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Impact of Corpus Luteum on *in vitro* Oocytes Recovery and Quality from Ovaries of Local Breeds of Cattle

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In vitro embryo production is critical for accelerating genetic improvement in livestock. Nigerian cattle breeds were not exposed to assisted reproductive technology other than artificial insemination techniques. The efficiency of producing embryos using *in vitro* technologies rarely exceeds the 40% threshold, indicating that the proportion of oocytes that fail to develop after *in vitro* fertilization and culture is significant. The aim of this study was to determined oocytes collection and grading. Seventy-five bovine ovaries were collected from Sokoto Modern Abattoir and transported in a thermos container maintained at 37-38ºC and processed in the laboratory. The ovaries were categorized based on the presence or absence of corpus luteum. The oocytes were recovered using aspiration technique. The oocytes transferred in minimum essential medium (MEM) and were graded using stereomicroscope based on cumulus investment and the nature of the cytoplasm into four grades: (I-IV). Greater numbers of oocytes were aspirated from ovaries without corpus luteum (CL- ovaries) 156 cumulus oocytes complex than those with corpus luteum (CL+ ovaries) (60 COCs). The CL- ovaries contributed higher normal oocytes (Grades I and II) than that of CL+ ovaries. It is highly recommended that ovaries without corpus luteum should be utilized for *in vitro* oocytes recovery and possible embryo production.

Keywords: Corpus luteum, Cattle, Grading, Ovaries, Oocyte.

1. Introduction

In vitro embryo production (IVEP) is a very promising area of reproduction. It offers a unique opportunity to study early mechanisms involving the control of reproductive physiology and significant benefits for its application within the livestock industry as well as in biomedical sciences (Rodrigo & Barros, 2020). Furthermore, (IVEP) is an indispensable assisted-reproductive technology which has numerous applications in production of superior genetic merit animals through *in vitro* maturation (IVM), *in vitro* fertilisation (IVF) and *in vitro* culture of presumptive zygote (Mukherjee et al., 2023). Reproductive biotechnologies encompass techniques such as estrous synchronisation, semen technology and analysis, ovum pick-up, superovulation, artificial insemination (AI), *in vitro* fertilisation (IVF), embryo transfer and methods for detecting and monitoring pregnancies. Preserving gametes from valuable, highly productive animals and endangered species offers significant benefits. These technologies have substantially advanced animal reproduction and driven genetic progress in the cattle industry

(Xu et al., 2020).Genetic advancement in cattle in Nigeria has seen limited progress over the years. The embryo transfer technique utilises exceptional cows to generate outstanding offspring (Eguizabal et al., 2019). This may result in an accelerated genetic gain hence complementing the AI program (Xu et al., 2020).

Over the last 10-15 years, after a dramatic development of cellular biology, a lot of research efforts have been moved towards the implementation of embryo technologies involving multiple ovulation and embryo transfer (MOET), in vitro production (IVP) of embryos, cloning and transgenesis to transfer a targeted number of embryos and among all, IVP of embryos has become a routine method of producing embryos from abattoir derived ovaries with minimal cost

(Mukherjee et al., 2023)
The ovaries are the main reproductive organs in bovines responsible for producing eggs. Structures like follicles and the corpus luteum are located on the ovary's surface. Following ovulation, the remaining follicle cells first form a corpus hemorrhagicum, which fills the ruptured follicle's cavity. Subsequently, under the influence of luteinizing hormone, the granulosa cells lining the cavity proliferate to develop into a corpus luteum. Based on this, ovaries can be categorized into two types: those with a corpus luteum (CL+) and those without (CL-). At birth, each ovary contains thousands of oocytes, most of which are lost over time due to atresia. The process of *in vitro* embryo production (IVEP) in cattle begins with the collection of oocytes from either live animals or those slaughtered (Wani, 2021).

A cow typically releases a single egg during ovulation, and if fertilization occurs naturally, it gives birth to one calf after an average gestation period of nine months. This process results in slow genetic progress (MAMY et al., 2017). *In vivo* matured oocytes can be obtained either by surgical or laparoscopic methods (Wani, 2021), but these methods are expensive and the number of oocytes recovered per ovary are very small (Polyzos et al., 2018).

Nigerian cattle breeds have primarily been exposed to artificial insemination (AI) as the sole form of assisted reproductive technology. The use of slaughterhouse ovaries for in vitro embryo production (IVEP) holds significant value, not only for the cost-effective mass production of cattle embryos for agricultural purposes but also as a research tool to enhance cattle genetic traits at an accelerated rate (Wani, 2021b). The aim of this study was to determined oocytes collection and grading in slaughterhouse bovine ovaries.

2. Materials and Methods

A total of 92 bovine ovaries were collected from a slaughterhouse and transported to the laboratory in a thermos container maintained at a temperature of 37-38°C. Processing was completed within two hours of collection. The mesovaria were trimmed using scissors, and the ovaries were disinfected with 70% alcohol before being washed three times in warm (37°C) physiological saline. The ovaries were then categorised into two groups based on the presence or absence of a corpus luteum: Group A included ovaries with a corpus luteum (46 ovaries), while Group B consisted of ovaries without a corpus luteum (46 ovaries).

2.1. Oocytes Recovery

Follicular fluid containing cumulus-oocyte complexes (COCs) was aspirated from antral follicles with diameter ranges between 4 and 8 mm using an 18-gauge needle attached to a 10 mL syringe. The aspirates were transferred into 15 mL tube containing 2.5 mL of minimum essential media (MEM). The tubes were stored in the incubator for 30 minutes to allow the oocytes to settle down at the bottom. The supernatant was aspirated and the oocytes were transferred into a Petri-dish containing 7 mL of MEM.

2.2. Oocyte Grading

The oocytes were observed under stereo-microscope and graded based on compactness, number of layers of cumulus cells and homogeneity of cytoplasm (Cognié et al., 2003).
Grade I: Oocytes with homogenous cytoplasm and surrounded by more than five layers of un-expanded cumulus cells.

Grade II: Oocytes surrounded by three to five layers of cumulus cells with homogenous cytoplasm.

Grade III: Oocytes surrounded by one to two layers of cumulus cells with non-homogenous cytoplasm.

Grade IV: Oocytes without cumulus cells (Denuded oocytes)

The oocytes were graded according to (Yahia Khandoker et al., 2001).

Quality grade I and II: normal (cultivable oocytes)

Quality grade III and IV: abnormal (non-cultivable oocytes)

Statistical Analysis

Descriptive statistic was used to analyse the data. Data was presented as percentage.

3. Results and Discussion

3.1. Results

In this study, a total of 216 oocytes were aspirated from 92 ovaries. Out of this, 60 (27.78%) oocytes were recovered from 46 ovaries with CL and 156 (72.22%) oocytes were found from 46 ovaries without CL.

The quality grades (G I-IV) of oocytes recovered from CL- ovaries are 40%, 24%, 19% and 17% respectively while those recovered from CL+ ovaries are 22%, 28%, 25% and 25% for G I-IV respectively (Table 4.1).

Table 1: Ovarian types and quality grades of oocytes recovered

Ovarian Type	No. Of ovaries	Total No. Of Cocs	***Cocs quality grades***			
CL +	46	60	I	II	III	IV
			13	17	15	15
CL _	46	156	63	38	29	26
Total	92	216	75	55	43	41

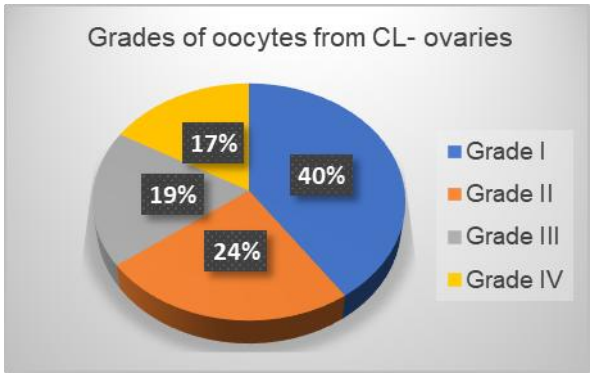

Figure 1: Showing the percentage of oocytes per quality grade recovered from ovaries without corpus luteum

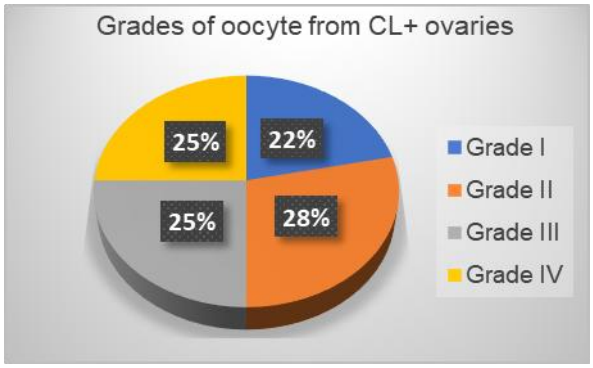

Figure 2: Showing the percentage of oocytes per quality grade recovered from ovaries with corpus luteum

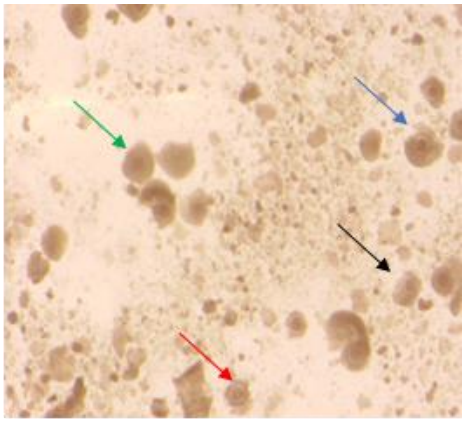

Plate 1: Green arrow: Grade I: Oocytes with homogenous cytoplasm and surrounded by more than five layers of un-expanded cumulus cells, Blue arrow; Grade II: Oocytes surrounded by three to five layers of cumulus cells with homogenous cytoplasm. Black arrow; Grade III: Oocytes surrounded by one to two layers of cumulus cells with non-homogenous cytoplasm and Red arrow; Grade IV: Oocytes without cumulus cells (Denuded oocytes)

3.2. Discussion

From the observation of this study, it was shown that among the 92 ovaries collected from abattoir, 46 ovaries were having no corpus luteum and 46 ovaries were having corpus luteum.

This result showed that more oocytes were recovered from the ovaries without corpus luteum. The higher number of oocytes found in ovaries without corpus luteum is likely as a result of hormonal changes. The presence of corpus luteum in cyclic ovary causes a surge in progesterone hormone production, giving a negative response to anterior pituitary gland for the restriction of gonadotrophin secretion which in turn leads to follicular atresia and inhibition of the development of large follicles (Hall, 2019). This result corroborates the findings of (Mondal et al., 1970)and also aligned with the work of Mondal et al. 2008 using goat ovaries.

Interestingly more quality grades I and II COCs were recovered from ovaries without corpus luteum than grades III and IV COCs compared to ovaries with corpus luteum (Table 4.1). When ovaries had a corpus luteum, the oocyte recovery rate decreased (Nandi et al., 2000). This is because there will be restriction of follicular development as lutein cells occupy most of the ovary (Kumar et al., 2004). Our findings were strongly supported by other researchers who have done their research in goat (MAMY et al., 2017; Yahia Khandoker et al., 2001). The quality grade of oocyte is affected by several factors such as nutritional status of the animal, hormonal imbalance and the stage of the oestrous cycle. In our study, the ovaries with corpus luteum are from animals at luteal phase, during this phase, the concentration of progesterone is high which in turn reduced the follicular activity, decrease estrogen and quality of the oocytes. The number of oocytes is also negatively affected due to negative feed-back sent by high progesterone surge. The emergence of dominant follicle inhibits further development of subordinate follicles thereby reducing the quality of follicular fluid and ultimately decreased in the quality of the oocytes.

4. Conclusion and Recommendations

4.1. Conclusion

Comparatively higher number and quality grades of COCs (I and II) were obtained from CL negative ovaries, indicating that ovaries without CL are more suitable for COCs recovery for initiation and optimization of *in vitro* embryo production.

4.2. Recommendations

Similar study should be conducted to cover other species of animals. Further research needs to be conducted on *in vitro* maturation, fertilization and culture of embryos; this is to bring out the full potentials of the local breed of cattle in the area of assisted reproductive technology

Authors' Contributions

Shehu Sidi conceived the ideal. Shehu Sidi, Muhammad Sanusi Yahaya, Shuaibu Musa Andarai and Adamu Umaru conducted the experiment. Shehu Sidi, Aliyu Ibrahim Musawa and Mahmud Abdullahi Saulawa prepared and reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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