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Ovarian and oocyte characteristics of abattoir-sourced ovaries from cattle breeds in Malawi and their implications for *in vitro* embryo production

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Abstract

This study evaluated the influence of animal and ovarian characteristics on antral follicle count (AFC) and oocyte quality in local cattle breeds for use in *in vitro* embryo production (IVP) in Malawi. A total of 106 ovaries were collected from Malawian zebu (MZ; n = 48), Brahman (n = 26), and Holstein Friesian (HF; n = 32) cows. Ovaries underwent morphometric analysis before oocyte recovery and qualitative grading. Average ovarian weight and volume were highest in HF (5.4g and 3.0 cm^3), intermediate in Brahman (4.4 g and 2.7 cm^3), and lowest in MZ (2.3 g and 2.0 cm^3). Younger cows (≤ 6 years) and those with good body condition score (BCS) had larger and heavier ovaries across all breeds. Average AFC was significantly higher in the HF (n = 35.4) than in Brahman (n = 20.9) and MZ (n = 18.4). However, the proportion of high-quality oocytes was higher in Brahman and MZ (72.8 and 75.3%) compared to HF (66%). Multivariate regression analysis showed that breed, younger age, and BCS were significant positive predictors of AFC and oocyte yield. However, logistic regression analysis showed no influence of these factors on oocyte quality across the breeds. We conclude that the *Bos indicus* Brahman and MZ have uncompromised oocyte quality despite lower ovarian morphometrics and AFC compared to the *Bos taurus* HF cows under Malawian conditions. Further, modifiable factors such as age and BCS could play a key role in donor selection to optimize success rates.

Introduction

In vitro embryo production (IVP) is one of the assisted reproductive technologies (ARTs) that has gained global recognition among prominent beef- and dairy-producing countries worldwide (Ferré et al. 2020; Maciel et al. 2025; Yusuf 2024). According to the International Embryo Technology Society (IETS), the total number of *in vitro*-derived embryos reported across several developed countries has surpassed that of *in vivo*-derived counterparts that used traditional Multiple Ovulation and Embryo Transfer (MOET) since 2016 (Viana et al. 2024). IVP involves oocyte collection, commonly via ultrasound-guided oocyte pickup or direct aspiration of oocytes from abattoir-sourced ovaries, followed by *in vitro* maturation (IVM), fertilization (IVF), and subsequent embryo culture (IVC) (Honneysett et al. 2021; Wani 2021). Embryos, typically at the late morula or prehatching blastocyst stage, can then be transferred to surrogate mothers or cryopreserved for future use (Souza-Fabjan et al. 2023). Accordingly, the use of IVP, together with recent advances in sex sorting (SS) and genomic selection (GS), is enabling accelerated genetic gain in cattle, as has been demonstrated in North America, South America, and Europe (Menchaca 2023). Moreover, a wider range of potential female donors is available for oocyte collection, including open-cycle females and those up to 3 months pregnant (Menchaca 2023; Yusuf 2024). In this context, IVP entails a reduced sperm requirement for embryo production (Claes and Stout 2022; Herrick 2019), while the associated SS technology increases the chances of obtaining the desired sex of offspring (Yodrug et al. 2025). In the end, the transfer of *in vitro*-derived blastocysts, which are more thermotolerant than earlier embryonic stages, reduces heat stress-induced pregnancy losses by bypassing earlier development in the oviduct (Gómez-Guzmán et al., 2024; Hansen, 2019, 2020), making it a legitimate strategy to mitigate the impact of heat stress on bovine reproduction.

Despite these attributes, IVP has been poorly adopted by many developing countries in the sub-Saharan African (SSA) region, a situation that, if overturned, could improve food security and resilience to heat stress in this region. Thus, developing countries in SSA should consider implementing these technologies, tailoring them to suit their environment and socioeconomic contexts. Although the lack of relevant infrastructure and resources poses a major bottleneck to implementing this technology (Nengovhela et al. 2021; Yusuf 2024), the suitability of locally available breeds, particularly in terms of their genetic traits, needs to be evaluated before implementing IVP. Indeed, physiological differences between *Bos indicus* and *Bos taurus* phenotypes, which translate into variations in follicular dynamics, size, and antral follicle count (AFC), are well established (Baruselli et al. 2017; Sartori et al. 2016; Velazquez 2023)

Several studies have demonstrated that *Bos indicus* breeds present superior IVP outcomes compared to the *Bos taurus* breeds in tropical environments.Pontes et al. (2010) reported that Gir (*Bos indicus*) cows yielded significantly more oocytes and embryos per ovum pick-up (OPU) session compared to Holstein cows. Similarly,Oliveira et al. (2019) found that Gyr donors had more oocytes per OPU and higher viable oocyte rates than Holstein donors. A comprehensive review byBaruselli et al. (2021) concluded that *indicus* cattle generally have more follicles, yield a greater number of oocytes, and have better IVP outcomes compared to *taurus* cattle. Furthermore,Ferreira et al. (2011) attributed this superiority to the greater heat tolerance of *Bos indicus* breeds, which enables them to maintain oocyte quality even under thermal stress conditions. While oocyte yield tends to be breed-dependent, embryo yield could further depend not only on breed and the number of recoverable viable oocytes (Lacerda et al. 2020; Pytel et al. 2024), but also on the quality of oocytes collected (Demetrio et al. 2020). The latter, which can be determined based on the surrounding cumulus cells and the nature of the cytoplasm, is particularly critical in IVP success, as it correlates with fertilization (Aguila et al. 2020; Emanuelli et al. 2018), maturation (Silva et al. 2021), and the developmental competency of bovine oocytes *in vitro* (Aguila et al. 2020; Chelenga et al. 2023; Pytel et al. 2024).

Malawi is a landlocked nation located in the SSA. The growth of the Malawian dairy and beef sectors has long been stagnant, partly due to inferior productivity and fertility traits of the local breeds, which are dominated by the Malawian Zebu (MZ), followed by Brahman breeds (Butkiewicz et al. 2024; Chawala et al. 2021), and the adaptation hurdles faced by the exotic *Bos taurus* breeds, i.e., Holstein-Friesian (HF) and Jersey, on the other hand (Ruane et al. 2023; Nanganga and Safalaoh 2020). *Bos indicus* cattle are well adapted to tropical environments, exhibiting reproductive resilience under heat stress and nutritional challenges, with relatively stable fertility performance that supports consistent calf production (Copley et al. 2024). These adaptive traits underscore their importance in sustainable beef productivity and make *Bos indicus* breeds valuable genetic resources for livestock improvement programs in tropical regions (Reddy and Anitha 2024). As the country embarks on a large-scale mega-farm program that partly aims to improve productivity and genetic gain in MZ and Brahman breeds, interest in IVP and the associated technologies (i.e., SS, GS, ET) has increased. While the MZ and Brahman breeds have proven to be hardy and well-adapted to the tropical environment (Burrow 2012; Nyamushamba et al. 2017; Rege, 2026), their

low fertility traits compared to HF (Baruselli et al. 2017; Khan et al. 2025) pose a critical question regarding their suitability for IVP programs in Malawi.

The objective of this study was to evaluate the suitability of common local cattle breeds for use in IVP programs by determining how animal-level traits, such as the breed, average body condition score (BCS), and age at the time of oocyte collection, influence ovarian morphometrics and oocyte quality in MZ and Brahman breeds, relative to the widely studied HF breeds. Specifically, the study aimed to determine 1) the influence of cow's breed, age, and BCS, as well as the presence of CL at the time of oocyte retrieval, on the ovarian morphometrics (i.e., weight and volume), 2) the average AFC, as well as the recovery rate and quality of oocytes collected from ovaries derived from MZ, Brahman, and HF breeds, and 3) the relationship between intrinsic animal-level traits and AFC, oocyte recovery, and quality. By generating this data, the study establishes a foundational understanding of the plausibility of implementing a successful IVP program for genetic improvement of local cattle breeds in Malawi.

Materials and Methods

Ethical approval

This study used slaughterhouse ovaries, and no ethical approval is required.

Study area and sample collection

The study was conducted in Lilongwe District, the central region of Malawi. Ovaries were obtained from slaughtered cows at Lilongwe Beef Companies, i.e., Cold Storage and Bwemba Abattoirs, which are the two major abattoirs in the district. Overall, 106 ovaries were collected from 53 cows, comprising 24 Malawian Zebu, 13 Brahman, and 16 Holstein Friesian cows. Breed identification was based on abattoir records and phenotypic traits. The age of the cows was determined using the data provided by the veterinary officers at the abattoir. The BCS of each cow was determined visually using a five-point scale, following the standardised body condition scoring method described by Wildman et al. (1982) and validated for use in tropical cattle (*Bos indicus* and *Bos taurus*) by Vall et al. (2025).

Ovary collection and handling

Ovaries were collected within 10 minutes after slaughter. Left and right ovaries from each cow were placed in different conical tubes containing 0.9% normal saline solution. Samples were transported to the Biotechnology Laboratory, Lilongwe University of Agriculture and Natural Resources (LUANAR), within two hours in an insulated container maintained at between 15 and 25 °C to prevent exposure to extreme temperatures (Speckhart et al. 2023). Discolored (brown or yellow) or cystic ovaries were excluded from analysis.

Ovarian morphometry determination

In the laboratory, excess utero-ovarian ligaments were trimmed off using a pair of scissors. The ovaries were washed twice in 0.9% normal saline solution. Each ovary was placed on absorbent tissue paper to remove excess moisture. Ovarian weight was measured using an Ohaus analytical balance. A digital vernier caliper was used to measure length, width, and circumference dimensions. Ovarian size was determined based on its volume (cm^3), estimated using the prolate ellipsoid formula V= 0.52× L×W×T, where L, W, and T represent the longitudinal, transverse, and anteroposterior dimensions, respectively (Pavlik et al. 2000). The coefficient 0.52 approximates π/6 (≈ 0.523), established in clinical and veterinary research for estimating the size of ellipsoid organs such as ovaries (Pavlik et al. 2000).

Determination of antral follicle count

All follicles visible on the surfaces were counted macroscopically under adequate illumination. Data were recorded on each separate ovary before oocyte retrieval. Low AFC is typically defined as **≤ 15 follicles and high AFC as ≥** 25 follicles (Burns et al. 2005; Ireland et al 2008; Morotti et al. 2017; Mossa et al. 2012; Mossa and Ireland 2019).

Oocyte collection

To retrieve the cumulus oocyte complexes (COCs), follicular fluid was aspirated from the surface of each visible follicle using a 21-gauge needle attached to a 10 mL sterilized syringe, following the procedure previously reported by Pitroda et al. (2021). Aspirates were transferred to 35 mm petri dishes and examined under a stereo microscope (10× magnification) at room temperature. The COCs were searched for and isolated from debris and then collected using a pipette for further evaluation.

Oocyte Grading

The recovered COCs were graded morphologically into four categories based on cumulus cell layers and ooplasm homogeneity under a stereo microscope (Table 1), according to Pitroda et al. (2021).

Table 1: Grading criteria of retrieved oocytes

Grade	Morphology of Cumulus oocyte complexes (COCs)
GI	Oocytes with ≥4 compact cumulus cell layers and uniformly granulated cytoplasm.
GII	Oocytes with 2-4 compact cumulus cell layers and evenly granulated cytoplasm.
GIII	Oocytes with 1-2 compact cumulus cell layers and evenly granulated cytoplasm.
GIV	Denuded oocytes or with incomplete/expanded cumulus cell layers, exhibiting dark or uneven granulated ooplasm.

GI = Grade I, GII = Grade II, GIII = Grade III, GIV = Grade IV

The overall quality (grade) of the recovered oocytes was determined as an index, and the oocyte quality index was calculated as:

$(GI * 1 + GII * 2 + GIII * 3 + GIV * 4)/ Total\ oocytes\ recovered$ (Acar et al. 2013).

Oocyte quality index values close to 1 indicate better overall quality.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 20.0 to generate descriptive and inferential statistics. Continuous variables were expressed as mean ± standard error (SE) throughout. Normality of residuals was assessed using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test was used to evaluate breed differences in ovarian morphometry, AFC, and oocyte yield and quality (grades). Independent-samples t-tests were performed to compare binary outcomes (age, BCS, and CL presence) within each breed. Multiple linear regression was applied to determine associations between predictor variables (breed, age, BCS, ovarian weight, size, and CL presence) and continuous outcomes (follicle count and oocyte quality). A logistic regression model was used to estimate the probability of retrieving high-quality oocytes (Grade I and II). Statistical significance was declared at $p < 0.05$.

Results

Influence of animal-level characteristics on ovarian morphometrics

Evaluation of the relationship between cow characteristics and ovarian morphology (weight and volume) showed that breed and age significantly influence ovarian traits (Table 2). Notably, the mean ovarian weight and volume were significantly higher in HF cows compared to Brahman, with MZ having the significantly lowest values (5.4 ± 0.3 g and 3.0 ± 0.1 cm^3 vs. 4.4 ± 0.35 g and 2.7 ± 0.13 cm^3 vs. 2.3 ± 0.1 g and 2.0 ± 0.06 cm, respectively; $p < 0.05$ for all pairwise comparisons across breeds for both metrics). Younger cows (≤ 6 years) had heavier and larger ovaries than older cows (>6 years) across all breeds ($p < 0.05$). Further, ovaries from cows with a "Good" BCS were significantly heavier and larger than those from cows with a "Poor" BCS across all the breeds. However, the presence of a corpus luteum (CL) was associated with greater ovarian weight (5.7 vs. 3.7g; $p < 0.05$) and volume (3.3 ± 0.11 vs. 2.5 ± 0.15; $p < 0.05$) in the Brahman breed. However, there were no differences in ovarian weight or volume between MZ and HF breeds with or without CL.

Effect of breed on antral follicle count, oocyte recovery rate, and oocyte quality

The effect of breed on AFC, oocyte recovery, and quality is shown in Table 3. Ovaries from HF cows had a significantly higher AFC than those from Brahman and MZ cows (35.4 ± 4.3 vs. 20.9 ± 2.1 and 18.4 ± 1.4, respectively; $p < 0.05$). However, the proportion of oocytes recovered from MZ was significantly higher compared to the recovery rates from both HF and Brahman (56.1 ± 1.7 vs 52.3 ± 1.7 and 49.6 ± 3.9, respectively; $p < 0.05$). Intriguingly, the recovered oocytes from MZ and Brahman had a significantly greater proportion of high-quality oocytes (GI + GII: 75.3 ± 2.3% and 72.8 ± 3.6%, respectively) compared to HF (66.0 ± 2.0%; $p < 0.05$).

Relationship between animal-level characteristics and antral follicle count and oocyte quality

Multivariable regression analyses were conducted to quantify how animal- and ovarian-level characteristics can be used to predict AFC and oocyte quality (Table 4). The Brahman and MZ breeds were associated with a 46.8% and 39.9% reduction in the AFC, respectively, in comparison to HF, while younger cattle (≤ 6 years) and a “Good” BCS were significant positive predictors for increased follicle count ($p < 0.05$). Ovarian weight was positively associated with a higher AFC ($p < 0.05$), while CL presence showed no significant influence ($p > 0.05$). Interestingly, none of the examined predictors, including breed, age, BCS, ovarian traits, or CL presence, showed a statistically significant association with oocyte quality (Grade I+II) in the logistic regression model ($p > 0.05$). Thus, although the unadjusted breed comparison suggested differences in the proportion of Grade I + II oocytes, breed was not a significant predictor of high-quality oocyte recovery after adjustment for other animal- and ovarian-level factors

Table 2. Relationship between cow characteristics and the ovarian weight and volume across three different breeds of cows

Factor	Brahman			HF			MZ		
	N	Wt. (g)	Vol. (cm^3)	N	Wt. (g)	Vol. (cm^3)	N	Wt. (g)	Vol. (cm^3)
Breed	26	4.4 ± 0.35^{x}	2.7 ±0.13^{A}	32	5.4 ± 0.3^{y}	3.0 ± 0.1^{B}	48	2.3 ± 0.1^{z}	2.0 ± 0.06^{C}
Age (years)									
>6	14	3.6 ± 0.36^{a}	2.5 ± .14^{a}	26	5.2 ± 0.32^{a}	2.7 ± 0.14^{a}	44	2.3 ± .10^{a}	2.5 ± 0.11^{a}
≤ 6	12	5.4 ± 0.35^{b}	3.2 ± .21^{b}	6	6.5 ± 0.37^{a}	3.8 ± 0.36^{b}	4	3.0 ± .14^{b}	3.3 ± 0.12^{b}
Body condition score									
Good	12	5.7 ± 0.13^{b}	3.3 ± 0.09^{b}	20	5.9 ± 0.31^{b}	3.3 ± 0.16^{b}	16	2.9 ± 0.07^{b}	3.2 ± 0.05^{b}
Poor	14	3.3 ± 0.09^{a}	2.3 ± 0.10^{a}	12	4.5 ± 0.40^{a}	2.2 ± 0.05^{a}	32	2.1 ± 0.09^{a}	2.2 ± 0.05^{a}
Corpus luteum									
Present	10	5.7 ± 0.15^{b}	3.3 ± 0.11^{b}	24	5.3 ± 0.35^{a}	3.0 ± 0.21^{a}	32	2.5 ± 0.09^{a}	2.7 ± 0.14^{a}
Absent	16	3.7 ± 0.34^{a}	2.5 ± 0.15^{a}	8	5.6 ± 0.63^{a}	2.5 ± 0.27^{a}	16	2.0 ± 0.21^{a}	2.3 ± 0.14^{a}

[ABC] Superscript indicates a significant difference in ovarian volume across the rows

[xyz] Superscript indicates a significant difference in ovarian weight across the rows

[abc] Superscript indicates a significant difference within a column

Table 3: Relationship between breed and follicular count, oocyte recovery rate, and oocyte quality

Breed (n)	AFC	Oocyte yield (%)	Oocyte quality (%)				
			GI	GII	GIII	GIV	GI + GII
HF (32)	35.4 ± 4.3[a]	52.3 ± 1.7[a]	33.3 ± 1.8[a]	43.8 ± 4.5[a]	25.0 ± 4.4[a]	24.0 ± 3.1[a]	66.0 ± 2.0[a]
Brahman (26)	20.9 ± 2.1[b]	49.6 ± 3.9[a]	37.8 ± 4.2[b]	26.6 ± 4.1[b]	17.2± 3.3[ab]	10.7 ± 3.3[b]	72.8 ± 3.6[b]
MZ (48)	18.4 ± 1.4[b]	56.1 ± 1.7[b]	38.0 ± 2.4 [b]	29.8 ± 3.0[b]	12.5 ± 2.4[b]	9.9 ± 2.3[b]	75.3 ± 2.3[b]

Values within the same column with different superscripts ([a, b]) differ significantly ($p < 0.05$).

Table 4: Influence of animal and ovarian characteristics on antral follicle count and oocyte quality

Predictor	AFC		Oocyte quality	
	% change (95% CI)	P	Odds Ratio (95% CI)	P
Animal characteristics				
Breed				
HF	Ref.	Ref.	Ref.	Ref.
Brahman	-46.8 (-58.58, -31.62)	0.003	2.19 (0.42, 11.28)	0.349
MZ	-39.9 (-51.31, -25.80)	0.001	1.05 (0.27, 4.05)	0.940
Age				
> 6 years	Ref.	Ref.	Ref.	Ref.
≤ 6 years	47.6 (20.50, 80.95)	0.002	0.59 (0.10, 3.37)	0.554
Body condition score				
Poor (< 2.5)	Ref.	Ref.	Ref.	Ref.
Good (≥ 2.5 < 4)	45.3 (15.97, 82.10)	0.001	0.78 (0.21, 2.87)	0.708
Ovarian characteristics				
Ovarian volume (cm^3)	-15.2 (-39.5, 18.8)	0.337	1.39 (0.41, 4.72)	0.602
Ovarian weight (g)	34.9 (20.5, 51.0)	0.002	0.83 (0.54, 1.28)	0.408
Corpus luteum presence				
Absent	Ref	Ref	Ref	Ref
Present	4.9 (-13.8, 27.6)	0.635	0.26 (0.03, 2.10)	0.207

Breed, age, body condition score and corpus luteum status were included as categorical predictors, with Holstein Friesian, age >6 years, poor body condition score and absence of corpus luteum serving as reference categories. Percent changes with 95% CI are shown for AFC. Odds ratios with 95% CI are shown for oocyte quality. Values with $p < 0.05$ were considered statistically significant.

Discussion

This study provides a foundational evaluation of how the animal- and ovarian-level factors may influence the outcomes of the IVP technology for genetic improvement of local and crossbred cattle in Malawi. Our findings reveal that young and well-conditioned cows have significantly greater ovarian weight, volume, and AFC. Although ovaries from MZ and Brahman were smaller and had lower AFC than those from HF cows, the unadjusted comparisons indicated a higher proportion of morphologically high-quality

oocytes in these *Bos indicus* breeds. However, this breed effect was not retained in the adjusted logistic regression model. These findings suggest that Malawian local breeds may be promising candidates for future IVP work, particularly where donor selection prioritizes younger animals with good BCS.

The observed variation in ovarian size across the three breeds, with HF being larger than Brahman and MZ recording the smallest ovaries, might be linked to a similar pattern observed in AFC in this study. However, it is not clear whether the differences in ovarian size could be due to breed predisposition or the genotype × environment interaction under the Malawian context. The link between ovarian size and AFC concurs with a previous study by Ireland et al. (2008), which showed that cows with low AFC had ovaries that were 60% smaller compared to cows with high AFC. The significance of AFC during IVP comes from a well-established positive correlation between ovarian size (Widodo et al. 2022; Ireland et al. 2008) and circulating Anti-müllerian hormone (AMH), which is a reliable fertility marker *in vivo* (Ireland et al. 2010). Using an *in vitro* growth culture system, Sakaguchi et al. (2018) showed that ovaries with low AFC produced poor-quality oocytes characterized by a low estrogen: progesterone ratio, as well as poor oocyte growth, maturation, and developmental competence during IVP. At first glance, the low AFC in the Brahman and MZ ovaries in the present study predicts low circulating AMH in these animals, which could entail poor oocyte quality, suggesting unsuitability for use in an IVP program in Malawi. However, two considerations may challenge that extrapolation. First, our findings contradict a large body of literature that reports higher ovarian follicle counts in *Bos indicus* than in *Bos taurus* breeds (Baldrighi et al. 2022; Delchiaro et al. 2022; Sartori et al. 2010, 2016; Vasconcelos et al. 2020). Therefore, large-scale studies that account for contextual parameters that could affect ovarian size and AFC, as well as AMH, to confirm the association with fertility, are required to validate these findings. Second, the finding is that oocyte quality is conserved in Brahman and MZ, even higher than that of HF, despite the smaller ovarian size and lower AFC observed in the present study.

The ability to isolate high-quality oocytes, which is defined as the competence to yield a blastocyst within an *in vitro* production system (Merton et al. 2003), is a critical element of IVP as it directly influences fertilizability and embryo yield (Nagai et al. 2015). The finding that about 73 and 75% of the oocytes derived from Brahman and MZ were of good quality, compared to the 66% from the HF, presents a promising prospect to produce high-quality embryos after IVP in Malawi. By leveraging this uncompromised oocyte quality in the Brahman and MZ under the Malawian context, oocytes derived from these breeds present a high probability of conferring high blastocyst yield and quality following IVP and IVC. This proposition is augmented by the existing evidence of a higher proportion of oocytes derived from *Bos indicus* breeds transitioning into high-quality blastocysts (Catal et al. 2012), even under heat stress conditions (Hernández-Cerón et al. 2004; Paula-Lopes et al. 2003; Satrapa et al. 2011), further pointing to higher thermotolerance *in vitro*. The identification of age and BCS as significant contributors to ovarian size and AFC suggests that oocyte quality and subsequent yield could be improved by the strategic selection of younger and in good condition cattle as the source of oocytes for IVP. The effect of age seems to be consistent with Alvarez et al. (2021), who used *Bos indicus* Nellore cows of different ages to establish that AFC and oocyte quality are lower in senescent compared with young cows. Similarly, selecting oocyte donors based on BCS, which indicates good energy balance in

cows, and with which a direct link with folliculogenesis and oocyte development competence has been established (Aguila et al. 2020; Bezdíček et al. 2020; Chrenek et al. 2015; Kubovičová et al. 2012).

Ovarian weight was positively associated with AFC, but ovarian volume and CL presence were not significant predictors in the adjusted multivariate regression model. Additionally, none of the ovarian-level traits significantly predicted high-quality oocyte recovery. The positive association between ovarian weight and AFC suggests that heavier ovaries may possess larger follicular reserve, consistent with previous reports in cattle (Ireland et al. 2008; Widodo et al. 2022). This further suggests that routine assessment of ovarian weight, volume, or presence of CL before IVP is unlikely to improve donor selection beyond visual BCS and age data. However, the lack of effect of the presence/absence of the CL on AFC and oocyte quality complicates our understanding of the influence of this structure, in which conflicting results have been reported during IVP. In one study, bovine oocytes isolated from ovaries bearing a functional CL had lower maturation, cleavage, and blastocyst rates than oocytes isolated from ovaries without a CL (Hajarian et al. 2016). Yet, other studies found the complete opposite effect of the CL on oocyte developmental competence *in vitro* (Argudo et al. 2020; Saad et al. 2019). Based on this controversy, the importance of selecting ovaries for oocyte isolation based on the presence or absence of the CL remains unresolved. It is therefore imperative that subsequent *in vivo* and *in vitro* studies should be conducted in Brahman and MZ breeds under the Malawian context to ascertain their relevance during oocyte selection.

Despite providing an initial assessment on the feasibility of improving the genetics and the overall productivity of the local cattle breeds in Malawi using IVP, a few relevant limitations of this study should be acknowledged. Firstly, this study was conducted using ovaries from an abattoir with smaller sample sizes, which precludes the tracking of individual animal history, exact age, or specific environmental factors they were exposed to before slaughter. Without a comprehensive understanding of the influence of these factors on oocyte quality, it is particularly difficult to develop an accurate model to predict the potential success rate of, or to identify the factors whose modification would improve IVP outcomes in Malawi, which are necessary to attract investment in this technology. Secondly, the study did not assess the maturational and developmental competency of oocytes, which is essential to fully assess IVP suitability using oocytes derived from these breeds, considering that oocyte yield/quality alone does not directly confer measurable and predictable embryo yield (Bahadur et al. 2023). Therefore, future *in vivo* and *in vitro* studies, where oocytes would be collected using OPU or would be tracked to slaughter for ovary collection, to which semen of confirmed fertility is used, are required to address these shortfalls.

This study provides foundational evidence that abattoir-sourced ovaries from Malawian *Bos indicus* cattle can serve as a potential source of morphologically acceptable oocytes for future IVP research, despite having smaller ovarian size and AFC than Holstein Friesian cattle raised under Malawian conditions. Modifiable factors such as age and body condition significantly influenced ovarian morphometrics and AFC and should therefore be considered in donor selection. However, further studies assessing *in vitro* maturation, fertilization, embryo development, and pregnancy outcomes are required

before firm conclusions can be made on the practical suitability of these breeds for IVP programs in Malawi and similar agro-ecological contexts.

Declarations

Author contributions

Liveness Banda and Madalitso Chelenga conceived and designed the research. Chifundo Masakasa performed material preparation, data collection, and analysis. The first draft of the manuscript was written by Chifundo Masakasa. Madalitso Chelenga, Liveness Banda, Daniel Chiumia, and Kingdom Kwapata reviewed the previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest Statement

The authors declare no conflict of interest

Data availability

The data that support the findings of this study are available on request from the corresponding authors.

Ethics approval

This study used slaughterhouse ovaries, and no ethical approval is required.

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