



Study of oocyte and embryonic quality of super precocious heifers (*Bos indicus*) aspirated prior to the breeding season in the municipality of Vilhena – RO, Brazil. Estudo da qualidade oocitária e embrionária de novilhas superprecoces (*Bos indicus*) aspiradas antes da estação reprodutiva no município de Vilhena – RO, Brasil.

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Abstract

Reproductive efficiency in females plays a fundamental role in the economic sustainability of beef cattle herds, mainly in characteristics related to the sexual precocity of heifers, with the increasing use of *in vitro* production (IVP) of embryos. The present study aimed to evaluate the quality of oocytes and embryos produced from Nelore heifers aged 11 and 12 months submitted to the precocity challenge, aspirated prior to the breeding season. The study was conducted in the months of September and October 2023, on a farm located in the municipality of Vilhena-RO. The animals were subjected to a pasture regime predominantly formed by *Brachiaria* and received protein supplementation with consumption of 0.6% of live weight added with Miyagi grass silage. Follicular aspirations took place in the morning, with the aim of not negatively influencing the ambient temperature. To conduct the research, 23 animals that underwent the follicular aspiration process were used. Subsequently, the oocytes were sent to the laboratory for selection, maturation, and fertilization. In the laboratory, these oocytes were selected to classify the viable ones and fertilize them. In total, 562 viable oocytes were collected, which converted into 129 embryos suitable for transfer. Oocyte conversion was 22.9%, a relatively considerable number compared to the conversion of adult matrices. It is evident, therefore, that follicular aspiration of super early heifers can be used in genetic improvement programs since the oocyte quality of these animals makes the process of genetic gain and generational advancement viable.

Keywords: Follicular aspiration. Embryo production. Reproduction.

Resumo

A eficiência reprodutiva desempenha nas fêmeas um papel fundamental na sustentabilidade econômica dos rebanhos de bovinos de corte, principalmente em características relacionadas a precocidade sexual de novilhas, com a crescente utilização da produção *in vitro* (PIVE) de embriões. O presente estudo teve como objetivo avaliar a qualidade oocitária e de embriões produzidos de novilhas nelore com idade de 11 e 12 meses submetidas ao desafio de precocidade, aspiradas previamente a estação de monta. O estudo foi realizado nos meses de setembro e outubro de 2023, em uma fazenda localizada no município de Vilhena-RO, os animais foram submetidos a um regime de pastagem predominantemente formada por *Brachiaria* e recebiam suplementação proteica com consumo 0,6% do peso vivo acrescido de silagem de capim Miyagi. As aspirações foliculares decorreram pela manhã, com intuito de não influenciar de forma negativa a temperatura ambiente. Para conduzir a pesquisa, foram utilizados 23 animais submetidos ao processo de aspiração folicular. Posteriormente, os oócitos foram encaminhados ao laboratório para seleção, maturação e fecundação. No laboratório, realizou-se a seleção desses oócitos para classificar os viáveis e fecunda-los, ao total foram coletados 562 oócitos viáveis que converteram em 129 embriões aptos a transferência. A conversão oocitária foi de 22,9% número relativamente considerável se comparado a conversão de matrizes adultas. É evidente, portanto, que a aspiração folicular de novilhas super precoce pode ser utilizada em programas melhoramento genético, visto que a qualidade oocitária desses animais viabiliza o processo de ganho genético e avanço de gerações.

Palavras-chave: Aspiração folicular. Produção de embriões. Reprodução.

Introduction

To meet market demands for quantity and quality, cattle breeders aiming to increase the reproductive efficiency of their herds invest in genetic improvement along with reproductive biotechnologies (FREITAS, 2023). In 1982, the first calf produced through the *in vitro* fertilization (IVF) process was born and developed normally (BRACKETT et al., 1982). As a result of funding from Beabisa Agricultura LTDA and Gretec Tecnologia de Embriões, commercialization began in 1998 (GALLI et al., 2003). The techniques of IVP in bovines are continuously enhanced due to the high demand for embryos in the livestock market, making biotechnology a standout in the global animal reproduction scenario, surpassing other reproductive biotechnologies (DAYAN, 2001). The use of IVP has shortened the genetic improvement timeline by up to 10 years, enabling the maximization of the reproductive potential of high-value bovine females, reducing intergenerational intervals, and increasing the number of offspring (SILVA et al., 2015; SOUZA; ABADE, 2019).

IVP comprises several stages that directly influence the outcome. These stages include follicular aspiration (OPU), selection of immature oocytes, *in vitro* oocyte maturation (MIV), fertilization of mature oocytes with capacitated and developed spermatozoa *in vitro* (FIV), and subsequently, the transfer of fresh or cryopreserved embryos. Oocyte harvest allows for the acquisition of a large quantity of oocytes (MACHATY et al., 2012). In cattle, oocyte retrieval can be performed through ovum pick-up, known as follicular aspiration (OPU), guided by ultrasound with the aid of a vaginal transducer that perforates the ovaries to collect the oocytes (GONÇALVES et al., 2008).

After oocyte harvesting, the following step is to track and classify the oocytes. The morphological aspect of the cumulus-oocyte complexes (COC) is evaluated for classification. Viana et al. (2004) described five grades of oocyte quality with specific criteria used to decide whether the oocyte can proceed to maturation and fertilization. The grades include: Grade I - COC with over three layers of cumulus cells, homogenous cytoplasm, and a clear nucleus; Grade II - COC with three or fewer layers of cumulus cells, slightly heterogeneous cytoplasm; Grade III - halo around the oocyte and partially naked; Grade IV - naked and expanded cumulus around the oocyte; Grade V - atretic, lack cumulus cells, and may exhibit granulations. Mariano et al. (2015) and Penitente Filho (2011) state that oocytes classified as Grade I and II are ideal for maturation and fertilization due to their higher likelihood of progressing to an embryo. Grade III can be used in cases with a low number of oocytes but has low viability, while Grade IV and V are discarded as non-viable.

There are numerous factors that can contribute to the reduction in the rate of *in vitro* embryo production, such as the collection of immature oocytes, donor age, transportation of oocytes to the laboratory, and poor oocyte selection (GOTTARDI; MINGOTI, 2009). Morphological classification is essential to select embryos suitable for fresh transfer or cryopreservation. Structures showing adequate growth are considered suitable for post-implantation development and have higher tolerance to freezing (SOUZA; ABADE, 2019). The criteria for classification are defined by the qualities and stages of embryo development, categorized accordingly (BATISTELA, 2023).

In this context, the present study aimed to assess the viability of utilizing super early heifers in the embryo aspiration and transfer program. Considering the quality of oocytes collected and embryos produced *in vitro* by these animals, the follicular aspiration technique (OPU), followed by oocyte selection and morphological classification, is crucial to ensure the viability of the produced embryos.

Material and methods

This study was conducted using retrospective data provided by a farm located in the municipality of Vilhena-RO. The animals were subjected to a pasture regimen predominantly consisting of *Brachiaria* and received protein supplementation with a consumption of 0.6% of their live weight, supplemented with Miyagi grass silage. The research utilized retrospective data from 23 animals aged 11 and 12 months who underwent follicular aspiration between September and October 2023. Following the aspiration, the oocytes were sent to the laboratory for selection, maturation, and fertilization. The follicular aspirations were performed using a portable ultrasound device equipped with a 7.5 MHz sectorial transducer adapted for intravaginal examination and guided by a biopsy needle. Disposable needles (20 G) were attached to a vacuum system with an approximate pressure of 50 mmHg or equivalent to a flow of 11 ml per minute. The follicular fluid and cumulus-oocyte complexes were aspirated into a 50 ml tube containing 10 ml of Dulbecco's PBS (DPBS), supplemented with 10% bovine fetal serum (Nutricell) and 100 IU of sodium heparin (Liquemine, Roche, Basel, Switzerland), heated to 36°C. All recovered oocytes were kept in cryotubes properly identified with buffered medium and transported to the laboratory within 3 to 12 hours at 37°C.

Counting and selection of oocytes

For classification, the methodology of Viana et al. (2004) was used. The oocytes were classified according to cytoplasm and the number of layers of cumulus cells based on the following criteria:

Grade I: The COC is compacted and has more than three layers of cumulus cells, and the oocytes have homogeneous cytoplasm. The nucleus shows a clear and apparent coloration.

Grade II: The COC is compacted and has three or fewer layers of cumulus cells, and the oocytes have slightly heterogeneous cytoplasm.

Grade III: Transparent halo around the oocyte, and partially denuded.

Grade IV: Denuded and with expanded cumulus around the oocyte.

Grade V: Atretic, lacking cumulus cells, and may show granulations.

In vitro embryo production

The *in vitro* maturation of COCs was performed in TCM 199 medium (Gibco) supplemented with 10% fetal bovine serum, FSH, LH, estrogen, pyruvate, and antibiotic, in an incubator at 38.8 °C, with 5% CO₂ in atmospheric air and 95% humidity, for 24 hours. The oocytes from each donor were placed in separate droplets in groups of 1 to 30 per droplet. The matured COCs were fertilized using proven bull semen. For sperm preparation, the Percoll gradient method (Invitrogen) was used. Fertilization was conducted in IVF medium containing heparin for approximately 18-22 hours, under the same atmospheric conditions as maturation. For the culture of presumed zygotes, CR4aa containing fetal bovine serum and BSA was used. On the third day, cleavage occurred, which is the separation of the structures where fertilization took place; the other structures that did not become zygotes were discarded. The embryos were co-cultured with granulosa cells under the same conditions as fertilization. On the seventh day of culture, the blastocysts were evaluated and classified according to their developmental stage (morula, early blastocyst, blastocyst, expanded blastocyst, and hatched blastocyst).

Results and discussion

Out of a total of 632 oocytes collected, 89% were viable, which corresponds to 562 oocytes subjected to the fertilization process. The oocytes classified as I to III proceeded to IVF, while those classified as IV and V were discarded. The data corresponds to the aspirated oocytes, viable oocytes, and total embryos produced (Table 1).

Table 1 - Representing the total collected and viable oocytes

Category	Total aspirated	Viable	Non-viable	% Non-viable
Heife	632	562	70	11,5%

Oocytes considered viable are grades I, II, and III; non-viable are grades IV and V, which are degenerated or expanded. The percentage of viable oocytes aspirated from heifers was 89%. In a study conducted by Batista et al. (2016), similar results were obtained, finding viability rates of 82% and 85% in two groups of evaluated animals. Furthermore, the quality of gametes in the studied category justifies greater viability compared to older animals.

Several factors influence the collection of oocytes, which are essential for maximizing the recovery rate. The quality and quantity of oocytes can be affected by breed, age, reproductive stage, climatic conditions, follicular characteristics, and experimental conditions involving the entire process of removing and transporting the ovaries to follicular aspiration, including technical skill and the time taken for the entire process (VASCONCELOS et al., 2022).

In a study conducted by Silva (2021), the production of oocytes in prepubertal heifers aged 11 months was evaluated, resulting in an average of 28.93 aspirated oocytes per animal, a value similar to the 27.47 found in this study. Furthermore, the viability of these oocytes was 20.60%, with a viability rate of 71%, which is lower than the 88.5% observed elsewhere. This difference is attributed to the nutrition of the animals; the author mentions that the body condition score of the evaluated animals influenced the results obtained. Regarding medium (5-8mm) and large (>8mm) follicles, fewer were observed in heifers compared to adult animals, according to literature data. This result can be explained by the immaturity of the hypothalamic- pituitary axis in prepubertal heifers, which causes a decrease in the concentration of hormones responsible for follicular growth (SANTOS et al., 2016), leading to underdevelopment. Therefore, prepubertal heifers tend to have smaller diameter follicles compared to older donors. A study conducted by Guerreiro (2015) evaluated the number of aspirated follicles from donors of different categories and did not observe any statistical difference. Similarly, Silva (2020) mentioned no difference between donor age and the number of produced oocytes, corroborating the results obtained in this research, where follicular production in heifers aged 11 and 12 months did not differ from literature regarding oocyte production in adult animals, supporting the use of this category in embryo transfer programs on farms for oocyte production. The data related to embryonic conversion are shown in Table 2.

Table 2 - Embryonary conversion

Category	Fertilized oocyte	Embryos obtained	% Conversion
Heifer	562	129	22,9%

According to Pires (2023), the embryonic conversion rate in Nelore cows was 30.8%, which demonstrates values above those found in the research conducted with heifers. This difference may be related to the collection method, culture conditions, semen quality used, or the age of the animal. According to Pinheiro et al. (2024), the average production of viable oocytes per donor was 29.94, and the conversion into embryos was 10.01 when involving adult animals of the Nelore and Senepol breeds.

These values are high and Camargo et al. (2005) reported that prepubertal heifers exhibited a lower blastocyst rate compared to cows. Therefore, the results observed in this study are consistent with the literature but can be interpreted positively, as the value obtained may make it feasible to use these animals as donors. As for the number of blastocysts produced and blastocyst rate, it indicates that using prepubertal or pubertal females will not affect *in vitro* embryo production. Consequently, using super-early heifers as donors in embryo transfer programs is a decision each farm can make or not, given that their embryonic production values are relatively lower. However, young donors allow for genetic testing of these animals at an early stage, increasing herd reliability.

Conclusion

The category of super early heifers has a lower rate of oocyte and embryonic production compared to that of cows. However, the strategic use of this category can be adopted in embryo transfer programs aiming for a shorter interval between generations.

Conflicts of interest

There was no conflict of interest between the authors.

Authors' contributions

Hemerson T. S. de Araújo e Lucas M. Roveda – data collection, interpretation of results and writing; Lucas W. de A. Soares – data collection and interpretation of results; Sallomão D. dos S. Lima – data collection; Carlos A. G. Leite – text review, guidance and corrections.

Financial support

Faculty of Veterinary Medicine Marechal Rondon – FARON.

Acknowledgments

The Faculty of Veterinary Medicine Marechal Rondon – FARON – and the companies that supply the inputs.

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Recebido em 30 de janeiro de 2025
Retornado para ajustes em 7 de junho de 2025
Recebido com ajustes em 10 de julho de 2025
Aceito em 25 de julho de 2025