Africa Journal of Animal and Biomedical Sciences
ISSN: 1819-4214

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The Potential of Ankole Cattle Abattoir Ovaries for *In Vitro* Embryo Production

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Abstract

A study was carried out to determine the potential of abattoir ovaries from Ankole cattle cows for *in vitro* embryo production. Ankole cattle cows (n = 109) due for slaughter at a local abattoir were selected. The body weight, age, body condition, pregnancy status and presence of corpus luteum (CL) for each animal were recorded. Pairs of ovaries were collected from the animals and transported in PBS supplemented with 0.5 mg/ml gentamycin. The ovaries were weighed and the number of vesicular follicles (2-10 mm) on the surface recorded. Oocytes were aspirated with 18G needle and a 5 ml syringe, washed with PBS, counted and the cumulus-oocyte complexes (COCs) graded. Data were analysed using SAS, 2003 and means were separated using Fisher's LSD at a significant level of 0.05. The mean live weight of Ankole cattle was cows 259.6 $\pm$ 43.4 kg. Live weight increased with age and improving body condition, and live weight was higher for pregnant and cows with CL than the non-pregnant and cows without CL. The average ovary weight of Ankole cattle was 4.6 $\pm$ 2.3 g. The left ovary weight and the ovary weight per cow increased significantly ($p < 0.05$) between the age groups. The right ovary weight was only different ($P < 0.05$) between animals less than four years and those four years and above. Pregnancy and presence of CL significantly influenced ($P < 0.05$) the left ovary and total ovary weight, while body condition did not. The average number of vesicular follicles per pair of ovaries was 14.7 $\pm$ 9.0 and only pregnancy influenced ($P < 0.05$) the number of vesicular follicles per animal. The mean oocyte yield per pair of ovaries was 8.02 ± 5.72, representing a recovery rate of approximately 54.6% for the method of follicular aspiration used. Grades I and II oocytes constituted 66% of the oocytes recovered and these were considered acceptable for *in vitro* embryo production. Grades II and III oocytes were different between the age-groups and pregnancy status, respectively. These results indicated a high potential for *in vitro* embryo production using abattoir ovaries of Ankole cattle. A further investigation of *in vitro* maturation, fertilization, culture and evaluation of the embryos for transfer into surrogate dams was recommended.

Key words: Ankole cattle, Ovary, Vesicular follicles, oocytes

Introduction

Several breeds of farm animals are threatened by extinction mainly due to their lack of reproductive competitiveness[1]. The Ankole cattle face extinction due to their low reproductive efficiency shown by late sexual maturity and long calving intervals. Due to their low production efficiency, there has been a deliberate shift to the more productive cattle breeds. As land becomes scarce, farmers have resorted to crossbreeding and/or keeping exotic breeds to produce more milk per unit area of land. A programme of crossbreeding and replacement of Ankole cattle with the exotic breeds has been practiced extensively and uncontrollably. The Ankole cattle, predominant in western Uganda, are kept mainly for meat and milk. This cattle breed has evolved in this environment over centuries and posses a certain degree of resistance to diseases that are very troublesome to the imported breeds and their crosses.

Although the productivity of indigenous breeds is inferior to the exotic breeds of cattle[2], their survivability and longevity under modest management systems outweigh the economic benefits of exotic breeds under similar management. Indigenous breeds such as the Ankole cattle should therefore be saved from any threat that may lead to their extinction despite their productive inferiority. Instead, the indigenous breed's biological efficiency within the management system most economical in their environment should be considered. There is also need to preserve the Ankole cattle breed through the establishment of a gene bank. Assisted reproductive technologies (ARTs) such as multiple ovulation and embryo transfer (MOET) and *in vitro* fertilization (IVF) could be applied in creating the gene bank as reported elsewhere[3].

Bovine ovaries contain hundreds of thousands of primodial follicles, but about 99% undergo atresia before ovulation and only a few are ovulated during the cow's lifespan[4]. Viable bovine oocytes can be collected from abattoir ovaries of females of all ages including foetuses[5]. These oocytes can be used to produce large numbers of embryos and live calves through IVF procedures[6]. Initial steps in bovine IVF using abattoir ovaries involves identification and selection of good quality ovaries, follicles and oocytes that are vital for a high rate of embryo production. Follicular quality is indicative of the oocyte quality and the oocyte's ability to develop into an embryo *in vitro*[7]. The objective of this study was to elucidate some characteristics of Ankole cattle, their ovaries and the quality of the follicles and oocytes to determine the suitability of Ankole cattle abattoir ovaries for *in vitro* embryo production.

Materials and Methods

Selection of Ankole cattle
Female Ankole cattle (n = 109) were identified using the described features[8,9,10]. The body condition of the cattle was assessed using a 1-5 scoring procedure[11]. After slaughter, the age of the animals was estimated basing on the dental formula[12]. From the carcass weights, the live weights were estimated using a conversion factor different for every carcass weight-range[13]. Physical examination and palpation of the uterus was used to determine the reproductive status of the slaughtered animals.

Collection of ovaries
Pairs of ovaries were obtained from the selected Ankole cattle after slaughter and tagged. The ovaries were washed once in phosphate buffered saline (PBS) supplemented with 0.5 mg/ml gentamycin and

transported to the laboratory in thermos flask containing PBS at 30 °C within 4 hours. In the laboratory, ovaries were washed twice with PBS and then kept in the saline in a water bath at 30 °C. Excessive tissues attached to the ovaries were trimmed and the ovarian tissue weighed using an electronic balance, and then vesicular follicles (2-10 mm in diameter) on the ovarian surface were counted.

Oocytes recovery and grading

Oocytes were recovered by aspiration from vesicular follicles (2- 10 mm in diameter) using an 18-gauge hypodermic needle and a 5 mL syringe[14,15]. The aspirated follicular fluid from individual ovaries was allowed to settle in separate test tubes and then the top fluid aspirated to leave the sediments at the bottom of the test tubes. The sediment was re-suspended four times in PBS for the medium to clear. The sediment was examined in 65 x 15 mm plastic Petri dishes using a stereoscope and cumulus–oocytes complexes (COCs) were isolated, washed in PBS and graded according to cumulus cells investment and cytoplasmic appearance[15].

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the SAS general linear model procedure[16]. Least square means (LSM) procedure was used to test significance among class variables and their interactions. Linear model used in data analysis is as follows:

$$Y_{ijklmn} = \mu + Age_i + BCS_j + PState_k + CL_l + e_{ijklmn} \quad N(0, \sigma_e^2)$$

Where:

Y_{ijklm} = Ovary parameter of the n^{th} cow of the i^{th} age and j^{th} body condition score, of k^{th} pregnancy state, with the l^{th} corpus luteum

μ = general mean of the ovary parameter

Age_i = effect of age of cow (i = 1,2,3)

BCS_j = effect of body condition score of cow (j = 1,2,3)

$PState_k$ = effect of pregnancy state of cow (k = 1,2)

CL_l = effect of corpus luteum (l = 1,2)

e_{ijklm} = random error term that is associated with the cow record.

Note: The interactions were not included in the model above since preliminary analysis showed no significant effect.

Results

Mean live weight of the cows was 259.6 ± 43.4 kg, but live weight varied between the age groups, body condition status, pregnancy status and presence of the corpus luteum (Table 1). Majority of Ankole cattle cows studied (71/109, 65.1%) were 4-5 years old. Most of these cows studied (62/109, 56.9%) were in good body condition. Mean live weight increased with improving body condition. Cows in the fat body condition status were heavier than the others. Up to 43.1% of the cows were pregnant. The pregnant cows had a mean live weight higher than those of the non-pregnant cows. Majority of the cows (79/109, 72.5%) had CL. The mean live weight of cows that had CL was higher than those without CL.

Table 1 Means and standard error of live weight of Ankole cattle cows in different age groups, body condition, pregnancy status and presence of corpus luteum.

Factor	Category	Number (%) of cows	Mean live wt (Kg)	Std error
Age-groups (years)	< 4	14 (12.9)	221.43	8.55
	4-5	71 (65.1)	254.38	3.60
	>5	24 (22.0)	289.71	9.88
Body condition	Thin	35 (32.1)	238.63	5.01
	Good	62 (56.9)	260.87	4.69
	Fat	12 (11.0)	299.00	14.65
Pregnancy	Pregnant	47 (43.1)	264.45	4.59
	Not pregnant	62 (56.9)	252.98	5.78
Corpus luteum	Present	79 (72.5)	263.57	3.89
	Absent	30 (27.5)	243.07	9.16

Table 2 shows the influence of age, body condition, pregnancy status and the presence of CL on the means and standard error of ovary weight and number of vesicular follicles of Ankole cattle. Mean ovary weight was 4.42 ± 2.20 g and 4.65 ± 2.32g for right and left ovaries, respectively, and 9.07 ± 2.57 g per pair of ovaries. The ovary weight significantly differed ($P < 0.05$) between the age-groups. The left ovary was generally heavier than the right ovary in all age-groups. Total ovary weight per animal increased with age from 5.50 ± 0.70 gm in the < 4 years age-group (n = 14), through 9.10 ± 0.31 gm in the 4-5 years age-group (n = 71) to 11.06 ± 0.57 gm in the > 5 years age-group (n = 24). Body condition did not influence ($P > 0.05$) the left, right or total ovary weight. There was a significant difference ($P < 0.05$) in ovary weight between the pregnant (n = 47) and non-pregnant (n = 62) cows. The pregnant cows had heavier ovaries than the non-pregnant cows. The presence of the corpus luteum (CL) influenced the left and the total ovary weight, but not the right ovary weight. Left ovary and ovaries per animal were significantly heavier for cows that had CL than those that did not have CL. Right ovary weight did not differ between cows that had and those that did not have CL.

Table 1 Means (±SE) values of ovary weight and follicles at different age, body condition, pregnancy status and presence of corpus luteum.

	Left ovary weight	Right ovary weight	Ovary weight per animal	Left follicles	Right follicles	Follicles per animal
Age yrs						
<4 (n=14)	2.77±0.41[a]	2.72±0.51[a]	5.50±0.70[a]	8.92±1.22 7.	64±0.94	15.28±2.15
4-5 (n=71)	4.64±0.24[b]	4.46±0.26[b]	9.10±0.31[b]	7.86±0.58 7.	91±0.67	15.21±1.14
> 5 (n=24)	5.77±0.51[c]	5.30±0.49[b]	11.06±0.57[c]	6.9±1.07	7.00±0.90	12.75±1.49
Body condition						
Thin (n=35)	4.71±0.45	4.10±0.35	8.80±0.56	7.53±0.59	6.59±0.70	13.29±1.13
Good (n=62)	4.60±0.27	4.56±0.30	9.16±0.40	7.98±0.74	8.33±0.73	15.53±1.31
Fat (n=12)	4.70±0.50	4.67±0.64	9.37±0.52	7.58±1.00	7.36±1.21	14.33±1.90
Pregnancy status						
Pregnant (n=47)	5.37±0.34[b]	4.70±0.34	10.07±0.36[b]	7.10±0.71	6.67±0.61	12.72±1.09[b]
Non- pregnant (n=62)	4.10±0.27[a]	4.22±0.28	8.31±0.42[a]	8.27±0.62	8.43±0.72	16.16±1.24[a]
$LSD_{0.05}$	0.7721		0.986			3.419
Corpus luteum						
Present (n=79)	5.24±0.24[b]	4.63±0.26	9.87±0.29[b]	7.42±0.56	7.20±0.51	13.78±0.94
Absent (n=30)	3.09±0.32[a]	3.88±0.40	6.97±0.61[a]	8.75±0.87	8.87±1.14	17.03±1.84
$LSD_{0.05}$	0.8561		1.0934			

a,b,c in the different column means significant difference (p < 0.05)

Mean number of vesicular follicles recorded (Table 2) was 7.67 ± 5.04 for right ovary, 7.79 ± 4.79 for left and 14.68 ± 8.91 for the pair of ovaries. Age, body condition and CL did not influence (P > 0.05) the number of vesicular follicles on the ovarian surface. However, the younger cows < 5 years old, the cows in good to fat body condition, and the non-pregnant cows tended to have more vesicular follicles than the other cows in their respective categories. Only pregnancy influenced (P < 0.05) the number of vesicular follicles, where the non-pregnant cows had more vesicular follicles than the pregnant one.

The mean number of oocytes recovered per animal was 8.02 ± 5.72, representing a recovery rate of approximately 54.6% for the method of follicular aspiration used (Table 3). Oocyte recovery from left, right and both ovaries per animal was not influenced by age, body condition, CL and pregnancy. Grade I (best) and grade IV (worst) oocytes were not influenced (P > 0.05) by age, body condition, CL and pregnancy. However, there was a significant difference (P < 0.05) in grade II oocytes between the age-groups. The grade III oocytes were higher in the young cows (< 5 years old) than the older cows (> 5 years old). There was also a significant difference (P < 0.05) in the grade III oocytes between the pregnant and the non-pregnant cows. The non-pregnant cows had more grade III oocytes than the pregnant ones. Grade II oocytes were not influenced by body condition, CL and pregnancy, while grade III oocytes were not influenced by age, body condition and CL.

Table 3 Means (±SE) values of oocyte number and grade at different age, body condition, pregnancy status and presence of corpus luteum

	Left ovary oocytes	Right ovary oocytes	Total oocyte recovery	Oocyte grades 1	2	3	4
Age yrs							
<4 (n=14)	5.00±0.89	4.85±0.88	8.79±1.66	3.00±0.59	4.50±0.67[a]	2.29±0.47	2.22±0.43
4-5 (n=71)	4.48±0.32	4.60±0.42	8.17±0.63	2.78±0.26	3.31±0.28[ab]	2.02±0.23	2.26±0.17
> 5 (n=24)	4.16±0.96	4.60±0.91	7.1 ±1.33	3.52±0.87	2.77±0.44[b]	1.86±0.46	2.40±0.34
Body condition							
Thin (n=35)	4.45±0.41	4.40±0.68	7.94±0.86	2.92±0.36	3.33±0.40	1.80±0.30	2.40±0.25
Good (n=62)	4.52±0.49	4.76±0.47	7.94±0.80	3.00±0.39	3.39±0.31	2.11±0.28	2.28±0.20
Fat (n=12)	4.42±0.63	4.64±0.91	8.67±1.26	2.80±0.57	3.10±0.48	2.11±0.39	2.11±0.31
Pregnancy status							
Pregnant (n=47)	3.76±0.32	4.16±0.53	6.85±0.68	2.44±0.32	3.26±0.35	1.46±0.10[b]	2.09±0.21
Non- pregnant (n=62)	4.96±0.45	4.96±0.45	8.92±0.80	3.31±0.36	3.39±0.30	2.46±0.30[a]	2.44±0.19
$LSD_{0.05}$						0.763	
Corpus luteum							
Present (n=79)	4.36±0.38	4.42±0.39	7.65±0.63	3.06±0.33	3.07±0.22	1.84±0.16	2.36±0.18
Absent (n=30)	4.79±0.51	5.19±0.77	8.97±1.09	2.72±0.39	4.08±0.57	2.56±0.57	2.14±0.21

a,b,c in the different column means significant difference ($p < 0.05$)

Discussion

The mean live weight of the female Ankole cattle was 259.6 ± 43.4 kg. This was within the lower range of the mean body weight of 292 and 341 kg reported for first and second or higher parities, respectively, in the same breed[17]. Mean ovary weight of Ankole cattle (4.42 ± 2.20 g and 4.65 ± 2.32.01 g for right and left ovaries, respectively) recorded in this study was lower than the 10-19 g[18] and 5-15 g[19] reported for Friesian cattle. The lower ovarian weight recorded in this study is due to breed difference. Ankole cattle with mean live weight of 259.6 ± 43.4 kg are indeed smaller than the Friesian cattle, and therefore their ovaries are bound to be smaller and lighter than those of Friesian cows. The size of the ovary also depends on the corpora lutea and the follicles present, nutrition and body weights[19]. In this study, ovary weight significantly differed ($P < 0.05$) between the age-groups and increased with age, because live weight also increased ($P < 0.05$) with age. The pregnant cows and cows with CL had heavier ovaries than the non-pregnant cows and those without CL, because presence of CL influences the size and thus weight of ovary[19].

Mean number of vesicular follicles recorded was 7.67 ± 5.04 for right ovary, 7.79 ± 4.79 for left and 14.68 ± 8.91 for a pair of ovaries was lower than those reported elsewhere for other breeds[20]. The vesicular follicle population was higher in cows up to 5 years old and those in good body condition than in other age groups and body condition status, though the differences were not statistically significant. The plane of nutrition, and thus body condition, affects follicular development. In previous studies, it was observed that animals fed on low energy diet possessed fewer follicles than those on a high plane of nutrition[15,21]. Underfeeding reduces the secretions of gonadotropins responsible for follicular development[19]. The effect of high fat diets was examined and found that increase in fat intake increased the population of medium sized (5-9.9 mm) follicles in heifers[22]. It was suggested that energy balance may represent an important signal factor leading to increased follicular estradiol production before first ovulation in postpartum dairy cows[23]. Vesicular follicle population in the age groups is probably associated with this the plane of nutrition and body condition.

The non-pregnant cows had a higher ($P < 0.05$) number of vesicular follicles than the pregnant cows. However, number of vesicular follicles was not influenced by the presence of CL, a finding that disagreed with that a report of a higher number of vesicular follicles and oocytes associated with ovaries with no copra lutea[15]. The difference may be attributed to consideration of 2-4 mm diameter follicles in the study[15], compared with 2-10 mm follicles in the current study. Although the presence of CL did not influence the follicular number, the difference in follicular number in pregnant and non-pregnant cows is due to the difference in hormonal profiles in pregnant and non-pregnant cows. The sustained high levels of progesterone in pregnant cows suppresses production follicle stimulating hormone (FSH) leading to low turnover of vesicular follicles[24]. In non-pregnant cows the alternating levels of progesterone and oestrogen during the cycles stimulates secretion of FSH resulting to development of a high number of recruited follicles to vesicular follicles[24].

Oocyte recovery rate of 54.6% attained with the aspiration technique in this study was within the range of 30-60% recovery rate earlier reported[15,25,26]. Oocyte recovery rate was not affected by age, body condition, pregnancy or presence of CL because it could have only been influenced by the method of oocyte recovery and/or technicians that recovered the oocytes, which did not vary. Grade I and II oocytes that would be accepted for *in vitro* embryo production were more in all age-groups, and body condition, pregnancy and CL status than grade III and grade IV oocytes. This

finding indicated that oocytes for IVF could be obtained from all these categories, and agreed with a report that viable bovine oocytes can be collected from abattoir ovaries of females of all categories including foetuses[6]. Age influenced grade II while pregnancy status influenced grade III oocytes. The young cows less than 4 years and the non-pregnant produced the highest number of oocytes that would be used in IVF. This finding showed that ovaries for oocyte retrieval should be collected mainly from Ankole cattle < 5 years old, especially those that are not pregnant. The high number of grades I and II oocytes in these categories of cows is likely to have high embryo yield, because there is a relationship between oocyte quality and embryo yield[15].

Conclusion

A great potential for application of ART in Ankole cattle using abattoir ovaries for multiplication, upgrading and conservation exists. Although ovaries for oocyte aspiration can be collected from all categories of animals, the young (< 5 years old) and non-pregnant cows have the greatest potential for *in vitro* embryo production. The majority (66%) of oocytes aspirated had morphology that would be acceptable for *in vitro* production of embryos, further studies on maturation, fertilization and culture should be conducted to evaluate *in vitro* embryo production and success rates after transfer into surrogate dams.

Acknowledgments*: The Kampala abattoir staff, particularly Mr. H. Kwizera for the assistance during ovary collection and Makerere University's Faculties of Agriculture for the financial support and Veterinary Medicine for the laboratory facilities.*

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