Evaluation of the follicular population and oocyte quality of zebu cows (*Bos indicus*) with anovulatory anestrus

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Keywords

Bos indicus, anestrus, ovarian follicles, ova, Cameroon

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Summary

The study aimed to evaluate the ovarian follicular population and oocyte quality of local zebus with anovulatory anestrus. It was conducted at Ngaoundere municipal slaughterhouse, Adamawa region in Cameroon. Of the 496 genital tracts examined, 145 (29.2%) presented an anovulatory anestrus. The mean number of follicles per cow was 37.5 ± 25.2. The number of small, medium and large follicles per cow were 21.7 ± 18.7, 14.9 ± 12.1 and 0.9 ± 1.1, respectively. The mean follicular populations of cyclic or in anovulatory-anestrus cows were 29.1 ± 18.1 and 41.9 ± 27.3 ($p < 0.05$), respectively. The number of oocytes per cow were 15.5 ± 4.5 and 15.6 ± 3.8 ($p > 0.05$) in cyclic and anestrus cows, respectively. The number of grade I, II, III and IV oocytes were 4.5 ± 4.1 (28.5%), 4.1 ± 3.7 (26.2%), 2.3 ± 2.4 (14.8%) and 4.8 ± 5.6 (30.5%), respectively. This study showed that anovulatory anestrus tended to enhance the follicular population but had no effect on the oocyte yield. The number of grade I and II oocytes acceptable for *in vitro* maturation were significantly lower in cows with anovulatory anestrus than in cyclic cows ($p < 0.05$).

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■ INTRODUCTION

Livestock plays a very important role in Cameroon agriculture, contributing about 9% (398.4 billion CFA) of the total agricultural production. In 2013, the national livestock population represented about 5.9 million cattle (Minepia, 2015). The productivity is very low because of many factors such as a poor genetic potential, diseases (pasteurellosis, foot-and-mouth, tick-borne and trypanosomosis), and reproduction in traditional systems with little or no livestock program (Ebangi et al., 2011). Reproductive technologies such as artificial insemination, embryo transfer and *in vitro* embryo production (IVP) were developed to minimize the effects of these factors and improve herd profitability (Morrell and Rodriguez-Martinez, 2010). IVP is widely used in the world and embryos can be produced from oocytes issued from ovaries collected at the slaughterhouse (Dauzet and Marquant-Le Guienne, 1994). The quality of these oocytes, IVP main limiting factor, is the result of multifactorial interactions involving the ovarian function, the surrounding cumulus complex, environmental factors such as heat stress, the genetic background, age and lactation status of donor animals (Boni, 2012). In a previous study, Kouamo et al. (2014) showed that zebu oocytes collected at a slaughterhouse have an average potential for IVP. In parallel, a high prevalence of genital tract abnormalities in donor cows (58.3%) has been reported. The predominant ovarian affection was anovulatory anestrus (Kouamo et al., 2016). To investigate further this common syndrome, the present study was carried out to evaluate the ovarian follicular population, oocyte recovery rate and oocyte quality of zebus with anovulatory anestrus.

■ MATERIALS AND METHODS

Study area

Samples were collected at Ngaoundere Municipal Slaughterhouse (NMSH) and analyzed at the Veterinary Laboratory of IRAD-Wakwa Regional Center (Physiology and Reproduction Biotechnology Department) in Adamawa Region, Cameroon. The cattle slaughtered at NMSH were from Vina Division (59.7%) and Mayo Rey Division (40.3%). Ngaoundere is located between 7° 19’ 39 N and 13° 35’ 4 E, and has an average annual rainfall of 1496.7 millimeters. Temperatures vary from 15.2 to 29°C with an average humidity of 58.2% (Tchotsoua, 2008).

Characteristics of the animals

Local cows (*Bos indicus*) of different breeds (Gudali, n = 270; White Fulani, n = 141; Red Fulani, n = 82; and Bokolo, n = 3) were randomly included in the study. The mean live body weight was estimated

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from the thoracic circumference (THC) as follows: $124.69 - 3.171 \times THC + 0.0276 \times THC^2$ (Njoya et al., 1997). The body condition score (BCS) was determined according to Ferguson et al. (1994), and the age according to Lucyna and Zdzisław (1984). Pregnant animals were discarded from the study.

Definition of ovarian troubles

After slaughter, genital tracts were examined to determine ovarian troubles. Only the cyclic cows (n = 351) and those presenting anovulatory anestrus (n = 145), as reported by Kouamo et al. (2016), were selected. The classification of types of anovulatory anestrus was based on the presence of follicles and/or cysts on the ovaries (Peter et al., 2009). Thus, three types of anovulatory anestrus were identified: Type I, presence on one ovary of 2–7 mm diameter follicles, absence in both ovaries of corpus luteum (CL) and cysts; Type II, presence on one ovary of at least one follicle larger than 7 mm in diameter, and 2–7 mm follicles in the absence of CL and cysts on both ovaries; Type III, presence on one ovary of a cyst in the presence or not of follicles larger than 7 mm in diameter, but in the absence of CL on both ovaries.

Ovary collection and handling

After determining the status of the cow, the left and right ovaries were collected and transported to the laboratory at 35–37°C within two hours after slaughter. They were then carefully trimmed off and weighed with an electronic scale (Mettler PC 2000 at 0.1 g precision; Laboratory Weighing, Greifensee, Switzerland).

Follicular population determination

The ovaries were washed with a medium (saline solution 0.9% supplemented with penicillin-streptomycin sulfate). For each ovary, visible follicles were counted and follicular diameters (Φs) measured with electronic Stainless Hardened calipers. Follicular diameters were classified into three categories as described by Duygu et al. (2013).

Recovery and grading of oocytes

The ovaries were placed in separate plastic Petri dishes containing Dulbecco's phosphate buffered saline (Sciencell Research Laboratory, CA, USA) and chopped into small pieces with a scalpel to release oocytes (Wang et al., 2007). Oocyte quality was evaluated under a stereoscope (× 10) and scored into four grades (G) according to the homogeneity of the cytoplasm and layers of cumulus cells (Figure 1) as described by Alves et al. (2014). The overall oocyte quality per cow was calculated as an index using the formula by Duygu et al. (2013): (G I × 1 + G II × 2 + G III × 3 + G IV × 4) / Total number of oocytes recovered.

Statistical analyses

Data were analyzed with Statgraphic Centurion version 15.0. Wilcoxon and Kruskal-Wallis tests were used to compare the follicular population and oocyte quality of cyclic or anovulatory anestrus cows. The chi-squared (and Fisher when n < 5) test was used for characterization (origin, breed, age, weight, BCS). Differences were significant at $p < 0.05$.

■ RESULTS

Characterization of cows and ovaries

Of the 496 female zebus examined, the mean (minimum-maximum) live weight, BCS and age were 324.1 ± 55.5 kg (166.7–536.4), 2.8 ± 0.37 (2–3) and 5.9 ± 2.2 (3–12) years, respectively. Age and BCS had an influence on the cow status ($p < 0.05$), whereas the origin and breed had no effect ($p > 0.05$). Cows with anovulatory anestrus were heavier than cyclic cows, whereas their ovarian weights were not significantly different (Table I). The mean weight of the ovaries was 4.4 ± 0.17 kg. The right ovaries (5.1 ± 4.3 g) were heavier than the left ones (3.8 ± 2.6 g) ($p = 0.03$).

Follicular population and oocyte recovery

From 992 ovaries (anestrus and cyclic cows), 15,799 follicles were counted. The mean number of follicles per cow was 37.5 ± 25.2. The numbers of small (Φ < 3 mm), medium (3 ≤ Φ ≥ 8 mm) and large (Φ > 8 mm) follicles per cow were 21.6 ± 18.7 (57.7%), 14.9 ± 12.1 (39.7%), and 0.9 ± 1.1 (2.6%), respectively.

The average number of oocytes recovered per cow was 15.6 ± 11.6 (n = 7687) with a recovery rate of 48.6% (number of oocytes / number of follicles present). The numbers of oocytes per ovary graded I, II, III and IV were 4.5 ± 4.1 (28.5%), 4.1 ± 3.7 (26.2%), 2.3 ± 2.4 (14.8%) and 4.8 ± 5.6 (30.5%), respectively. The number of good IVP quality oocytes (grades I and II) per cow was 8.6 ± 6.5 (55.1%). The overall oocyte index was 2.5 and was significantly lower in cyclic females (2.41) than in anovulatory anestrus cows (2.57, $p = 0.002$). The number of follicles and the mean number of collected oocytes were higher on the right ovary (19.5 ± 13.8 and 8.1 ± 6.9) than on the left one (18.1 ± 13.2 and 7.7 ± 5.8, respectively) ($p < 0.05$).

Follicular population and oocyte quality in anestrus

In cows with anovulatory anestrus, the average number of follicles was significantly higher than that in cyclic females ($p < 0.05$) (Table II). The number of follicles in females with anovulatory anestrus type 1 (54.2 follicles; n = 42) was significantly higher than that in cows in anestrus type 2 (39.3 follicles; n = 62) and type 3 (33.3 follicles; n = 41) ($p = 0.0005$) (Table II).

The oocyte yield of females, cyclic or with anovulatory anestrus, was not significantly different ($p > 0.05$), whereas the number of oocytes

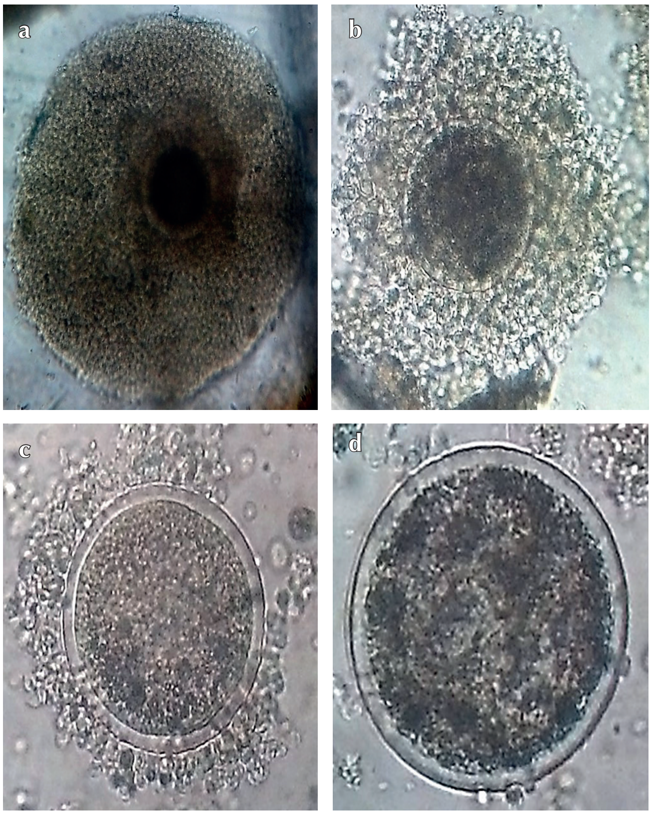

Figure 1: *Oocyte quality; (a) grade I, (b) grade II, (c) grade III, (d) grade IV (stereoscope x 10).*

selected for *in vitro* embryo production of cows with anovulatory anestrus was significantly lower than that of cyclic cows (p = 0.005) (Table III). The oocyte yield and number of oocytes selected for IVP from cows with type-3 anovulatory anestrus were significantly lower than those with type 1 and 2 anovulatory anestrus (p < 0.05) (Table III).

■ DISCUSSION

Anovulatory anestrus is the main problem in cattle that affects fertility and thus causes major economic losses to farmers (Lafi et al., 1992; Vanholder et al., 2006). Kouamo et al. (2016) reported the predominance of anovulatory anestrus in zebu cows in the same environment. Breed, age, level of milk production, feeding, nutritional deficiencies are factors that influence the prevalence of anovulatory anestrus in these breeding conditions (Noakes et al., 2002).

Very few studies have been conducted to assess the ovarian follicular population and oocyte quality of local zebus with anovulatory anestrus. In the present study, the follicular population differed significantly between anestrus and cyclic cows. The number of large ovarian follicles was significantly higher in cows with anovulatory anestrus. Similarly, Gümen et al. (2003) reported 14% and 22% for the small follicles, and 10% and 20% for the large follicles in cyclic and anestrus cows, respectively. Nogueira et al. (2015) reported a higher number of small follicles and total number of follicles in follicular

Table I

Characteristics of zebu cows according to the cyclic or anovulatory anestrus state at Ngaoundere Municipal Slaughterhouse, Cameroon

		Anovulatory anestrus	Cyclic	Test used and statistical difference
District	Vina	72 (14.5%)	224 (45.2%)	χ2 = 3.89; df = 1
	Mayo Rey	73 (14.7%)	127 (25.6%)	p = 0.51
Breed	Akou	34 (6.9%)	107 (21.6%)	Fischer; df = 3
	Bokolo	3 (0.6%)	0 (0%)	p = 0.41
	Djafoun	28 (5.6%)	54 (10.9%)	
	Goudali	80 (16.1%)	190 (38.3%)	
Age (years)	[3–5]	85 (17.1%)	151 (30.4%)	χ2 = 14.32; df = 2
	]5–9]	45 (9.1%)	195 (39.3%)	p = 0.0008
	≥ 10	15 (3.1%)	5 (1%)	
Cow weight (kg)	Mean ± SE	330.7 ± 55.6	311.3 ± 53.4	Wilcoxon test
	Min–Max	[166.7–536.4]	[205.8–505.8]	p = 0.005
Body condition score	[1–2]	35 (7.1%)	0 (0%)	Fischer; df = 1
	3	110 (22.1%)	351 (70.8%)	p = 0.001
Ovary weight (g)	Mean ± SE	4.7 ± 5.2	4.0 ± 1.5	Wilcoxon test
	Min–Max	[0.9–7.9]	[0.9–9.8]	p = 0.1
	Left	3.8 ± 2.9	3.8 ± 1.9	Wilcoxon test
	Min–Max	[0.9–25.1]	[1–9.8]	p = 0.6
	Right	5.6 ± 9.1	4.2 ± 1.8	Wilcoxon test
	Min–Max	[1.7–7.9]	[0.9–9.4]	p = 0.01

Min: minimum; Max: maximum; SE: standard error; df: degree of freedom

Table II

Follicular populations (mean ± standard deviation) in zebu cows according to cyclic/anestrus characteristics, and anestrus types at Ngaoundere Municipal Slaughterhouse, Cameroon

	N	Follicular population			Average num. follicles / cow
		Small	Medium	Large	
Characteristics of cows					
Cyclic	351	14.6 ± 13.1[a]	13.8 ± 10.7[a]	0.7 ± 0.9[a]	29.1 ± 18.1[a]
Anovulatory anestrus	145	25.3 ± 20.1[b]	15.4 ± 12.7[a]	1.1 ±1.1[b]	41.9 ± 27.3[b]
P		0.002	0.2	0.001	0.03
Anovulatory anestrus					
Type 1	42	32.2 ± 21.2[a]	21.9 ± 16.7[a]	0 ± 0[a]	54.2 ± 30.3[a]
Type 2	62	22.9 ± 17.7[a]	14.9 ± 9.3[b]	1.5 ± 0.9[b]	39.3 ± 22.9[b]
Type 3	41	22.1 ± 21.3[b]	9.6 ± 9.4[c]	1.7 ± 1.2[b]	33.3± 26.5[b]
P		0.0001	0.00002	0.0001	0.0005

[a,b,c] In each column different letters indicate significant differences between groups (p < 0.05).

Table III

Oocyte quality (mean ± standard deviation) in zebu cows according to cyclic/anestrus characteristics, and anestrus types at Ngaoundere Municipal Slaughterhouse, Cameroon

	N	Average num. oocytes / cow	Oocyte quality				Good IVP Oocytes I & II (%)
			I	II	III	IV	
Characteristics of cows							
Cyclic	351	15.5 ± 4.5[a]	4.5 ± 3.8[a]	4.8 ± 3.9[a]	2.9 ± 2.8[a]	3.3 ± 3.1[a]	9.3 ± 6.7 (59.9)[a]
AAnestrus	145	15.6 ± 3.8[a]	4.5 ± 4.1[a]	3.7 ± 3.6[b]	2.1 ± 2.2[b]	5.5 ± 6.2[b]	8.2 ± 6.4 (52.1)[b]
P		0.8	0.3	0.01	0.002	0.00005	0.005
Anovulatory anestrus							
Type 1	42	24.2 ± 5.3[b]	5.8 ± 4.7[a]	5.3 ± 4.1[a]	3.1 ± 2.9[b]	10.1 ± 8.6[c]	11.1 ± 7.2 (45.7)[a]
Type 2	62	14.9 ± 2.8[a]	5.1 ± 4.1[a]	3.9 ± 3.7[c]	1.8 ± 1.5[a]	4.2 ± 3.6[b]	9.1 ± 5.9 (60.1)[a]
Type 3	41	8.3 ± 2.6[c]	2.1 ± 2.5[b]	1.9 ± 1.8[b]	1.2 ± 1.8[c]	3.1 ± 3.7[a]	3.9 ± 3.5 (48.3)[b]
P		0.0003	0.00001	0.000007	0.0003	2.2×10^{-6}	8.1×10^{-8}

IVP: *in vitro* embryo production; AAnestrus: anovulatory anestrus
[a,b,c] In each column different letters indicate significant differences between groups ($p < 0.05$).

waves in anestrus females, but the number of large follicles was higher during the breeding season. Indeed, during the anovulatory anestrus phase, many follicles are arrested during the phase of independence to gonadotropins and only a small number of follicles is able to progress because of low follicle stimulating hormone (FSH) and/or luteinizing hormone (LH) concentrations (Nogueira et al., 2015). On the other hand, marked follicular dominance and formation of corpus luteum in females during the breeding season cause follicular atresia and might explain the limited population of small follicles. Bartlewski et al. (1998) reported a higher number of small and medium-sized follicles during the anestrus period, but follicles rarely reached dominance because of the scarcity of fluctuations of progesterone and the discretion of LH peaks, which could not allow attaining dominance.

In the present study, the follicular population of cystic anestrus cows (type 3) differed from types 1 and 2 anestrus. The interval between waves of follicular growth is longer for cows with cysts than for cows with normal estrus cycles (Garverick, 1997). The mechanical pressure that a cyst exerts on the ovarian tissue and hormonal perturbation reduces the growth surface of the follicles, as well as blood pressure in the ovary (Qublan et al., 2006; Eryilmaz et al., 2012). In fact, Gonzalez et al. (1999) reported that females with cystic ovaries and no signs of estrus present large follicles (16–28 mm,) and few new follicles, whereas normal cyclic cows have follicles that normally evolve throughout the cycle.

The oocyte number of cows, cyclic or with anovulatory anestrus, did not differ significantly, but the number of grades I and II oocytes that could be selected for IVP was significantly lower in females with anovulatory anestrus than in cyclic ones. Similarly, Mara et al. (2013) found that during production of blastocysts, the number of good quality oocytes (grades I and II) was lower during the seasonal anestrus than during the breeding season. This study indicated that cystic anestrus (type 3) significantly reduced the number and quality of oocytes compared to other anestrus types (1 and 2). These results are similar to those reported by authors who showed that the presence of a cyst might be responsible for the reduction in the number, quality and fertilization rate of oocytes (Qublan et al., 2006; Eryilmaz et al., 2012). Koji et al. (1998), Silvia et al. (2002), and Peter (2004) reported that the primary physiological defect leading to the formation of an ovarian cyst is the inability of the hypothalamic-pituitary complex to cause the necessary LH peak following the positive feedback of estradiol.

■ CONCLUSION

The present study showed that anovulatory anestrus increased the follicular population, but decreased the number of good quality oocytes usable for IVP compared to those in cyclic cows. Moreover, cystic anestrus decreased the number of good quality oocytes compared to that of other types of anestrus. In order to improve the efficacy of the IVP process of oocytes collected from local zebus, donors must be selected without ovarian pathologies including anovulatory anestrus.

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Résumé

Kouamo J., Zangue C.T., Zoli A.P. Evaluation de la population folliculaire et de la qualité des ovocytes des vaches zébus (*Bos indicus*) en anœstrus anovulatoire

L'étude, menée à l'abattoir municipal de Ngaoundéré, région de l'Adamaoua au Cameroun, visait à évaluer la population folliculaire et la qualité des ovocytes des vaches zébus locales en anœstrus anovulatoire. Sur 496 voies génitales examinées, 145 (29,2 %) présentaient un anœstrus anovulatoire. Le nombre moyen de follicules était de 37,5 ± 25,2 par vache. Le nombre de petits, moyens et grands follicules par vache était respectivement de 21,7 ± 18,7, 14,9 ± 12,1 et 0,9 ± 1,1. Les populations folliculaires des vaches cycliques ou en anœstrus anovulatoire étaient respectivement de 29,1 ± 18,1 et 41,9 ± 27,3 ($p < 0,05$). Le nombre d'ovocytes par vache était respectivement de 15,5 ± 4,5 et 15,6 ± 3,8 ($p > 0,05$) chez les vaches cycliques et en anœstrus anovulatoire. Le nombre d'ovocytes de grades I, II, III et IV était respectivement de 4,5 ± 4,1 (28,5 %), 4,1 ± 3,7 (26,2 %), 2,3 ± 2,4 (14,8 %) et 4,8 ± 5,6 (30,5 %). Cette étude a montré que l'anœstrus anovulatoire avait tendance à augmenter la population folliculaire mais n'avait aucun effet sur le nombre d'ovocytes récoltés. Le nombre d'ovocytes de grades I et II utilisables pour la maturation *in vitro* était significativement plus faible chez les vaches en anœstrus anovulatoire que chez les vaches cycliques ($p < 0,05$).

Mots-clés : *Bos indicus*, anestrus, follicule ovarien, ovule, Cameroun

Resumen

Kouamo J., Zangue C.T., Zoli A.P. Evaluación de la población folicular y la calidad de ovocitos en vacas cebú (*Bos indicus*) con anestros anovulatorios

El objetivo del estudio fue evaluar la población folicular y la calidad de los ovocitos en el ovario de cebúes locales con anestros anovulatorios y fue conducido en el matadero municipal de Ngaoundere, región de Adamawa en Camerún. De los 496 tractos genitales examinados, 145 (29,2%) presentaron un anestro anovulatorio. El número promedio de folículos por vaca fue de 37,5 ± 25,2. El número promedio de folículos pequeños, medianos y grandes por vaca fue 21,7 ± 18,7, 14,9 ± 12,1 y 0,9 ± 1,1, respectivamente. Las poblaciones foliculares promedio en vacas cíclicas o con estros anovulatorios fue 29,1 ± 18,1 y 41,9 ± 27,3 ($p < 0,05$), respectivamente. El número de ovocitos por vaca fue 15,5 ± 4,5 y 15,6 ± 3,8 ($p > 0,05$) en vacas cíclicas y en anestro, respectivamente. El número de ovocitos de grados I, II, III y IV fue 4,5 ± 4,1 (28,5%), 4,1 ± 3,7 (26,2%), 2,3 ± 2,4 (14,8%) y 4,8 ± 5,6 (30,5%), respectivamente. Este estudio mostró que los anestros anovulatorios tendieron a fomentar la población folicular pero no tuvieron efecto en el rendimiento de ovocitos. El número de grados I y II de ovocitos aceptables para la maduración *in vitro* fue significativamente menor en vacas con anestros anovularios que en las vacas cíclicas ($p < 0,05$).

Palabras clave: *Bos indicus*, anestro, folículos do ovário, óvulo, Camerún