

Determinants of the follicular population and oocyte quality of Cameroonian native ewes

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Abstract

The present study was conducted to evaluate the ovarian potential of 87 local ewes from Ngaoundere, Adamawa region (Cameroon) for *in vitro* oocyte production. The ovaries were excised, submerged in normal saline solution (0.9%) and transported to the laboratory for a detailed evaluation. Follicles on each ovary were counted, their diameters (Φ) measured and were grouped into 3 categories: small ($\Phi < 2$ mm), medium ($2 \leq \Phi \leq 4$ mm) and large ($4 > \Phi \leq 10$ mm). Each ovary was then sliced into a petri dish; the oocytes were recovered in Dulbecco's phosphate buffered saline, examined under a stereoscope (x10) and graded into four groups based on the morphology of cumulus oophorus cells and cytoplasmic changes of the oocytes. The effects of both ovarian (ovarian localization, corpus luteum, size and weight of ovary) and non-ovarian factors (breed, age, body condition score (BCS) and pregnancy status) on the follicular population and oocyte recovery rate were determined. The average follicular population was 8.80 ± 2.97 follicles per ovary. The number of small, medium and large follicles were 6.13 ± 2.90 ; 1.60 ± 0.49 and 1.06 ± 0.05 , respectively. Oocyte recovery rate were 6.04 ± 1.01 per ovary. Oocytes graded I, II, III and IV were 1.20 ± 0.18 (11%); 1.23 ± 0.23 (14%); 1.13 ± 0.12 (7%) and 2.47 ± 0.93 (68%), respectively. The oocyte quality index was 1.98. Younger, non-pregnant ewes with a body condition score of 3 and large ovaries presented higher number of follicles and oocytes. Oocytes (grades I and II) acceptable for *in vitro* embryo production (IVEP) constituted 25% of the harvest. This study indicates that some factors such as age, body condition score, pregnancy status, ovarian localization, weight of ovary and corpus luteum must be taken into account to increase the potential of the ovary for IVEP.

Introduction

In Cameroon, the number of small ruminants is estimated at around 7 million heads, including 3.2 million sheep [1]. The sheep breeds encountered are among others the Djallonke, fulani of the Wayla and Ouda varieties, Mayo Kebbi and Kirdi. They are commonly called "the poor man's meat" [2]. However, this breeding is confronted with many problems in particular the reproduction remaining natural without any assistance of biotechnologies in particular artificial insemination, *in vitro* fertilization and embryo transfer [3]. The advantage of using these reproductive biotechnologies, is that it considerably increases the size of the herd while contributing to the preservation of the genetic heritage of sub fertile or dead animals [4].

Indeed, the ovaries can be considered as a sustainable and inexpensive source in which can be used to study the development and application of biotechnology of reproduction, conservation and cell freezing [5]. Embryos can be produced and transferred to recipient females from oocytes collected from the ovaries of slaughtered females. However, the development and application of assisted reproductive technologies such as *in vitro* embryo production (IVEP) through *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture can revolutionize research on domestic livestock [6]. In addition, the production of a good embryo necessarily requires better quality oocytes [7]. The initial and most important step in *in vitro* fertilization is the selection of viable oocytes capable of being matured *in vitro* [8]. In sub-Saharan Africa, the recovery rate of oocytes is low and the cost of embryo production *in vitro* is high [9]. To our knowledge, no study has been done on the characterization of follicles and oocytes of native ewes in Cameroon. Consequently, the present study was carried out with the aim of determining the ovarian potential of the local sheep

for the production of oocytes *in vitro* in Cameroon. Specifically, we will determine the follicular population and oocytes recovery rate; assess the influence of ovarian and non-ovarian parameters on follicular population, yield and quality of oocytes.

Materials and methods

Study area

The present study was carried out using a samples of ewes taken at the small ruminant slaughterhouse Bantaï and analyzed at the biochemical analysis laboratory (ADAM'S LABO) in the Adamawa region of Cameroon. Ngaoundere, chief town of the Adamawa region is situated between Latitude $7^{\circ}19'39''\text{N}$ and Longitude $13^{\circ}35'4''\text{E}$ and have an average annual rainfall of 1496.7 mm. The temperatures varied from 15.2°C to 29°C with an average humidity of 58.2%. The study was conducted from November 2019 to January 2020. The slaughtered ewes were from the Vina Division (62.1%), Mayo Rey Division (31%) and Mbam and Kim (6.9%).

Characteristics of animals

A total of 87 local ewes of different breeds [Djallonke (67), Peul (8), Kirdi (8) and Mayo Kebbi (4)] were selected for this study. The average

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live weight of the animals was measured using a Mechanical Pocket Balance 100Kg of precision 1 Kg. The body condition score (BCS) and age were determined as described by Russel *et al.* [10] and Salami [11], respectively. For pregnant ewes, the fetal age was determined by the formula $X = 2.1 (y + 17)$, y represented the cranio-caudal length in cm and X the duration of pregnancy in days [12] and the length of pregnancy was classified in three groups: ≤ 50 days, 51-100 days and >100 days.

Ovary collection and handling

After slaughter, the left and right ovaries were removed using scissors and placed in separate conical tubes containing Washed Medium (WM) and transported to the laboratory at 35-37°C within next 2 hours after slaughter. All cystic ovaries (Φ of the follicles >10 mm) were excluded from studies [13].

Determination of the weight and the size of the ovary

In the laboratory, the ovaries were removed from the tubes and placed in a petri dish, excessive tissues attached to the ovaries were carefully trimmed off and ovaries were weighed using an electronic Digital Scale with a precision of 0.01. The length, width and thickness of the ovaries were measured using a digital caliper like Carbon Fiber Composites Digital Caliper and the ovaries were thereafter allocated into two size groups ($<1.70 \times 1.26 \times 0.75$ and $>1.70 \times 1.26 \times 0.75$). Three types of corpus luteum have been identified: white, hemorrhagic and yellow.

Determination of follicular population

The ovaries were washed with washing medium. For each ovary, visible follicles were counted and follicular diameters (Φ s) was measured with digital caliper. Follicular Φ s were classified into 3 categories: small (<2 mm), medium ($[2$ to $4]$ mm) and large ($[4$ to $10]$ mm) as described by Mohamed *et al.* [14].

Recovery and grading of oocytes

The ovaries were placed in separate plastic Petri dishes containing Dulbecco's Phosphate Buffered Saline (DPBS) and the slicing technique was used to collect oocytes [13]. Oocyte quality was evaluated under a stereoscope (x10) and scored into four grades (G) according to the homogeneity of the cytoplasm and layers of cumulus cells as described by Khandoker *et al.* [15]. Grade I (GI): Oocytes with more than 4 layers of bunch of compact cumulus cells mass with evenly granulated cytoplasm; grade II (GII): oocyte with at least 2–4 layers of compact cumulus cell mass with evenly granulated cytoplasm; grade III (GIII): Oocyte with at least one layer of compact cumulus cell mass with evenly granulated cytoplasm; grade IV (GIV): Denuded oocyte with no cumulus cells or incomplete layer of cumulus cell or expanded cells and having dark or unevenly granulated cytoplasm. The overall quality was calculated as an index using the formula $[(G I \times 1 + G II \times 2 + G III \times 3 + G IV \times 4) / \text{Total number of oocytes recovered}]$ as described by Baki Acar *et al.* [16]. Index values that approach one reflected good quality oocytes.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Sciences) Version 25.0. The analysis of variance and Duncan's test statistics were used to analyze appropriate data sets. Differences were significant at $P < 0.05$.

Results

Characteristics of slaughtered ewes

The mean live weight (Kg), BCS and age (years) of the ewes were 27.25 ± 4.16 ; 2.44 ± 0.54 and 2.71 ± 1.33 , respectively. A pregnancy rate of 68% was recorded and the average age of the fetal was around 72 days.

The mean weight (g) of the ovaries was 1.16 ± 0.49 . The right ovaries (1.18 ± 0.45 g) were heavier than the left (1.15 ± 0.55 g). The length, wide and thickness (cm) of the ovaries were 1.7 ± 0.25 , 1.26 ± 0.18 and 0.75 ± 0.14 , respectively. The ovaries of pregnant ewes were significantly ($P < 0.05$) larger and heavier than those of non-pregnant ones (Table 1).

Follicular population

From 174 ovaries, 1,194 follicles were counted. The mean number of follicles per ovary recorded was 8.80 ± 2.97 . Small ($\Phi < 2$ mm), medium ($2 \geq \Phi \leq 4$ mm) and large ($4 > \Phi \leq 10$ mm) follicles represented 87%, 11% and 2% of the follicular population, respectively.

Yield and quality of oocytes

The average number of oocytes recovered per ovary was 6.04 ± 1.01 ($n = 607$) with a recovery rate of 65%. The quality of the oocytes graded I, II, III and IV (Figure 1) were 1.20 ± 0.18 (11%), 1.23 ± 0.23 (14%) 1.13 ± 0.12 (7%) and 2.47 ± 0.93 (68%), respectively. Selected oocytes for in vitro embryo production (IVEP) (G I and II) represented 2.43 ± 0.41 (25%) per ovary. The oocyte index was 1.98.

Influence of ovarian parameters (ovarian localization, corpus luteum, size and weight of ovary) on the follicular population, number and grade of the oocytes

The right ovary tended to have more follicles than the left ($P > 0.05$). However, the left ovary tended to have more oocytes ($P > 0.05$). The total number of follicles increased with the weight (g) and size (cm) of the ovary; however, the yield and quality of the oocyte decreased with the weight and size of the ovary. On the other hand, ovaries with a corpus luteum had fewer follicles and oocytes acceptable for IVEP (Tables 2 and 3).

Influence of non-ovarian parameters (breed, age, BCS, state and pregnancy length) on the follicular population, number and grade of the oocytes

The total number of follicles and oocytes was higher in ewes less than 3 years old and with a BCS of 3. However, non-pregnant ewes or those whose pregnant was found in the first three months presented also a large number of follicles and a good yield of oocytes (Tables 4 and 5).

Discussion

The characteristics of the breeds of ewes in this study indicate that a large number of Djallonke breeds were slaughtered, which is contrary to that observed by Kouamo *et al.* [17] at the municipal slaughterhouse in Maroua with a dominance of Fulani sheep. However, the average age of slaughtered ewes was slightly higher than those recorded by Benchaib [18] and Kouamo *et al.* [17]. Indeed, the average live weight of ewes (27.25 ± 4.16 kg) was higher than those reported by Ngona *et al.* [19] in the Democratic Republic of Congo (20.50 ± 4.96 kg) and Kouamo *et al.* [17] (23.00 ± 2.90 kg). The difference could be explained by the large number of pregnant ewes studied.

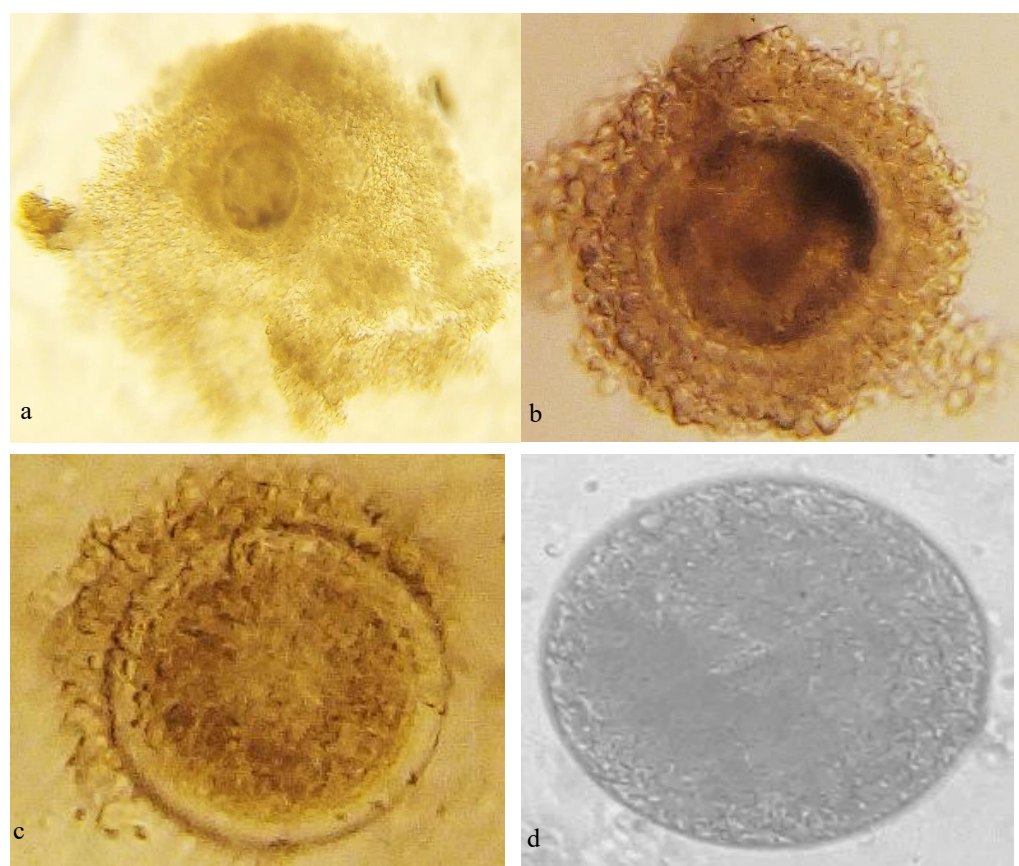


Figure 1. a= Oocyte grade I; b= Oocyte grade II; c= Oocyte grade III; d= Oocyte grade IV

Table 1. Means \pm SD values of the weight and size of the ovaries, breed, BCS, age, pregnancy status and corpus luteum

Parameters	Variables	N	Left ovary weight (g)	Right ovary weight (g)	Ovary weight (g) per animal	Ovary length (cm)	Ovary width (cm)	Ovary thickness (cm)
Breed	Djallonke	67	1.13 \pm 0.53 ^{ab}	1.19 \pm 0.44 ^a	1.16 \pm 0.38 ^{ab}	1.69 \pm 0.24 ^a	1.27 \pm 0.17 ^b	0.75 \pm 0.14 ^{ab}
	Kirdi	8	0.78 \pm 0.51 ^a	0.97 \pm 0.46 ^a	0.87 \pm 0.46 ^a	1.51 \pm 0.18 ^a	1.07 \pm 0.05 ^a	0.64 \pm 0.06 ^a
	Mayo Kebbi	4	1.47 \pm 0.30 ^b	0.95 \pm 0.44 ^a	1.21 \pm 0.34 ^{ab}	1.71 \pm 0.16 ^a	1.20 \pm 0.13 ^b	0.74 \pm 0.10 ^{ab}
	Peul	8	1.52 \pm 0.59 ^{ab}	1.48 \pm 0.45 ^a	1.50 \pm 0.41 ^b	2.01 \pm 0.15 ^b	1.48 \pm 0.17 ^c	0.85 \pm 0.15 ^b
	P-value		0.045	0.095	0.039	0.001	0.000	0.019
BCS	1 (Thin)	2	1.60 \pm 0.01 ^a	1.64 \pm 0.05 ^a	1.62 \pm 0.03 ^a	1.83 \pm 0.04 ^a	1.35 \pm 0.05 ^a	0.72 \pm 0.11 ^a
	2 (Medium)	45	1.13 \pm 0.54 ^a	1.18 \pm 0.42 ^a	1.16 \pm 0.39 ^a	1.71 \pm 0.21 ^a	1.27 \pm 0.19 ^a	0.73 \pm 0.14 ^a
	3 (Good)	40	1.14 \pm 0.57 ^a	1.16 \pm 0.50 ^a	1.15 \pm 0.42 ^a	1.68 \pm 0.29 ^a	1.26 \pm 0.18 ^a	0.78 \pm 0.13 ^a
	P-value		0.235	0.320	0.224	0.521	0.590	0.083
Age (years)	< 1	10	1.21 \pm 0.31 ^{ab}	1.15 \pm 0.29 ^{ab}	1.18 \pm 0.17 ^b	1.74 \pm 0.19 ^b	1.37 \pm 0.21 ^b	0.77 \pm 0.08 ^a
	[1-2]	14	0.78 \pm 0.41 ^a	0.90 \pm 0.36 ^a	0.84 \pm 0.34 ^a	1.49 \pm 0.20 ^a	1.13 \pm 0.12 ^a	0.72 \pm 0.10 ^a
	[2-3]	7	1.11 \pm 0.65 ^{ab}	1.04 \pm 0.41 ^{ab}	1.07 \pm 0.47 ^{ab}	1.62 \pm 0.21 ^{ab}	1.22 \pm 0.14 ^{ab}	0.72 \pm 0.12 ^a
	[3-4]	56	1.23 \pm 0.57 ^b	1.28 \pm 0.47 ^b	1.26 \pm 0.41 ^b	1.76 \pm 0.24 ^b	1.29 \pm 0.18 ^b	0.76 \pm 0.16 ^a
	P-value		0.034	0.028	0.005	0.005	0.006	0.657
Pregnancy status	Non pregnant	28	1.05 \pm 0.44 ^a	0.99 \pm 0.42 ^a	1.02 \pm 0.41 ^a	1.64 \pm 0.25 ^a	1.20 \pm 0.20 ^a	0.72 \pm 0.16 ^a
	Pregnant	59	1.19 \pm 0.59 ^a	1.27 \pm 0.44 ^b	1.23 \pm 0.39 ^b	1.73 \pm 0.24 ^a	1.30 \pm 0.17 ^b	0.77 \pm 0.13 ^a
	P-value		0.298	0.005	0.026	0.078	0.004	0.060
Corpus luteum	Absent	15	0.90 \pm 0.44 ^a	0.98 \pm 0.43 ^a	0.94 \pm 0.41 ^a	1.61 \pm 0.17 ^a	1.12 \pm 0.17 ^a	0.65 \pm 0.11 ^a
	Present	72	1.20 \pm 0.56 ^a	1.23 \pm 0.45 ^b	1.21 \pm 0.39 ^b	1.72 \pm 0.26 ^a	1.30 \pm 0.17 ^b	0.77 \pm 0.14 ^b
	P-value		0.064	0.047	0.028	0.131	0.000	0.002

a,b,c In each column different letters indicated significant difference between group ($p < 0.05$)

N=number of ewes

SD=standard deviation

Table 2. Effects of ovarian factors on follicular population. a,b, In each column different letters indicated significant difference between group (p<0.05) N=number of ewes

Parameters	Variables	N	Number of follicles			Average number of follicles /ovary
			Small (<2 mm)	Medium ([2-4] mm)	Large ([4-10] mm)	
Ovary localization	Left	87	5.86 ± 3.03 ^a	1.45 ± 0.48 ^b	1.00 ± 0.00 ^b	8.28 ± 3.15 ^a
	Right	87	6.30 ± 3.91 ^a	1.72 ± 0.77 ^a	1.05 ± 0.21 ^a	9.06 ± 3.92 ^a
	P-value		0.420	0.007	0.085	0.148
Corpus luteum	Absent	15	6.07 ± 3.08 ^a	1.83 ± 0.20 ^b	1.00 ± 0.00 ^a	8.90 ± 3.08 ^a
	Present	72	6.15 ± 2.89 ^a	1.58 ± 0.53 ^a	1.07 ± 0.05 ^b	8.81 ± 2.98 ^a
	P-value		0.995	0.000	0.000	0.901
Ovary weight (g)	<1	27	6.55 ± 3.47 ^a	1.50 ± 0.21 ^a	1.00 ± 0.00 ^a	9.05 ± 3.51 ^a
	[1-1.50]	41	5.73 ± 2.66 ^a	1.62 ± 0.52 ^a	1.00 ± 0.00 ^a	8.35 ± 2.77 ^a
	>1.50	19	6.70 ± 2.38 ^a	1.71 ± 0.68 ^a	1.10 ± 0.11 ^b	9.51 ± 2.39 ^a
	P-value		0.340	0.146	0.000	0.185
Ovary size (cm ³)	<1.70x1.26x0.75	50	5.94 ± 2.84 ^a	1.64 ± 0.44 ^b	1.00 ± 0.00 ^a	8.58 ± 2.88 ^a
	>1.70x1.26x0.75	37	6.39 ± 3.02 ^a	1.56 ± 0.57 ^a	1.07 ± 0.08 ^b	9.02 ± 3.13 ^a
	P-value		0.656	0.000	0.000	0.673

Table 3. Effects of ovarian factors on oocyte number and grade

Parameters	Variables	N	Average number of oocytes /ovary	Oocyte grades				Selected oocytes for IVEP. I and II
				I	II	III	IV	
Ovary localization	Left	87	6.09 ± 1.30 ^a	1.17 ± 0.24 ^b	1.42 ± 0.44 ^b	1.03 ± 0.11 ^a	2.46 ± 1.16 ^a	2.59 ± 0.49 ^b
	Right	87	5.90 ± 1.31 ^a	1.14 ± 0.22 ^a	1.11 ± 0.20 ^a	1.16 ± 0.25 ^b	2.49 ± 1.26 ^a	2.25 ± 0.29 ^a
	P-value		0.244	0.00	0.000	0.000	0.874	0.000
Corpus luteum	Absent	15	5.59 ± 0.72 ^a	1.27 ± 0.24 ^b	1.25 ± 0.23 ^a	1.00 ± 0.00 ^a	2.07 ± 0.75 ^a	2.52 ± 0.44 ^a
	Present	72	6.14 ± 1.06 ^a	1.19 ± 0.17 ^a	1.23 ± 0.24 ^a	1.17 ± 0.14 ^b	2.55 ± 0.96 ^a	2.42 ± 0.25 ^a
	P-value		0.170	0.003	0.055	0.000	0.051	0.193
Ovary weight (g)	<1	27	6.20 ± 1.24 ^a	1.16 ± 0.09 ^b	1.38 ± 0.23 ^b	1.00 ± 0.00 ^a	2.66 ± 1.12 ^a	2.54 ± 0.24 ^c
	[1-1.50]	41	5.82 ± 0.84 ^a	1.30 ± 0.20 ^c	1.17 ± 0.21 ^a	1.13 ± 0.12 ^b	2.21 ± 0.83 ^a	2.47 ± 0.31 ^b
	>1.50	19	6.46 ± 0.91 ^a	1.15 ± 0.21 ^a	1.21 ± 0.26 ^a	1.33 ± 0.19 ^c	2.76 ± 0.73 ^a	2.36 ± 0.31 ^a
	P-value		0.089	0.000	0.001	0.000	0.087	0.003
Ovary size (cm ³)	<1.70x1.26x0.75	50	6.15 ± 1.10 ^a	1.21 ± 0.19 ^a	1.25 ± 0.25 ^b	1.23 ± 0.16 ^b	2.46 ± 1.03 ^a	2.46 ± 0.32 ^a
	>1.70x1.26x0.75	37	5.97 ± 0.89 ^a	1.26 ± 0.18 ^b	1.21 ± 0.22 ^a	1.00 ± 0.00 ^a	2.49 ± 0.79 ^a	2.47 ± 0.25 ^a
	P-value		0.614	0.000	0.000	0.000	0.908	0.916

a,b,c In each column different letters indicated significant difference between group (p<0.05) N=number of ewes

Table 4. Effects of non-ovarian factors on follicular population

Parameters	Variables	N	Number of follicles			Average number of follicles / ovary
			Small (<2mm)	Medium ([2-4] mm)	Large ([4-10] mm)	
Breed	Djallonke	67	5.79 ± 2.80 ^{ab}	1.54 ± 0.43 ^b	1.00 ± 0.00 ^a	8.32 ± 2.90 ^{ab}
	Kirdi	8	9.25 ± 2.30 ^c	0.75 ± 0.19 ^a	1.00 ± 0.00 ^a	11.00 ± 2.35 ^b
	Mayo Kebbi	4	3.38 ± 1.75 ^a	2.00 ± 1.35 ^b	1.50 ± 0.00 ^b	6.88 ± 1.60 ^a
	Peul	8	6.69 ± 3.31 ^{bc}	1.63 ± 0.38 ^b	1.00 ± 0.00 ^a	9.31 ± 3.31 ^{ab}
	P-value		0.003	0.000	0.000	0.049
BCS	1	2	8.50 ± 1.41 ^a	0.00 ± 0.00 ^a	0.50 ± 0.00 ^a	9.00 ± 1.41 ^a
	2	45	6.12 ± 3.29 ^a	1.70 ± 0.59 ^b	1.17 ± 0.06 ^b	8.99 ± 3.28 ^a
	3	40	5.73 ± 2.83 ^a	1.44 ± 0.33 ^b	1.00 ± 0.00 ^c	8.16 ± 2.94 ^a
	P-value		0.098	0.000	0.000	0.658
Age (years)	<1	10	6.00 ± 2.52 ^a	2.19 ± 0.65 ^b	0.50 ± 0.00 ^b	8.69 ± 2.56 ^{ab}
	[1-2]	14	7.50 ± 3.28 ^a	1.24 ± 0.20 ^a	1.00 ± 0.00 ^c	9.74 ± 3.34 ^b
	[2-3]	7	5.50 ± 3.34 ^a	1.50 ± 0.29 ^a	0.00 ± 0.00 ^a	7.00 ± 3.48 ^a
	[3-4]	56	5.83 ± 2.87 ^a	1.48 ± 0.45 ^a	1.10 ± 0.06 ^d	8.41 ± 2.92 ^{ab}
	P-value		0.132	0.000	0.000	0.246
Pregnancy status	Non pregnant	28	6.90 ± 3.10 ^a	1.56 ± 0.43 ^a	0.72 ± 0.08 ^a	9.18 ± 3.24 ^a
	pregnant	59	5.78 ± 2.76 ^a	1.64 ± 0.53 ^a	1.10 ± 0.06 ^b	8.52 ± 2.81 ^a
	P-value		0.140	0.028	0.000	0.403
Pregnancy length in days	[1-50]	19	6.26 ± 3.28 ^a	1.51 ± 0.38 ^b	1.00 ± 0.00 ^a	8.78 ± 3.51 ^a
	[51-100]	27	5.62 ± 2.72 ^a	1.86 ± 0.68 ^c	1.13 ± 0.08 ^b	8.61 ± 2.72 ^a
	>100	13	5.38 ± 1.84 ^a	1.00 ± 0.00 ^a	1.00 ± 0.00 ^a	7.38 ± 1.84 ^a
	P-value		0.374	0.000	0.000	0.172

a,b,c,d In each column different letters indicated significant difference between group (p<0.05) N=number of ewes

Table 5. Effects of non-ovarian factors on oocytes number and grade

Parameters	Variables	N	Average number of oocytes/ovary	Oocyte grades				Selected oocytes for IVEP. I and II
				I	II	III	IV	
Breed	Djallonke	67	5.92 ± 1.01 ^a	1.22 ± 0.19 ^b	1.21 ± 0.23 ^{ab}	1.08 ± 0.08 ^b	2.40 ± 0.95 ^a	2.44 ± 0.30 ^b
	Kirdi	8	6.62 ± 0.89 ^a	1.00 ± 0.00 ^a	1.00 ± 0.00 ^a	1.25 ± 0.32 ^c	3.37 ± 0.83 ^b	2.00 ± 0.00 ^a
	Mayo Kebbi	4	4.54 ± 0.63 ^b	1.00 ± 0.00 ^a	1.00 ± 0.00 ^a	0.66 ± 0.23 ^a	1.87 ± 0.48 ^a	2.00 ± 0.00 ^a
	Peul	8	6.11 ± 0.66 ^a	1.33 ± 0.12 ^b	1.37 ± 0.33 ^b	1.00 ± 0.00 ^b	2.50 ± 0.46 ^{ab}	2.61 ± 0.34 ^b
	P-value		0.00	0.022	0.00	0.020	0.00	0.008
BCS	1	2	4.25 ± 0.35 ^a	0.50 ± 0.00 ^a	1.00 ± 0.00 ^a	0.00 ± 0.00 ^a	2.75 ± 0.35 ^a	1.50 ± 0.00 ^a
	2	45	6.17 ± 0.35 ^b	1.61 ± 0.18 ^b	1.27 ± 0.24 ^b	1.15 ± 0.11 ^b	2.59 ± 1.00 ^a	2.43 ± 0.25 ^b
	3	40	5.76 ± 0.98 ^b	1.14 ± 0.12 ^b	1.22 ± 0.24 ^{ab}	1.11 ± 0.15 ^b	2.30 ± 0.91 ^a	2.35 ± 0.28 ^b
	P-value		0.041	0.000	0.204	0.000	0.442	0.000
Age (years)	<1	10	5.45 ± 0.72 ^{ab}	1.00 ± 0.00 ^a	1.00 ± 0.00 ^a	1.00 ± 0.00 ^b	2.45 ± 0.72 ^a	2.00 ± 0.00 ^a
	[1-2]	14	5.68 ± 1.17 ^b	1.27 ± 0.23 ^b	1.20 ± 0.15 ^b	0.50 ± 0.00 ^a	2.71 ± 1.05 ^a	2.47 ± 0.26 ^b
	[2-3]	7	5.83 ± 1.12 ^b	1.00 ± 0.00 ^a	1.42 ± 0.25 ^c	1.00 ± 0.00 ^b	2.42 ± 1.02 ^a	2.42 ± 0.25 ^b
	[3-4]	56	4.79 ± 1.00 ^a	1.22 ± 0.20 ^b	1.28 ± 0.26 ^{bc}	1.18 ± 0.15 ^c	2.42 ± 0.94 ^a	2.50 ± 0.31 ^b
	P-value		0.154	0.000	0.001	0.000	0.489	0.000
Pregnancy status	Non pregnant	28	6.04 ± 0.98 ^a	1.43 ± 0.24 ^b	1.25 ± 0.26 ^a	1.18 ± 0.20 ^b	2.17 ± 0.79 ^a	2.68 ± 0.37 ^b
	Pregnant	59	6.00 ± 1.06 ^a	1.04 ± 0.06 ^a	1.22 ± 0.23 ^a	1.13 ± 0.08 ^a	2.61 ± 0.98 ^a	2.26 ± 0.23 ^a
	P-value		0.852	0.000	0.518	0.045	0.067	0.000
Pregnancy length in days	[1-50]	19	6.22 ± 1.27 ^b	1.00 ± 0.00 ^a	1.29 ± 0.33 ^b	1.00 ± 0.00 ^a	2.92 ± 1.10 ^b	2.29 ± 0.33 ^b
	[51-100]	27	6.12 ± 0.98 ^b	1.23 ± 0.18 ^b	1.15 ± 0.15 ^{ab}	1.13 ± 0.08 ^b	2.62 ± 0.94 ^{ab}	2.38 ± 0.22 ^b
	>100	13	5.36 ± 0.68 ^a	1.00 ± 0.00 ^a	1.08 ± 0.13 ^a	1.17 ± 0.12 ^b	2.12 ± 0.71 ^a	2.08 ± 0.13 ^a
	P-value		0.000	0.000	0.023	0.003	0.072	0.054

a,b,c In each column different letters indicated significant difference between group ($p < 0.05$)
N=number of ewes

The pregnancy rate (68%) was higher than that observed by Manjeli *et al.* [20] at the Garoua and Maroua slaughterhouse (38.60 %); Benchaib [18] in Algeria (26.00 %); Nana *et al.* [21] in the city of Dschang (49%) and Kouamo *et al.* [17] at the municipal slaughterhouse in Maroua (45.30 %). On the other hand, this rate is lower than that of Pitala *et al.* [22] in Togo (80.10 %). The difference could be related to the study period and the breed [17]. As reported by Manjeli *et al.* [20]; Pitala *et al.* [22] in Togo and Nana *et al.* [21] in Cameroon, the majority of pregnant ewes slaughtered were within the first three months of gestation. This could be explained on the one hand by the fact that the slaughter of pregnant females is not only due to ignorance of the physiological state of the animals because their physical appearance clearly indicates their physiological condition; but more because of the socio-economic context of small ruminant farming in our country which is considered as a subsistence farming to resolve urgent needs whatever the physiological state of the animals [20]. In fact, by slaughtering pregnant females, humans attack reproduction at its root, by exterminating future reproducers [21].

The average weight of the ovaries (1.16 ± 0.49 g) was lower than that reported by Mohamed *et al.* [14] (1.30 ± 0.23 g) and higher than that of Islam *et al.* [23] (0.69 ± 0.01 g) in goats. This difference may be due to the effect of the breed. The average dimensions of the ovaries were slightly larger than those of Islam *et al.* [23] and Mohamed *et al.* [14]. This result shows that the presence of the corpus luteum had a positive effect on the dimensions of the ovary because the corpus luteum formed from the follicle which ovulated develops in all direction on the surface of the ovary. However, the difference between the left and right ovaries has also been reported by Islam *et al.* [23], Mohamed *et al.* [14] and Asad *et al.* [7]. In fact ovulation was more marked in the right ovary. This greater activity of the right ovary would be responsible for its weight.

The average number of follicles (8.80 ± 2.97) was higher than that of Mohamed *et al.* [14] (4.9 ± 0.89) and Wani *et al.* [24] (7.46 ± 0.14). However, the number of follicles with diameters less than 2 mm was higher than those with diameters greater than 2 mm as reported by

Mohamed *et al.* [14]. The slicing technique allows the recovery of all oocytes present in all follicles regardless of their location on the ovarian cortex. The average oocyte yield per ovary was 6.04 ± 1.01 oocytes. This value is higher than that observed by Wani *et al.* [24] (5.87 ± 0.08 oocytes) and Rameez *et al.* [6] (4.51 ± 0.25 oocytes) in goats in India. This difference may be due to the collection technique used. However, greater follicular activity was recorded in the right ovary, with a larger number of large follicles ($P < 0.05$) [14]. The total number of follicles was higher in the right ovaries [14,25,26]. However, ovaries without a corpus luteum had a higher number of oocytes acceptable for IVEP compared to ovaries with a corpus luteum [7,14,27]. This could be due to limited follicular development because lutein cells occupy a significant part of the ovary [6]. It can also arise from the fact that the corpus luteum could inhibit the growth of follicles and increase their atresia [28].

The total number of follicles acceptable grade of oocytes was higher in the ovaries of non-pregnant ewes than others [29]; however, Eias *et al.* [25] obtained a high number of follicles with pregnant female animals in the buffalo.

Conclusion

In conclusion, this study indicated that the ovaries of the native ewes in Cameroon have a low potential to produce acceptable oocytes for IVEP. Future studies must take into account certain parameters such as BCS, age and physiological status to maximize success of IVEP.

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