaining offspring of a predetermined sex in beef cattle in the same study. Therefore, the objective of this study was to evaluate the pregnancy rates and proportion of male calves produced using IVF of oocytes obtained from slaughterhouse ovaries or using TAI, both performed using sexed-sorted sperm.

2. Materials and methods

2.1. Location, animals, and management

This study was conducted during the breeding season in South America at latitude 07° 05′ 42″ and longitude 49° 56′ 45″ using primiparous and multiparous cows *Bos indicus* and *Bos taurus* crossbred females (n = 1948). The cows were maintained in an extensive pasture that allowed for continuous grazing of *Brachiaria* spp. and were given *ad libitum* access to mineralized salt and water.

The females (48–180 months) used in this study were selected on the basis of their having an adequate body condition score (BCS) and postpartum period, normal estrous cycles, and a normal health status. The lactating cows were 30 to 70 days postpartum (average 50 days), and only females with a BCS between 2.5 and 4.0 (average 3 ± 0.6) on a scale of 1 to 5 [17] were selected to receive a conventional ovulation-synchronization treatment for TAI or fixed-time embryo transfer (TET) on a single commercial beef operation located in northern Brazil.

2.2. Experimental design and performance of TAI and TET

On a random day of the estrous cycle (Day 0), 974 recipients designated for embryo transfer (TET group; primiparous = 390 and multiparous = 584) and 974 females designated for insemination (TAI group; primiparous = 323 and multiparous = 651) underwent a conventional ovulation-synchronization treatment, which consisted of inserting an intravaginal device containing 1.9 g of progesterone (P4; CIDR; Zoetis, Hamilton, New Zealand) and administration of an im injection of 2 mg of estradiol benzoate (Estrogin; Farmavet, São Paulo, Brazil). On Day 8, the CIDR devices were removed, and the animals received im injections of 150 μg of cloprostenol (Preloban; Intervet Schering-Plough, São Paulo, Brazil), 300 IU of equine chorionic gonadotropin (Novormon; MSD Animal Health, São Paulo, Brazil), and 1 mg of estradiol cypionate im (EC; ECP; Zoetis, São Paulo, Brazil). All the females designated for TAI were inseminated 60 hours after the removal of the P4 devices by a single experienced inseminator using male-sexed-sorted sperm (freeze-thawed Y-chromosome-bearing sperm) obtained from one Angus bull. Before embryo transfer (Day 17), the ovaries of each potential recipient were examined using transrectal palpation and ultrasonography (Aloka SSD 500 with 5-MHz linear transducer; Tokyo, Japan) to confirm the presence and size of the corpus luteum (CL). Only recipients with a CL ≥13 mm in diameter received an embryo [18,19].

2.3. Collection of ovaries

A total of 250 Nelore females with known histories (progeny of genetic improvement) were slaughtered in a slaughterhouse located 25 km from the IVF laboratory. Immediately after slaughter, the ovaries of each animal were collected (n = 500) and placed in Dulbecco's phosphate-buffered saline (Nutricell, Campinas, São Paulo, Brazil) at room temperature for transport to the laboratory. In the laboratory, each ovary was evaluated, and the visible follicles were aspirated using a disposable 19-gauge × 12 mm hypodermic needle (Becton Dickinson, Curitiba, Paraná, Brazil) connected to a 50-mL conical tube (Corning, Acton, MA, USA) via silicon tubing (0.8 m; 2 mm inner diameter). The follicular aspirations were performed using a vacuum pump (WTA, Watanabe, São Paulo, Brazil) with a negative pressure of 75 mm Hg. The collection medium used was TCM 199 medium (Gibco Life Technologies, Grand Island, NY, USA) supplemented with 25-mM HEPES (Sigma H-0763), 5% fetal calf serum (FCS), 50-μg/mL gentamicin sulfate (Schering-Plough, São Paulo, São Paulo, Brazil), and 10,000 IU/L sodium heparin (Sigma H-3149).