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Quality of Oocytes Obtainded from Abattoir-Derived Ovaries Kept at 37ºC and 5ºC

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ABSTRACT

Oocyte quality is a determinant of successful *in vitro* oocyte maturation. This study was conducted to determine quality of oocytes obtained from ovaries of cows slaughtered at Ubakala abattoir, Umuahia, South East Nigeria and stored at 37ºC and 5ºC from time of ovary collection at the abattoir to the time of processing in the laboratory. Oocytes' aspiration was done according to standard technique. The aspirated oocytes were qualified following a standard grading system (I-IV). Ten ovaries were processed for both storage systems. The number of Grade 1 oocytes in the ovary kept at 37ºC was 6 while that kept at 5ºC was 9. With grade II oocytes, an equal number of seven oocytes were recorded for the two storage systems. The number of Grade III oocytes obtained in ovaries stored at 37ºC and 5ºC were 23 and 30, respectively. However, there was no significant difference (P>0.05) in the number of oocytes of various grades obtained from ovaries kept at 37ºC and 5ºC immediately after animal slaughtered to the time of aspiration in the laboratory. On the aggregate, a greater number of oocytes were obtained from ovaries kept at 5ºC than those kept at 37ºC, though more quality oocytes (I and II) were obtained with 37ºC. Generally, the study showed low number of oocytes obtained from the two systems primarily due to malnourishment and old age of the cows slaughtered in the abattoir. Having yielded relatively more quality and cultivable oocytes, storage at 37ºC is preferred to storage at 5ºC.

Keywords: Abattoir, cow, oocytes, ovaries, Umuahia.

INTRODUCTION

Animal proteins are preferred to plant protein because they contain essential amino-acids which are absent in plant protein while these amino-acids are essentially required of body growth and repair. Unfortunately, credible data from Food and Agriculture Organization indicated low consumption of animal protein in developing countries with associated malnutrition especially in children (FAO 2013).

Livestock industry apart from being a major source of animal proteins for human consumption in form of meat, milk and egg, it also provides means of livelihood for millions of people directly or through associated value chain, creates wealth and perhaps, contributes significantly to national gross domestic product (Herrero *et al*., 2013). The urbanization of livestock production in developing countries has failed to add value chain to broaden the concept of food security beyond supply (Assem *et al*., 2019).

Livestock population in Nigeria is among the highest in Africa. It comprises about 34.5 million goats, 22.1 million sheep and 13.9 million cattle (Lawal-Adebowale, 2012). Diseases, malnutrition and poor breeds and lack of improved breeding technique to improve the indigenous breeds are some of the factors limiting cattle production. Higher percentage of cattle population lies with the Fulanis whose mode of rearing is nomadic system that has constituted a serious impediment to adaptation of progressive biotechnologies into cattle breeding (Ameh, 2017).

Nigerian breeds of cattle exhibit reproductive inefficiency expressed as low fertility, prolonged age of puberty, low milk production (for dairy) and low rate of feed to meat conversion as well as longer generation intervals in farm animals. Unfortunately, several assisted reproductive technologies (ARTs) that have been developed and capable of correcting these anomalies have not been integrated into livestock breeding programme in

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Nigeria. Such ARTs include artificial insemination (AI), *in vitro* embryo production (IVEP), multiple ovulations, and embryo transfer (MOET). Only AI has been utilized in Nigeria to a greater extent, others are not even at the experimental level due partially to lack of technical expertise and high cost of facilities to conduct them (Raheem *et al*. 2023). Allan *et al.* (2024) has earlier identified small number of studies directed at reproductive performance of cattle in Nigeria.

Specifically for cattle production in developed countries, globally acknowledged meat and dairy producers, *in vitro* fertilization (IVF) and embryo transfer (ET) are two associated reproductive biotechnologies that have been used extensively to improve cattle reproductive capacity with a marked over a million number of *in vitro* produced embryos in 2022 (Viana, 2022). Brazil is one of them and remains a global leader in the exportation of meat due to adequate utilization of these technologies namely IVF and ET (Rodrigues, 2018). *In vitro* oocyte maturation is a requisite technology for the conduct of IVF. Good oocyte quality is the first and most important step in achieving successful *in vitro* oocyte maturation. Meanwhile, these oocytes are obtained from the ovary through ovum picked up in life animals or abattoir –derived ovaries immediately post slaughter (Boni, 2012).

To the best of our knowledge, there is dearth of information on the quality of oocytes that are obtainable from abattoir-derived ovaries in Nigeria. Therefore, the objective of the present study was to determine the quality of oocytes aspirated from cow ovaries stored at two different temperatures (37ºC and 5ºC) after cow slaughter to the time of processing in the laboratory. The study would also show the potential of using these oocytes for further reproductive biotechnologies such as *in vitro* oocytes maturation and *in vitro* fertilization.

MATERIALS AND METHODS

Study Area

This study was conducted during the period from February to October 2018 at Umuahia, South east Nigeria. The ovaries were collected from Ubakala abattoir, Umuahia, Abia State, Nigeria and taken to the Theriogenology laboratory of Department of Theriogenology, Michael Okpara University of Agriculture, Umudike for further processing. Abia State is in the South-eastern part of Nigeria. Umuahia, the capital of Abia State is located at longitude 7.5º and latitude 5.4º and has a total land mass of 5,243.7 km^2. The State has about 2.8 million people according to National Population Commission (2006) and has many agrarian communities.

Oocyte collection and handling

Immediately after slaughtering of the cow, the ovaries were removed by section of the broad ligament using scissors as described in previous study (Marei *et al.,* 2017). Then, they were individually introduced into tapered tubes containing isotonic saline (0.9% NaCl) supplemented with penicillin-streptomycin (100 IU / ml penicillin and 100 µg/ml streptomycin) and labeled with identification. After, the samples were divided into two groups according to the preservation temperature. The first group tubes had the collection medium warmed to 35-37℃ in an isothermal container. The tubes of the second group were placed in an isothermal container at a temperature of 5°C using ice block transported to the laboratory and kept in the refrigerator at 5ºC until ready for processing. The samples were transported to the laboratory within 90 mins post slaughter. A total of 20 ovaries were collected. Three visitations were made to get the required number of ovaries and during each visitation, equal numbers of oocytes were processed for each temperature during each visitation.

Oocyte aspiration grading

On getting to the laboratory, a few tissue papers were spread on the table and sprayed with 70% ethanol. With the aid of a sterile disposable 10ml syringe and 18–19-gauge needles, follicular fluid (containing oocytes) was aspirated as was described in previous study (Marei *et al.,* 2017). All visible antral follicles which ranged between 2-8 mm in diameter were aspirated before leaving the ovary. To avoid leaking of the follicular fluid, puncturing from the top was avoided; rather, ovary was punctured from behind the follicle from underneath. The negative pressure maintained on the ovary by pressing it between the fingers holding it facilitated the

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sucking of the follicular fluid into the syringe such that produced air sucking sound when the needle was taken out of the follicle. The syringe contained a small volume of washing media (*In vitro* Bioscience, UK) before the commencement of aspiration. The amount in the syringe was transfer into 50 ml sterile conical centrifuge tube to settle for about 5 minutes before oocyte grading.

Oocyte Grading

The follicular fluid was poured into a petri dish and observed under a microscope in search of oocytes at the lowest magnification of microscope (×4). The Stereomicroscope is the ideal microscope to use. The binocular was impoverished by removing the other objective lens but leaving only × 4 lens to create enough space for picking the sighted oocyte with a micropipette into four different micro drops of washing media already placed in another petri dish.

The oocytes were selected and counted into four grades based on their morphology especially the cumulus oophorus complex (COC) as described previously (de Loos *et al*., 1989, Fouladi-Nashta *et al*., 2007). Grade I COCs were characterized by dark homogenous ooplasm and more than four compact layers of cumulus cells. Grade II COCs comprised dark homogenous cytoplasm and more than four layers of less compact cumulus cells starting to expand peripherally. Poor quality COCs of grades III contained COCs with heterogeneous granulated ooplasm and grade IV had oocytes completely or partially denuded of cumulus cell (Fouladi-Nashta *et al*., 2007).

Percentage cultivable oocytes (PCO)

Only grades I and II oocytes are usually used for *in vitro* maturation while grades III and IV are rated poor quality and discarded during this technology (Kouamo *et al.,* 2014). Therefore, the percentage cultivable oocytes represented the percentage of the number of oocytes of grade I and II and was calculated and expressed as a percentage to the total number of oocytes grades (grades I-IV).

$$PCO = \frac{\text{Number of grades I + II} \times 100}{\text{Total number of grades I+II+III+IV}}$$

RESULTS

Oocytes' grading obtained from ovaries kept at 37^{o}C and 5^{0}C

On the aggregate, a greater number of oocytes were obtained from ovaries kept at 5^{o}C than those kept at 37^{o}C (Table I). The number of Grade 1 oocyte in the ovary kept at 37^{o}C was 6 while that kept at 5^{o}C was 9 representing 13.1% and 12.75% respectively. An equal number of seven oocytes were recorded for the two storage systems. The number of Grade III oocytes obtained in ovaries stored at 37^{o}C and 5^{o}C were 23 and 30 respectively. In the same trend with the former, the ovaries kept at 37^{o}C yielded more Grade IV oocytes that those ovaries kept 5^{o}C. However, there was no significant difference (P>0.05) in the number of oocytes of various grades (Figure I) obtained from ovaries kept at both 37^{o}C and 5^{o}C immediately after animal slaughtered to the time of aspiration in the laboratory.

Table I. Oocytes grading obtained from ovaries kept at 37^{o}C and 5^{o}C

	Ovary kept at 37^{o}C N=10		**Ovary kept at 5^{o}C N=10**		**P values**
	no	%	no	%	
Grade 1	6	13.1	9	12.7	0.133
Grade II	7	15.2	7	9.9	0.177
Grade III	10	21.7	25	35.2	0.182
Grade IV	23	50.0	30	42.2	0.131
Total number	46	100	71	100	
Average number of oocyte per cow	4.6		7.1		

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Quality Index and percentage quantifiable

The quality index of the oocytes obtained from ovaries kept at 37°C was 3, while that of oocytes obtained from ovaries kept at 5ºC was 2.5. The percentage oocyte cultivable in ovary stored at 37°C was 28.2% while that of ovaries stored 5°C was 22.5%.

Table 4.2. Quality index of oocytes obtained from ovaries kept at 37°C and 5°C

	Ovary kept at 37ºC	**Ovary kept at 5ºC**
Quality index	3	2.5
Percentage Cultivable oocytes	28.3%	26.3%

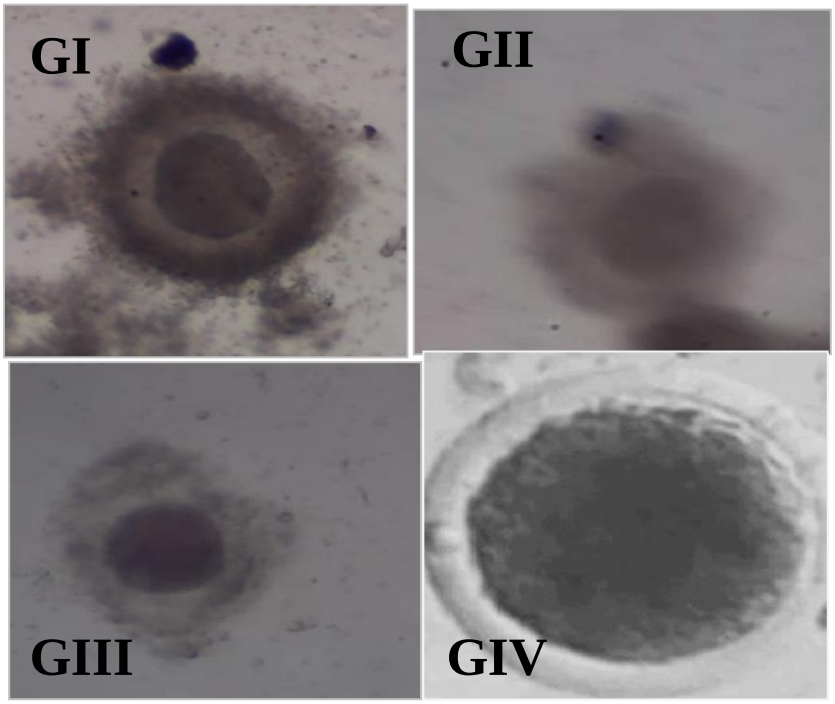

Figure 1. Representative Grade I-IV oocytes obtained from cow ovaries stored at 37ºC and 5ºC. Acronyms: COC- cumulus oophorous complex, o-oocyte

DISCUSSION

There was low level of oocytes obtained from the ovaries through the two storage systems used in the present study. This is contrary to report of Dorice *et al*. (2019) where 1822 oocytes were harvested from 3888 follicles collected from 95 cows. The disparity is attributable to different geographical location of the study (Nigeria verses Cameroon), different breeds of cattle involved, age, body condition score, and nutritional status of cows slaughtered at the abattoirs where the ovaries were collected. The aforementioned altogether affect the ovarian dynamics (Kątska & Smorąg, 1984; Kouamo *et al.*, 2015; Adams & Singh, 2021) and subsequently, the viable and number of oocytes present in the follicular fluid.

Characteristically, the number of oocytes obtained from an ovary is a function of the number of follicles in that ovary which is directly related to the stage of oestrus of the cow just prior to slaughter (Adams & Singh, 2021). Oogenesis is the development of oocyte from primordial germ cell to secondary oocytes and occurs simultaneously within the follicle and along with folliculogenesis (Diop & Cisse, 1994), however, studies of Eppig *et al*. (2002) showed that the number of oocytes actually determined the follicular development and amount of follicular fluid.

The percentage oocyte cultivable in ovary stored at 37°C was 28.3% while that of ovaries stored 5°C was 22.5%. These results are far lower than 58% cultivable oocytes obtained in precious study reported by Natumanya *et al.* (2013) using Ankole breeds of cattle. The essence of this technique is essentially to obtain

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good quality oocytes that could be further cultured and used for *in vitro* maturation. Despite producing a higher aggregate number of oocytes at 5ºC, higher oocyte quality percentage was obtained with storage at 37ºC and hence, using the latter temperature in future studies that have to do with *in vitro* maturation of oocyte is reasonable.

The age of the cow brought to the abattoir for slaughter was not determined; however, the body condition showed they were highly malnourished and old in age. Reproduction is a secondary characteristic and hence such cows may no longer be cycling or cycling with low rate of folliculogenesis that culminates into formation of antral follicles and subsequently fewer number oocytes (Boland *et al.,* 2001).

There are limited studies on this theme like other reproductive biotechnologies in Nigeria. Low utilization of ARTs was identified as one of the major factors responsible for low productivity in dairy and beef cattle breeding and production in Nigeria (Oguejiofor 2019). The only study obtained was done in goat experimentally infected with Trypanosome (Leigh 2019). Apathy in this line of research was the motivation to design this research.

Ovaries collected from the abattoir is favoured compared to others sources because the method is most convenient and abundant source of primary oocytes for large scale production of embryo through *in vitro* maturation and IVF (Kouamo *et al*., 2014). However, IVF studies that required *in vitro* maturation of oocyte and hence oocyte aspiration of abattoir-derived ovary are lacking in Nigeria specifically and to a lesser extent in Africa generally. This is further buttressed by a reliable data provided by International Embryo Technology Society, where no single abattoir-derived embryo was recorded for Africa in 2022 (Viana 2022). Crucial to abattoir-derived embryo technology is oocytes aspiration and quality assessment to choose grade I and II oocytes for *in vitro* maturation.

There are other methods of oocytes' collection from the ovaries. During the earliest development of this technique, slicing, follicle puncture and follicle aspiration were methods of choice and have been compared (Das *et al.,* 1996). Ovum pick up was a later technique and used in life animal but required special instrument for transvaginal ultrasound-guided follicular puncture that are not readily available in resource-limited settings. Higher percentage of oocytes used for IVF and subsequently embryo transfer is derived from ovum pick-up (Chinarov, 2024). However, aspiration reported yielded moderate oocyte count of high-quality oocyte and good embryo production post IVF than slashing and slicing (Saleh, 2017) and remains a desirable technique in low resource settings.

The underlying reason of aspirated oocyte in this study is for possible use for *in vitro* maturation and subsequently IVF, therefore, oocytes aspiration with ovaries stored at 37ºC is justifiable in future studies. And aspiration is favoured due to its simplicity and nonrequirement of any sophisticated instrument such as ultrasound.

The stage of oestrus determines the structure found on the ovary at every point in time. During luteal phase, the predominant structures on the ovary are the corpus luteum while follicles are predominant during the follicular phase. Ovary harvested during the later stage is certainly going to yield more follicles and hence more oocytes than the former. This coupled with collection method can influence the amount of cumulus oophorus complex obtainable from such ovary (Khandoker *et al.,* 2016). Most of the cows slaughtered in the abattoir were cachectic, aged and almost going off their reproductive age. These factors are responsible for the low level of oocytes derived from the ovary in the present study when compared to similar studies elsewhere (Dorice *et al.,* 2019; Kouamo *et al.,* 2014)

CONCLUSION

There was no significant difference in the quality of oocytes obtained from ovaries stored at 37ºC and 5ºC after cow slaughter to the time of processing in the laboratory. Generally, the number of oocytes obtainable in the cows during the period of the study was quite low and is attributable to the malnourished status and old age of the cow slaughtered during this period. Limitation of a small sample size was recognized and further studies with larger population size are suggested.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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