CHAPTER 47

Fetal sexing in bovine embryos for in vitro production – literature review.

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ABSTRACT

The genetic improvement in animal production has been expanding day by day. Cattle farming is an area that increasingly needs the use of biotechnologies because commercially, the birth of genetically improved calves has been essential to ensure the quality of the herd. The early diagnosis of the fetus has helped producers to guide their strategies along the production chain, whether in dairy or beef cattle. Cattle farming is efficient regarding the use of biotechnologies for genetic improvement. The agricultural sector has been seeking to revolutionize animal production, for sustainable use, prioritizing animal welfare without harming profits. Factors such as nutrition, management, and health have been implemented in dairy farms to help these animals express their genetic potential (BECHER et al., 2018).

Keywords: Fetal sexing, bovine, in vitro production.

1 INTRODUCTION

The genetic improvement in animal production has been expanding day by day. Cattle farming is an area that increasingly needs the use of biotechnologies because commercially, the birth of genetically improved calves has been essential to ensure the quality of the herd. The early diagnosis of the fetus has helped producers to guide their strategies along the production chain, whether in dairy or beef cattle.

Cattle farming is efficient regarding the use of biotechnologies for genetic improvement. The agricultural sector has been seeking to revolutionize animal production, for sustainable use, prioritizing animal welfare without harming profits. Factors such as nutrition, management, and health have been implemented in dairy farms to help these animals express their genetic potential (BECHER *et al.*, 2018).

Assisted reproductive biotechnologies have been evolving vehemently. These can be classified into four generations of these technologies in cattle breeding. The first generation was marked by the use of Artificial Insemination and Cryopreservation of gems and embryos, while in the second generation, superovulation and Embryo Transfer are observed, sperm and embryonic sexing and *in* *vitro* production of embryos (PIV/PIVE) mark the third generation, and Cloning represents the fourth generation (BECHER *et al.*, 2018).

1.1 IN VITRO PRODUCTION

It is of great commercial interest to genetically improve the stock ensuring better quality, whether meat or milk. There is also a need for females to be better genetically and more so that they are fertile, generate healthy calves and that have better milk production or feed conversion, that is, produce more at less cost (BECHER *et al.*, 2018).

In *Vitro* Embryo Production (IVP/PIVE) has made this reality accessible through embryo sexing. Brazil is a leader and reference in the use of PIVE being responsible for 50% of world production (BECHER *et al*, 2018). In 1998, the technological innovation project financed by the companies Beabisa Agricultura Ltda and Gertec Tecnologia de embriões initiated the use of this technique in the country (SOUZA; ABADE, 2018).

According to IETS (2017), in 2016 the production of in vitro embryos surpassed the production of embryos in vivo, reaching 666,215 sexed embryos. PIV is extremely advantageous to other techniques because a cow that in all its useful life would have 4 to 5 pregnancies can produce between 30 to 40 offspring with in vitro production (ROCHA; SAINTS, 2007; SOUZA; ABADE, 2018). In addition, the PIV enables the genetic improvement of the herd, accelerating the progress in cattle herds, and reducing the interval between generations (SOUZA; ABADE, 2018). In Vitro, Production enables the contact of the oocyte of the female with the sperm of the male forming a new individual outside the reproductive tract (SOUZA; ABADE, 2018; GONCALVES; FLOWERS; GASPERIN, 2021). IVP has four stages: oocyte collection, in vitro maturation, in vitro fertilization, and in vitro culture (SOUZA; ABADE, 2018). The collection can be done with oocytes rescued from the ovaries of slaughterhouse cows or through follicular aspiration with the aid of ultrasonography (SOUZA; ABADE, 2018; GONCALVEZ; FLOWERS; GASPERIN, 2021).

The first step consists of the collection of oocytes from females through follicular puncture with a needle attached to a transvaginal probe, the follicles are observed on the ultrasound screen and thus are punctured. The ideal size of oocytes to be aspirated from the follicles should be 2 to 8 mm in diameter. Non-standard sizes become unfeasible. The oocytes are recovered along with the follicular fluid into a collecting tube through a vacuum pump system fixed to the puncture needle. The selection of oocytes is made based on the characteristics of the cumulus cells and oocyte morphology according to their number of layers, being grade 1 - excellent, grade 2 - good, grade 3 - regular, and cytoplasm irregular and degenerate-denuded (SOUZA; ABADE, 2018; GONCALVEZ; FLOWERS; GASPERIN, 2021).

After selection, the oocytes are transported to the laboratory where the second stage of in vitro maturation will be performed. The most common means for maturation used in laboratories is TCM 199, which consists of the use of fetal bovine serum (SFB) and gonadotrophins (Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and 17β Estradiol), as well as amino acids, vitamins, and antibiotics for oocyte supplementation that are maintained in controlled conditions from 18 to 24 hours, which is the period necessary for the maturation of oocytes of the bovine species (SOUZA; ABADE, 2018).

After maturation, the third stage begins, which is in vitro fertilization, which consists of the cultivation of mature oocytes with sperm and the combination of the genetic material of the gametes, which are fertilized generating a zygote, which will develop until the blastocyst phase. The fourth phase is the in vitro culture of the embryos which consists of the evolution of the zygote that will undergo cleavage, activation of the embryonic genome, compaction of the blastomeres, differentiation of the embryonic distinction, formation and expansion of the blastocele and rupture of the zona pellucida, until reaching the blastocyst stage. For in vitro cultivation to be completed the means used in laboratories must be similar to the environment and fluids of a cow's uterus in early gestation (SOUZA; ABADE, 2018).

Despite many advances regarding in vitro production, some limitations affect the quality of the embryo produced in the laboratory about the one produced in vivo, the first being generally inferior having a darker coloration and lower cytoplasmic density, due to its lipid content, and a more fragile zona pellucida, being more susceptible to chromosomal abnormalities (ROCHA; SAINTS, 2007; BECHER *et al.*, 2018; SOUZA; ABADE, 2018; GONCALVEZ; FLOWERS; GASPERIN, 2021).

1.2 FETAL SEXING

Over time, applied ultrasonography has been potentiated in Veterinary Medicine, offering improvement mainly aimed at reproduction. The ultrasound examination has great value, allowing for visualization of the reproductive tract of females in different phases, both in the gestational phase, in the reproductive organs of the mother, and the envelopes, in the embryo since its formation and until it is possible to diagnose fetal sexing (GASPERIN *et al.,* 2017).

Fetal sexing in cattle has been used by many farmers to provide improvements to the herd. The method used by Veterinarians is a modern and non-invasive examination, which will provide a fast, safe diagnosis that will not cause stress in the animal. To diagnose fetal sex, it is necessary to visualize the genital tubercle, identical between both sexes, which presents as a bilobulated structure, with an appearance similar to two oval and hyperechoic parallel bars (GASPERIN *et al.*, 2017). The genital tubercle has the function throughout pregnancy to form a penis in males and a clitoris in females, so the diagnosis is based on the differentiated location between both (GASPERIN *et al.*, 2017; GONCALVEZ; FLOWERS; GASPERIN, 2021).

First, the fetus should be located before introducing the ultrasound device, after postponement locate the posterior region of the fetus and then identify the genital tubercle. In females, the genital tubercle is ventral the insertion of the tail, in males the genital tubercle is caudal the insertion of the umbilical cord (GASPERIN *et al.*, 2017; GONCALVEZ; FLOWERS; GASPERIN, 2021).

The diagnosis of sexing is best performed on the 55th to 70th day of gestation because the tuber has greater echogenicity, ensuring greater accuracy. Before 50 days of gestation the diagnosis may be erroneous because the tuber and the reference points are lower, after 70 days of gestation the fetus will be a little larger and thus hindering the visualization of the tubercle, and at this stage may be observed the presence of scrotum in the male and mammary gland in the female (GASPERIN *et al*, 2017).

It is extremely advantageous for the producer to know in advance the sex of the progeny so that he can plan by concentrating the males for the beef herds and the females for the milk herds and replacement of matrices. In addition, one can also add commercial value to pregnant women by selling sexed mothers. Both in vitro production and fetal sexing are allied to maximize the financial return of livestock in Brazilian agribusiness (GASPERIN *et al*, 2017).

This study aims to describe the fetal sexing procedure in vitro production of bovine embryos, according to the literature.

2 CONCLUSIONS

It is extremely advantageous for the producer to know in advance the sex of the progeny so that he can plan by concentrating the males for the beef herds and the females for the milk herds and replacement of matrices. The uses of biotechnologies have made it easier to perform these procedures, both with animals and with the control of the herd in the management part. In addition, one can also add commercial value to pregnant women by selling sexed mothers. Both in vitro production and fetal sexing are allied to maximize the financial return of livestock in Brazilian agribusiness.

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