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Investigation of Ovarian and Non-ovarian Associated Factors Related to Follicular Population and Oocyte Maturation of Chadian Cattle Breeds

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ABSTRACT

A cow can give birth to an average of 6-7 calves in her entire reproductive period. The remaining oocytes could be used for the *in vitro* production of embryos. The present study was conducted to evaluate the effects of ovarian and non-ovarian factors on the follicular population and oocyte maturation of three Chadian cattle breeds (Arab, Kouri, and Toupouri). For this purpose, the ovaries of 166 cycled cows were collected at the Farcha slaughterhouse of Chad and placed individually in labeled conical tubes containing 0.9% NaCl and 0.5 mg/ml penicillin-streptomycin. After clearing the ovaries of tissue debris, they were weighed, and the follicles were counted. The diameter of each follicle was measured and classified into three categories. A total of 2734 oocytes were collected in 28 days with a minimum of 97 per day by the slicing method using a 10X stereoscope. They were then classified into four groups according to the structure of their cumulus oophorus. Immature oocytes (class 1 and 2 [1455]) were placed in different culture media consisting of Minimum Essential Medium (MEM) alone, MEM with 10% follicular fluid, and MEM with 50% follicular fluid for oocyte maturation. The results indicated that the mean follicular population and mean oocyte yield were 24.71 ± 0.88 and 11.65 ± 0.94, respectively. The mean oocyte index and the number of cultivable oocytes for *in vitro* embryo production (class 1 and 2) were 1.03 ± 0.23 and 1.65 ± 0.94, respectively. The number of follicles observed in the age group of 6-9 years was higher than in other age groups. Oocyte yield was significantly higher in cows with a body condition score of 4-5 compared to average and lean cows. Among the different culture media used for oocyte maturation, the medium consisting of MEM plus 10% follicular fluid recorded a higher maturation rate than the other culture media. Cows aged 6-9 years had a higher maturation rate than other age groups. In conclusion, the good follicle (follicle that produced oocyte) and appropriate oocyte performance were observed in cows with body condition score 3-5 and an age range of 6-9 years.

Keywords: Age, Breed, Cattle, Maturation, Oocyte

INTRODUCTION

Livestock significantly influences the economies of sub-Saharan African countries (Tacher and Letenneur, 1999). It can reduce poverty and increase food disponibility. In Chad, the livestock sector, with more than 20 million ruminants, including cattle, supports 40% of the population's needs for meat (MDPPA, 2011). With more than 10 million heads, the cattle sector plays a vital role in national animal production (PNDE, 2017). It provides almost 87,000 tons of meat and 89% of the milk supply annually (FAO and CEEAC 2021). Despite this high representativeness of cattle and their contribution to the bioavailability of meat in Chad, their numerical productivity remains very low, and the oocyte reserve of a heifer at birth is estimated at around 100,000 oocytes (Hanzen et al., 2000). A cow can give birth to an average of 6-7 calves in during her entire reproductive period. The remaining oocytes could be used for the *in vitro* production of embryos.

In this regard, there are new modern reproduction techniques, such as embryo *in vitro* production and transfer to the recipient mother (Manik et al., 2003; Huang and Rosenwarks, 2012). These new techniques contribute to the intensification of the genetic improvement of the herd (Chukwuka et al., 2010) and the usefulness of slaughtered pregnant cows' oocytes.

The production of embryos *in vitro* and transfer to recipient females act as alternatives to artificial insemination (Huang and Rosenwarks, 2012). These techniques allow the preservation of the genetic potential of sub-fertile or dead animals (Deuleuze et al., 2009) through the creation of a gene bank (Ducos et al., 2021) and the production of embryos from the oocytes of slaughtered animals to promote or multiply of this species (Guignot, 2005). Ovaries collected from animals after slaughter are the greatest source of inexpensive primary oocytes that could be incubated until maturation, followed by *in vitro* fertilization (Agrawal et al., 1995). This technique has not yet been sufficiently explored in Chad

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although many diverse cattle species exist. This study was conducted to highlight the use of the ovarian oocytes of slaughtered cows at the slaughterhouse.

MATERIALS AND METHODS

Ethical approval

The work was conducted based on the ethical rules of the National Institute of Science and Technology of Abeche, Chad.

Study site

The study was carried out from October 2020 to the end of September 2021 in the Farcha slaughterhouse, Chad, and the laboratory of the Institute of Livestock Research for Development in the Chari baguirmi region, in the peri-urban area of Ndjamena, Chad (13°49'59''N and 20°50'05''E). Arid climate and rainfall are nil for five months, from November to March, while July and August are well watered, with 144 mm and 175 mm, respectively.

Animal

A total of 166 cycled cows were divided into three breeds, namely Arab (n = 59), Kouri (n = 57), and Toupouri (n = 50). In this study, 40 pregnant cows were grouped into Arab (n = 11), Kouri (n = 15), and Toupouri (n = 14). The study was conducted in two seasons, a dry season (October to June) and a rainy season (July to September).

Age determination

The age of each animal (pregnant or not) was determined by simultaneous analysis of the dentition and the horn (Garba et al., 2013, Table 1). When a female was pregnant, the approximate age of the fetus was determined using the following formula: $Y= X(X+2)$

where, X represents the number of months of gestation, and Y denotes the nape-rump length in centimeters (Santos et al. 2013).

For age by horns, the following formula was used: Age (in years) = N + 2,

where, N represents the number of furrows and 2 is constant (Garba et al., 2013).

Table 1. Method of determining the age of the cows (Arab, Kouri, and Toupouri) in Chad

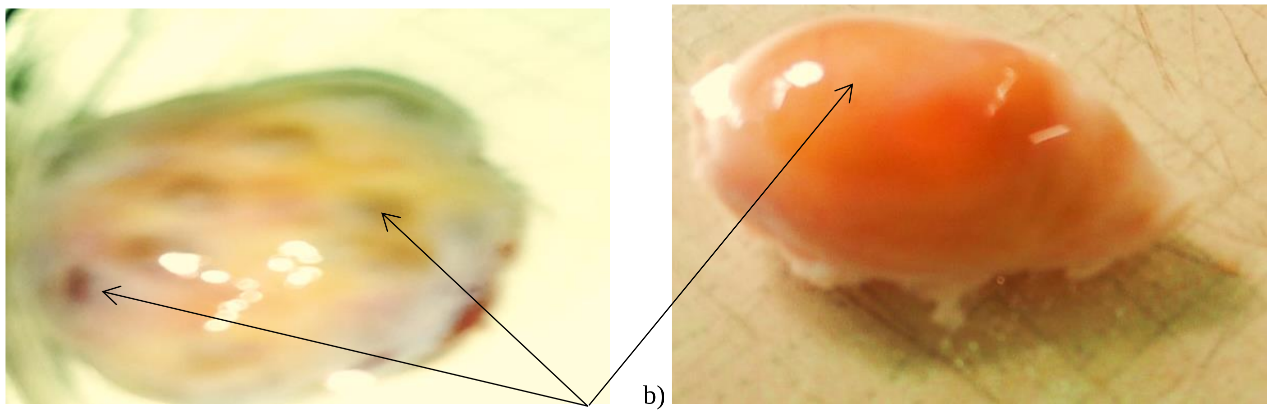
Teething	Age of the cow
Full development of the first intermediate pair of permanent incisors and corners	3-5 years
Permanent pinchers show noticeable wear	6 -9 years
Corners show dental stars	10 and more

Body condition score

The body condition score (BCS) was determined before the animal was slaughtered according to a study by Vall et al**.** (2004). As proposed by Natumanya et al. (2008), the cows were classified into three categories. Scores 1-2 represent lean, score 3 refers to average, and scores 4-5 are for fat.

Ovary collection

After the slaughter of the animal, the two ovaries were removed separately by incising the broad ligament with a chisel. These were identified (left and right ovary) and individually introduced into tubes containing 0.9% NaCl isotonic medium and penicillin-streptomycin (0.5 mg/ml). The samples (ovaries) were placed in an isothermal container at a temperature of 30-32°C and transported immediately to the laboratory in less than 20 minutes, as the slaughterhouse and the laboratory were situated in the same company. Ovaries with pathologies (cysts) were excluded (Wang et al., 2007).



a) b)

Follicules

Figure 1. Ovaries bearing follicles in a cow. **a**: small and medium; **b**: large (Source: Azafack, 2019)

Determination of follicular population

In the laboratory, ovaries were cleaned 4 times using 0.9 % of sodium chloride and cleared of their tissue debris using a chisel. Each ovary was weighed. The surface area of each ovary was observed, the present follicles were counted and their diameters (Φ) were measured using electronic stainless hardened calipers, then classified into three categories of small ($\Phi < 3$ mm), medium ($3 \leq \Phi \leq 8$ mm), and large ($\Phi > 8$ mm) following a study by Duygu et al. (2013). The color and shape of the corpus luteum were observed to determine the stage of each cow's sexual cycle, as described by Houmadi (2007).

Oocyte collection and classification

Ovaries were incised using the slicing technique (Wang et al., 2007). Into a petri dish containing 5ml of 0.9% NaCl solution to collect oocytes.

Morphological evaluation of oocyte quality

The collected oocytes were examined and counted under stereoscope at (10X) objective and then classified into four qualities taking into account the homogeneity of the cytoplasm or layers of cumulus oophorus cells according to Alves et al. (2014). Quality 1 entails compacted cumulus having more than three layers with homogeneous cytoplasm. Quality 2 entails compacted cumulus with one or two layers with homogeneous cytoplasm. Quality 3 encompasses cumulus not very compacted with irregular cytoplasm with dark zones, and quality 4 includes no cumulus with irregular cytoplasm for those of quality 1 and 2, these oocytes were recovered with a pipette for *in vitro* maturation. The average oocyte yield per cow was determined as the ratio of the total number of oocytes to the total number of cows. The oocyte index (In) was calculated using the following formula:

In = ([quality I x 1+ quality II x 2 + quality III x 3 + quality IV x 4] / total number of oocytes) to assess the overall oocyte quality (Duygu et al., 2013). A value tending towards 1 reflects good overall oocyte quality.

Maturation of oocytes

Media used for maturation

The different culture media used for oocyte maturation were composed. Medium 1 included minimum essential medium (MEM), Medium 2 contained MEM + 10% follicular fluid, and Medium 3 consisted of MEM + 50% follicular fluid.

Follicular fluid

Follicular fluid was collected by puncturing the antral follicles, as described by Fahiminiya et al. (2010).

Oocyte collection and culture

After examination, counting under a stereoscope, and classification of the oocytes, the cultivable oocytes (class 1 and 2) were removed with a pipette and placed in a multi-well dish with 5-6 oocytes per well. Each well contained 1.5 ml of culture medium. The dish was wrapped in a plastic bag and incubated in an oven at 38°C under 5% CO_2 for 24 hours.

Oocyte reading

After 24 hours of incubation, the oocytes were observed in their wells using an inverted microscope at 400 X magnification to observe the maturation rate. Oocytes considered mature were those with expanded cumuli (Figure 1). On the other hand, those whose cumuli persisted were considered immature (Figure 2).

Statistical analysis

All data were entered into Excel® and subjected to multifactor analysis of variance (multifactor ANOVA) using SPSS (Statistical data analysis software Package for Social Scientists, USA), version 20. Duncan's t-test was used to separate the means where there was a difference ($p < 0.05$).

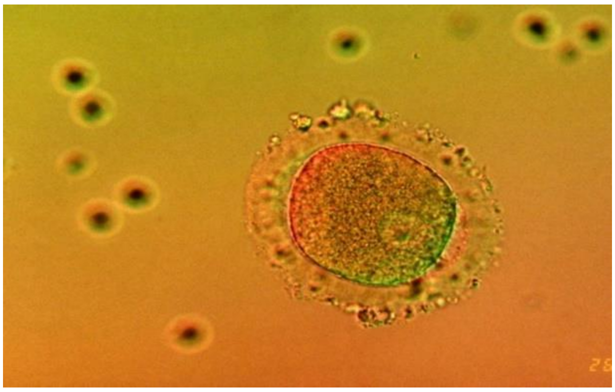


Figure 2. Mature oocyte in a cow

Figure 3. Immature oocytes in a cow

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RESULTS

Characterization of cows according to breed, age, body condition score, and physiological status

According to Table 2, the rate of pregnant cows was 13%. Regarding BCS, 31.66 of the slaughtered cows had BCS 3. The average age of the cows in the current study was 8 ± 0.88.

Determination of the follicular population

Figure 4 shows the follicle population in different classes. It can be seen that the number of follicles was inversely higher according to their size (small follicles: 14.32 ± 0.72, medium follicles: 10.25 ± 0.43, and large follicles: 0.61 ± 0.04).

Determination of oocyte class yield

Table 3 present the number of oocytes per class. As it can be seen, the number of oocytes was not spread out in different classes although a slight increase was observed in class I.

Table 2. Distributions of cows by breed, age, body condition score, and physiological status in Chad

Factors	Breed	Arab	Kouri	Toupouri	Average
BCS	[1-2]	17	19	15	17
	[3]	34	38	23	31.66
	[4-5]	19	15	26	20
	Total	70	72	64	-
Age (years)	[3-5]	42	43	39	41
	[6-9]	20	21	18	19.66
	[10 and more]	8	8	7	7
	Total	70	72	64	-
Physiological status	Not pregnant	59	57	51	55.66
	Pregnant	11	15	13	13
	Total	70	72	64	-

BCS: Body Condition Score

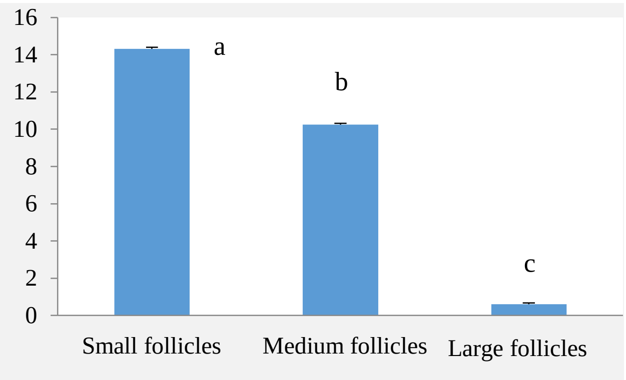

Figure 4. Determination of a follicular population of cows (Arab, Kouri, and Toupouri) in Chad as a function of size.

Table 3. Determination of oocyte class I, II, III, and IV yields of cows (Arab, Kouri, and Toupouri) in Chad

Oocyte class	Number
Class I	766
Class II	689
Class III	626
Class IV	653

Effects of breed and stage of the sexual cycle on follicle number

The breed of cattle did not significantly ($p > 0.05$) affect the average number of follicles. Furthermore, the follicle numbers recorded in metestrus and diestrus were comparable but significantly ($p < 0.05$) higher than the values recorded in proestrus and estrus independently of the breed of animals (Table 4).

Determination of oocyte yield by breed and stage of the sexual cycle

Table 5 shows the oocyte number according to breed and stage of the sexual cycle of the cows. The breed and stage of the sexual cycle did not significantly affect the oocyte average number ($p > 0.05$). Regardless of the sexual cycle stage, the comparison of the breeds indicated that Arab and Toupouri breeds recorded lower numbers of the oocyte in

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diestrus ($p < 0.05$) in reference to that recorded in Kouri. On the other hand, the number of oocytes recorded a significantly lower value in the Toupouri breed during the estrus stage when sexual cycle stages were considered regardless of breed ($p < 0.05$).

Determination of oocyte classes by breed and stage of the sexual cycle

Table 6 summarizes the effects of race and sexual cycle stage on oocyte classes. It is shown that race and stage of the sexual cycle did not significantly affect oocyte classes ($p > 0.05$). The number of oocytes generally increased with the evolution of the sexual cycle of the cows, but the difference was not significant ($p > 0.05$).

Effects of breed and body condition score on egg yield in cows

Table 7 shows the effects of breed and BCS on egg yield. The result indicated that breed and BCS did not significantly affect egg yield ($p > 0.05$). Cows with medium and fat BCS had significantly higher oocyte yields than lean cows ($p < 0.05$).

Effects of breed and body condition score on oocyte class

The effects of breed and body condition score on the oocyte class of cows are presented in Table 8. The breed and body condition scores did not significantly affect the oocyte class ($p > 0.05$). Cows with medium and high body reserves had significantly higher numbers of class I and IV oocytes, compared to lean cows ($p < 0.05$).

Effects of breed and body condition score on egg quality

The effects of breed and body condition score on egg quality is in Table 9. According to the table, the breed and body condition scores did not significantly affect oocyte quality ($p > 0.05$). Good quality oocytes (class I and II) were compared to poor ones (class III and IV, $p > 0.05$).

Effects of breed and age on egg yield

Table 10 shows the effects of breed and age on the egg yield of cows. The results revealed that breed did not significantly affect average egg yield ($p > 0.05$). However, cows aged 6-9 and 10-15 years had a significantly higher oocyte count than the 3-5 age group ($p < 0.05$).

Effects of breed and age on egg yield by class

The effects of breed and age on egg yield with regard to cow class, breed, and age are presented in Table 11. As it can be seen, breed and age did not significantly affect oocyte yield ($p > 0.05$). However, class II oocytes were significantly higher in Arab cows aged 10-15 years compared to other age groups ($p < 0.05$).

Number of oocytes collected for oocyte maturation

Table 12 shows the number of follicles and oocytes collected for oocyte maturation. Of 14, 9, and 13 ovaries selected from the Arab, Kouri, and Toupouri breeds, 210, 126, and 205 follicles were counted, respectively. From these follicles, a total of 113, 84, and 130 oocytes were recovered for oocyte maturation in the Arab, Kouri, and Toupouri breeds, respectively.

Distribution of oocytes by breed and class

The distribution of oocytes collected from a cow ovary sample by breed and class is listed in Table 13. In all breeds studied, good-quality oocytes (class I and II) were generally more than poor-quality oocytes (class III and IV). However, this rate was slightly higher with Arabian cows (56.97%), compared to Kouri and Toupouri (59.05% and 51.53%, respectively).

Oocyte maturation rate by breeds

Table 14 shows the oocyte maturation rate by breed and according to the different culture media. The culture medium of MEM + 10% follicular fluid showed a higher maturation rate for all breeds. This value was higher in the Arabian breed than in the Kouri and Toupouri breeds.

Effects of race and age on oocyte maturation rate

The effects of breed and age on oocyte maturation rate are shown in Table 15. It can be seen that the oocyte maturation rate was not significantly affected by breed ($p > 0.05$). On the other hand, cows belonging to the age group of 6-9 years showed a significantly higher oocyte maturation rate, compared to other age groups ($p < 0.05$).

Effects of breed and Body condition score on oocyte maturation rate

The effects of breed and body condition score on oocyte maturation rate are presented in Table 16. It is seen that the maturation rate was not significantly affected by breed and BCS ($p > 0.05$).

Relationships between different cow parameters

Positive and significant correlations were found between BSC and average ovarian weight ($r = 0.67$, $p < 0.01$) and between the total number of follicles and the total number of small follicles ($r = 0.93$, $p < 0.01$).

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Table 4. Effects of breed and sexual cycle phases on the number of follicles of cows (Arab, Kouri, and Toupouri) in Chad

	Races	**Stage of the sexual cycle**					**p-value**
		Prestrus (n = 35)	**Estrus (n = 50)**	**Metestrus (n = 43)**	**Diestrus (n = 38)**	**Mean ± Standard deviation**	
Total follicles	Arab (59)	23.93 ± 3.31^{aA}	25.67 ± 4.95^{abAB}	25.00 ± 17.79^{abA}	30.25 ± 4.16^{bA}	$25.98 \pm 1.89^{\alpha}$	0.02
	Kouri (57)	24.14 ± 2.39^{aA}	30.17 ± 3.50^{aB}	28.18 ± 3.45^{aAB}	30.63 ± 3.16^{aA}	$27.42 \pm 1.71^{\alpha}$	0.30
	Toupouri (50)	19.25 ± 2.64^{abA}	15.65 ± 1.62^{aA}	33.88 ± 3.02^{cB}	24.56 ± 1.90^{bA}	$24.47 \pm 13.86^{\alpha}$	0.04
	Mean ± Standard deviation	$21.81 \pm 1.68^{\alpha}$	$23.88 \pm 2.18^{\alpha}$	$29.91 \pm 1.81^{\beta}$	28.63 ± 1.54^{ß}	$25.37 \pm 11.36^{\alpha}$	-
	P-value	0.44	0.02	0.01	0.40	-	-

$^{a\ b\ c}$ Values with the same letter in a row do not differ significantly ($p > 0.05$). $^{A\ B\ C}$ Values with the same letter in a column do not differ significantly ($p > 0.05$). $^{\alpha\ \beta}$ Values with the same letter in a column or a row do not differ significantly ($p > 0.05$).

Table 5. Total number of oocytes according to breed and stage of the sexual cycle of cows (Arab, Kouri, and Toupouri) in Chad

	Races	**Stage of the sexual cycle**					**p-value**
		Prestrus (n =38 35)	**Estrus (n = 50)**	**Metestrus (n = 43)**	**Diestrus (n = 38)**	**Mean ± Standard deviation**	
Total oocyte	Arab	13.71 ± 1.77^{abA}	12.93 ± 2.45^{aAB}	12.33 ± 1.02^{aA}	14.17 ± 1.56^{bA}	$13.19 \pm 0.89^{\alpha}$	0.02
	Kouri	12.36 ± 1.00^{aA}	17.44 ± 2.24^{aB}	14.18 ± 1.58^{aAB}	16.13 ± 2.26^{aA}	$15.04 \pm 0.95^{\alpha}$	0.3
	Toupouri	11.25 ± 1.00^{aA}	9.12 ± 0.97^{aA}	17.25 ± 1.68^{cB}	13.33 ± 1.80^{bA}	$12.22 \pm 0.76^{\alpha}$	0.03
	Mean ± Standard deviation	$12.64 \pm 0.98^{\alpha}$	$13.26 \pm 1.22^{\alpha}$	$13.98 \pm 0.84^{\alpha}$	$14.63 \pm 1.67^{\alpha}$	$13.37 \pm 11.36^{\alpha}$	-
	P-value	0.44	0.01	0.20	0.4	-	-

$^{a\ b\ c}$ Values with the same letter in a row do not differ significantly ($p > 0.05$). $^{A\ B\ C}$ Values with the same letter in a column do not differ significantly ($p > 0.05$). $^{\alpha\ \beta}$ Values with the same letter in a column or a row do not differ significantly ($p > 0.05$).

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Table 6. Variation in oocyte class according to breed and stage of the sexual cycle of cows (Arab, Kouri, and Toupouri) in Chad

Classes	Race	Stage of the sexual cycle				Mean ± Standard deviation	p-value
		Proestrus (n = 35)	Eostrus (n = 50)	Metoestrus (n = 43)	Diestrus (n= 38)		
Class I	Arab	8.29 ± 1.16^{aB}	9.07 ± 1.97^{bAB}	8.28 ± 0.85^{aA}	9.43 ± 0.96^{bA}	$8.71 \pm 0.63^{\alpha}$	0.03
	Kouri	8.00 ± 0.85^{aAB}	11.22 ± 1.60^{aB}	9.29 ± 1.31^{aA}	9.80 ± 0.57^{aA}	$9.67 \pm 0.69^{\alpha}$	0.3
	Toupouri	6.88 ± 0.93^{aA}	6.00 ± 0.72^{aA}	10.88 ± 1.44^{aA}	8.89 ± 0.82^{aA}	$7.92 \pm 0.51^{\alpha}$	0.23
	Mean ± Standard deviation	$7.86 \pm 0.59^{\alpha}$	$8.80 \pm 0.86^{\alpha}$	$9.16 \pm 0.88^{\alpha}$	$9.26 \pm 0.51^{\alpha}$	$8.88 \pm 2.41^{\alpha}$	0.42
	P-value	0.03	0.01	0.23	0.3	-	-
Class II	Arab	3.36 ± 0.70^{aB}	3.27 ± 0.70^{aA}	3.33 ± 0.44^{aA}	2.53 ± 0.46^{aA}	$3.15 \pm 0.23^{\alpha}$	0.03
	Kouri	3.21 ± 0.28^{aAB}	4.28 ± 0.80^{aA}	3.35 ± 0.46^{aA}	4.63 ± 1.37^{aB}	$3.77 \pm 0.35^{\alpha}$	0.3
	Toupouri	2.88 ± 0.93^{aA}	3.18 ± 0.68^{aA}	4.38 ± 0.46^{aA}	3.23 ± 0.61^{aAB}	$3.33 \pm 0.35^{\alpha}$	0.23
	Mean ± Standard deviation	$3.19 \pm 0.34^{\alpha}$	$3.60 \pm 0.42^{\alpha}$	$3.53 \pm 0.22^{\alpha}$	$3.29 \pm 0.41^{\alpha}$	$3.88 \pm 2.41^{\alpha}$	0.42
	P-value	0.03	0.01	0.23	0.03	-	-
Class III	Arab	2.59 ± 0.58^{aA}	2.67 ± 0.53^{abAB}	2.61 ± 0.26^{aA}	3.27 ± 0.63^{bA}	$2.73 \pm 0.24^{\alpha}$	0.03
	Kouri	3.36 ± 0.55^{aA}	4.44 ± 0.66^{aB}	3.71 ± 0.57^{aAB}	2.23 ± 0.33^{aA}	$3.67 \pm 0.22^{\alpha}$	0.3
	Toupouri	3.25 ± 0.70^{bcA}	2.41 ± 0.43^{aA}	4.38 ± 0.42^{cB}	3.26 ± 0.63^{bcA}	$3.16 \pm 0.29^{\alpha}$	0.02
	Mean ± Standard deviation	$3.00 \pm 0.30^{\alpha}$	$3.22 \pm 0.32^{\alpha}$	$3.37 \pm 0.27^{\alpha}$	$3.08 \pm 0.37^{\alpha}$	$3.27 \pm 3.26^{\alpha}$	0.2
	P-value	0.40	0.02	0.03	0.69	-	-
Class IV	Arab	3.50 ± 0.65^{aB}	2.60 ± 0.57^{aAB}	3.17 ± 0.39^{aA}	3.75 ± 0.58^{aA}	$3.22 \pm 0.27^{\alpha}$	0.34
	Kouri	2.50 ± 0.56^{aAB}	4.00 ± 0.56^{bB}	3.82 ± 0.72^{baA}	5.35 ± 0.86^{cA}	$3.77 \pm 0.34^{\alpha}$	0.3
	Toupouri	2.00 ± 0.50^{aA}	1.710 ± 0.28^{aA}	4.00 ± 0.50^{bA}	3.61 ± 0.46^{bA}	$2.72 \pm 0.25^{\alpha}$	0.23
	Mean ± Standard deviation	$2.78 \pm 0.35^{\alpha}$	$2.80 \pm 0.30^{\alpha}$	$3.58 \pm 0.34^{\alpha}$	$4.02 \pm 0.34^{\alpha}$	$3.38 \pm 0.21^{\alpha}$	0.03
	P value	0.02	0.17	0.22	0.41	-	-

$^{a\ b\ c}$ Values with the same letter in a row do not differ significantly ($p > 0.05$). $^{A\ B\ C}$ Values with the same letter in a column do not differ significantly ($P > 0.05$). $^{\alpha\ \beta}$ Values with the same letter in a column or a raw do not differ significantly ($p > 0.05$).

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Table 7. Effects of breed and body condition score on egg yield of cows (Arab, Kouri, and Toupouri) in Chad

Egg yield	Race	Body condition score			Mean ± Standard deviation	p-value
		[1-2] n = 51	[3] n = 95	[4-5] n = 60		
Total egg	Arab	9.97 ± 1.23^{aA}	14.80 ± 1.29^{bA}	(14.83 ±1.78^{bA}	13.13 ± 0.89$^{\alpha}$	0.02
	Kouri	8.50 ± 1.51^{aA}	13.39 ± 1.55^{bA}	15.45 ±1.10^{bA}	12.43 ± 1.10$^{\alpha}$	0.04
	Toupouri	7.44 ± 1.13^{aA}	11.41 ± 0.91^{bA}	16.19 ± 1.16^{cA}	12.22 ± 0.78$^{\alpha}$	0.03
	Mean ± SD	10.39 ± 0.87$^{\alpha}$	13.90 ± 0.73$^{\beta}$	13.62 ± 0.89$^{\beta}$	11.71 ± 0.19$^{\alpha}$	-
	p-value	0.2	0.36	0.21	-	-

$^{a\ b\ c}$ Values with the same letter in a row do not differ significantly (P > 0.05). $^{A\ B\ C}$ Values with the same letter in a column do not differ significantly (P > 0.05). $^{\alpha\ \beta}$ Values with the same letter in a column or a raw do not differ significantly (P > 0.05), SD: Standard deviation

Table 8. Effects of breed and body condition score on oocyte class of cows (Arab, Kouri, and Toupouri) in Chad

Size of the follicles	Race	Body condition score			Mean ± Standard deviation	p-value
		[1-2] n = 51	[3] n = 95	[4-5] n = 60		
Class I	Arab	5.56 ± 0.70^{aA}	10.17 ± 0.99^{bA}	10.10 ± 1.09^{bA}	8.793 ± 0.68$^{\alpha}$	0.03
	Kouri	8.88 ± 0.70^{abB}	9.91 ± 1.1^{aAB}	10.37 ± 1.4^{aA}	9.67 ± 0.69$^{\alpha}$	0.64
	Toupouri	4.78 ± 0.66^{aA}	7.85 ± 0.64^{bB}	9.96 ± 0.69^{bA}	7.95 ± 0.52$^{\alpha}$	0.04
	Mean ± SD	6.51 ± 0.50$^{\alpha}$	9.24 ± 0.55$^{\beta}$	10.04 ± 0.64$^{\beta}$	8.91 ± 11.89$^{\alpha}$	-
	p-value	0.03	0.02	0.36	-	-
Class II	Arab	2.78 ± 0.43^{aA}	3.37 ± 0.44^{aAB}	3.39 ± 0.45^{aA}	3.12 ± 0.42$^{\alpha}$	0.78
	Kouri	3.79 ± 0.82^{aA}	3.57 ± 0.43^{aA}	4.20 ± 0.44^{aA}	3.79 ± 0.51$^{\alpha}$	0.47
	Toupouri	2.00 ± 0.54^{aA}	3.15 ± 0.58^{abB}	4.38 ± 0.54^{bA}	3.33 ± 0.31$^{\alpha}$	0.03
	Mean ± SD	2.91 ± 0.33$^{\alpha}$	3.35 ± 0.26$^{\alpha}$	± 0.33$^{\alpha}$	3.52 ± 0.21$^{\alpha}$	-
	p-value	0.98	0.02	0.31	-	-
Classe III	Arab	1.77 ± 0.23^{aA}	3.27 ± 0.49^{bA}	3.00 ± 0.48^{bA}	2.73 ± 0.24$^{\alpha}$	0.02
	Kouri	3.34 ± 0.39^{aB}	3.70 ± 0.35^{aA}	3.80 ± 0.29^{aA}	3.64 ± 0.79$^{\alpha}$	0.76
	Toupouri	2.00 ± 0.45^{aA}	2.85 ± 0.36^{aA}	4.31 ± 0.46^{bA}	3.17 ± 0.28$^{\alpha}$	0.02
	Mean ± SD	2.37 ± 0.22$^{\alpha}$	3.28 ± 0.23$^{\alpha}$	3.72 ± 0.31$^{\alpha}$	3.41 ± 0.19$^{\alpha}$	-
	p-value	0.02	0.36	0.21	-	-
Class IV	Arab	2.67 ± 0.48^{aAB}	3.29 ± 0.40^{bA}	3.78 ± 0.43^{bA}	3.28 ± 0.29$^{\alpha}$	0.02
	Kouri	3.50 ± 0.68^{abB}	4.04 ± 0.54^{bB}	3.47 ± 0.55^{aA}	3.77 ± 0.30$^{\alpha}$	0.03
	Toupouri	1.63 ± 0.35^{aA}	2.87 ± 0.35^{abA}	3.34 ± 0.51^{bA}	2.78 ± 0.28$^{\alpha}$	0.52
	Mean ± SD	2.73 ± 0.29$^{\alpha}$	3.42 ± 0.23$^{\beta}$	3.51 ± 0.22$^{\beta}$	3.37 ± 0.92$^{\alpha}$	-
	P-value	0.01	0.03	0.06	-	-

$^{a\ b\ c}$ Values with the same letter in a row do not differ significantly (P > 0.05). $^{A\ B\ C}$ Values with the same letter in a column do not differ significantly (P > 0.05). $^{\alpha\ \beta}$ Values with the same letter in a column do not differ significantly (P > 0.05), SD: Standard deviation

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Table 9. Effects of breed and body condition score on egg quality of cows (Arab, Kouri, and Toupouri) in Chad

Egg quality	Race	Body condition score			Mean ± Standard deviation	p-value
		[1-2] n = 51	[3] n = 95	[4-5] n = 60		
Class I and II	Arab	16.54 ± 2.90^{aA}	15.47 ± 1.89^{aA}	16.15 ± 1.89^{aA}	$15.93 \pm 1.38^{\alpha}$	0.46
	Kouri	17.93 ± 2.60^{bA}	16.71 ± 2.3^{abA}	13.27 ± 2.49^{aA}	$16.12 \pm 2.39^{\alpha}$	0.04
	Toupouri	8.22 ± 1.55^{aA}	10.40 ± 1.04^{bA}	13.96 ± 1.22^{cA}	$11.65 \pm 0.92^{\beta}$	0.04
	Mean ± SD	$14.60 \pm 2.18^{\alpha}$	$14.26 \pm 1.41^{\alpha}$	$14.73 \pm 1.46^{\alpha}$	$14.51 \pm 1.59^{\alpha}$	-
	P-value	0.43	0.25	0.36	-	-
Class III and IV	Arab	6.78 ± 0.83^{bA}	10.27 ± 1.94^{aA}	10.79 ± 1.75^{aA}	$9.32 \pm 0.62^{\alpha}$	0.78
	Kouri	9.79 ± 1.12^{aA}	13.00 ± 1.19^{bA}	10.87 ± 1.51^{aA}	$11.65 \pm 1.21^{\alpha}$	0.47
	Toupouri	6.69 ± 1.04^{aA}	10.69 ± 1.06^{bA}	13.00 ± 0.60^{cA}	$10.67 \pm 0.64^{\alpha}$	0.03
	Mean ± SD	$8.31 \pm 1.63^{\alpha}$	$11.46 \pm 1.56^{\alpha\beta}$	$11.57 \pm 1.53^{\beta}$	$10.32 \pm 0.21^{\alpha}$	-
	P-value	0.98	0.34	0.31	-	-

$^{a\ b\ c}$ Values with the same letter in a row do not differ significantly (P > 0.05). $^{A\ B\ C}$ Values with the same letter in a column do not differ significantly (P > 0.05). $^{\alpha\ \beta}$ Values with the same letter in a column or a row do not differ significantly (P > 0.05), SD: Standard deviation

Table 10. Effects of breed and age on egg yield of cows **(**Arab, Kouri, and Toupouri) in Chad

Oocyte yield	Race	Age (years)			Mean ± Standard deviation	p-value
		[3-5] n = 123	[6-9] n = 60	[10-15] n = 23		
Total oocyte	Arab	12.00 ± 0.99^{aA}	13.76 ± 1.24^{abA}	18.25 ± 1.69^{bB}	$13.72 \pm 0.72^{\alpha}$	0.03
	Kouri	13.21 ± 0.99^{abB}	17.45 ± 1.79^{bA}	12.00 ± 0.70^{aA}	$14.34 \pm 0.84^{\alpha}$	0.02
	Toupouri	11.33 ± 0.74^{aA}	14.44 ± 1.51^{bA}	13.00 ± 2.70^{abA}	$13.43 \pm 0.64^{\alpha}$	0.03
	Mean ± SD	$12.23 \pm 0.53^{\alpha}$	$15.20 \pm 0.78^{\beta}$	$14.34 \pm 1.00^{\beta}$	-	-
	P-value	0.98	0.34	0.02	-	-

$^{a\ b\ c}$ Values with the same letter in a row do not differ significantly (p > 0.05). $^{A\ B\ C}$ Values with the same letter in a column do not differ significantly (P > 0.05). $^{\alpha\ \beta}$ Values with the same letter in a row do not differ significantly (P > 0.05). $^{\alpha,\ \beta}$ Values with the same letter in a column do not differ significantly (p > 0.05). SD: Standard deviation

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Table 11. Effects of breed and age on egg yield by class of cows **(**Arab, Kouri, and Toupouri) in Chad

Oocyte class	Race	Age (years)			Mean ± Standard deviation	p-value
		[3-5] n = 123	[6-9] n = 60	[10-15] n = 23		
Class I	Arab	3.33 ± 0.38^{aA}	6.75 ± 1.19^{bB}	4.69 ± 0.59^{abA}	$4.27 \pm 0.48^{\alpha}$	0.03
	Kouri	3.64 ± 0.50^{abA}	4.32 ± 0.47^{bA}	3.17 ± 0.69^{aA}	$3.78 \pm 0.39^{\alpha}$	0.64
	Toupouri	3.00 ± 0.29^{aA}	3.00 ± 0.40^{aA}	3.75 ± 0.92^{aA}	$3.05 \pm 0.22^{\alpha}$	0.42
	Mean ± Standard deviation	$3.28 \pm 0.28^{\alpha}$	$4.30 \pm 0.341^{\alpha}$	$4.51 \pm 0.74^{\alpha}$	$3.94 \pm 0.29^{\alpha}$	-
	P-value	0.43	0.25	0.36	-	
Class II	Arab	3.00 ± 0.39^{abA}	4.50 ± 0.56^{bAB}	4.50 ± 0.56^{bB}	$3.10 \pm 0.62^{\alpha}$	0.02
	Kouri	3.31 ± 0.38^{aA}	4.95 ± 0.69^{bB}	$2.91 \pm .48^{aA}$	$3.75 \pm 0.21^{\alpha}$	0.47
	Toupouri	3.02 ± 0.34^{aA}	4.44 ± 0.60^{bA}	4.00 ± 0.70^{bAB}	$3.30 \pm 0.24^{\alpha}$	0.03
	Mean ± Standard deviation	$3.11 \pm 0.225^{\alpha}$	$3.87 \pm 0.37^{\alpha}$	$3.65 \pm 0.33^{\alpha}$	$3.82 \pm 0.31^{\alpha}$	-
	P-value	0.98	0.04	0.03	-	-
Class III	Arab	2.61 ± 0.23^{aA}	3.00 ± 0.39^{aA}	3.13 ± 0.62^{aB}	$2.83 \pm 0.37^{\alpha}$	0.52
	Kouri	3.14 ± 0.30^{aA}	3.77 ± 0.14^{aA}	2.82 ± 0.45^{aA}	$3.31 \pm 0.26^{\alpha}$	0.76
	Toupouri	2.75 ± 0.20^{abA}	4.44 ± 0.39^{bA}	1.79 ± 0.86^{aA}	$2.97 \pm 0.26^{\alpha}$	0.02
	Mean ± Standard deviation	2.86 ± 0.16	$3.52 \pm 0.29^{\alpha}$	$2.76 \pm 0.33^{\alpha}$	$2.871 \pm 0.23^{\alpha}$	-
	P-value	0.12	0.36	0.21	-	-
Class IV	Arab	3.06 ± 0.35^{aA}	2.97 ± 0.380^{aAB}	4.13 ± 0.38^{bA}	$3.14 \pm 0.24^{\alpha}$	0.69
	Kouri	3.07 ± 0.32^{aA}	4.47 ± 0.62^{bB}	3.09 ± 0.63^{aA}	$3.50 \pm 0.20^{\alpha}$	0.03
	Toupouri	2.82 ± 0.25^{aA}	2.54 ± 2.90^{aA}	3.53 ± 0.51^{aA}	$2.82 \pm 0.38^{\alpha}$	0.52
	Mean ± Standard deviation	2.96 ± 0.18α	3.43 ± 0.32α	3.53 ± 0.32α	3.57 ± 0.52α	-
	P-value	0.6	0.02	0.6	0.5	-

$^{a\,b\,c}$ Values with the same letter in a row do not differ significantly ($p > 0.05$). $^{A\,B\,C}$ Values with the same letter in a column do not differ significantly ($p > 0.05$). $^{\alpha\,\beta}$ Values with the same letter in a column do not differ significantly ($p > 0.05$).

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Table 12. Number of follicles and oocytes recovered per breed of cows (Arab, Kouri, and Toupouri) in Chad

Breed of cows	Number of ovaries	Number of follicles	Number of oocytes
Arab	04	59	21
	06	88	52
	04	63	40
Total	**14**	**210**	**113**
Kouri	02	36	25
	04	51	39
	03	39	20
Total	**09**	**126**	**84**
Toupouri	03	55	31
	07	102	79
	03	48	20
Total	**13**	**205**	**130**

Table 13. Distribution of collected oocytes by race and class of cows (Arab, Kouri, and Toupouri) in Chad

Race	Oocyte numbers	Oocytes class 1	Oocytes class 2	Oocytes class 3	Oocytes class 4	Oocytes For cultivation (Class 1 and 2)	Proportion (%)
Arab	21	07	06	05	03	13	61.90
	52	15	17	13	07	32	61.52
	40	08	11	09	12	19	47.5
Total	**113**	**30**	**34**	**27**	**22**	**64**	**56.97**
Kouri	25	8	6	8	3	14	56
	39	7	11	9	12	18	46.15
	20	6	9	1	4	15	75
Total	**84**	**21**	**26**	**18**	**19**	**47**	**59.05**
Toupouri	31	12	4	7	8	16	51.61
	79	19	21	22	17	40	50.63
	21	6	5	5	5	11	52.38
Total	**131**	**37**	**30**	**34**	**30**	**67**	**51.53**

Table 14. Oocyte maturation rates per breed and according to the different culture media of cows (Arab, Kouri, and Toupouri) in Chad

Race	Composition of medium	Number of oocytes in culture	Number of expanded oocytes	Number of immature oocytes	Rate of maturation (%)
	MEM only	13	4	9	30.76
Arab	MEM + 10% follicular liquid	32	13	19	40.62
	MEM + 50% follicular liquid	19	3	16	15.78
Kouri	MEM only	14	3	11	35.71
	MEM + 10% follicular liquid	18	7	11	38.88
	MEM + 50% follicular liquid	15	4	11	26.66
Toupouri	MEM only	16	4	12	25
	MEM + 10% follicular liquid	40	12	28	30
	MEM + 50% follicular liquid	11	3	8	27.27

MEM: Minimum essential medium

Table 15. Effects of breeds and age on oocyte maturation rate of cows (Arab, Kouri, and Toupouri) in Chad

Maturation	Breeds	Age (years)			Mean ± Standard deviation	p-value
		[3-5] n=12	[6-9] n=12	[10-15] n=12		
Rate of maturation (%)	Arab	12.50 ± 7.50^{aA}	43.75 ± 6.20^{bB}	31.25 ± 11.66^{abA}	$28.17 \pm 6.02^{\alpha}$	0.04
	Kouri	25.00 ± 10.43^{aAB}	37.50 ± 7.20^{aAB}	25.00 ± 9.35^{aA}	$29.37 \pm 5.19^{\alpha}$	0.64
	Toupouri	31.25 ± 11.25^{aB}	25.00 ± 7.21^{aA}	31.25 ± 6.95^{aA}	$29.17 \pm 5.78^{\alpha}$	0.3
	Mean ± SD	$22.92 \pm 7.78^{\alpha}$	$35.47 \pm 6.541^{\beta}$	$29.17 \pm 7.44^{\alpha\beta}$	$29.27 \pm 5.79^{\alpha}$	-
	P-value	0.04	0.02	0.36	-	-

$^{a, b\, c}$ Values with the same letter in a row do not differ significantly (P > 0.05). $^{A, B\, C}$ Values with the same letter in a column do not differ significantly (P > 0.05). $^{\alpha\,\beta}$ Values with the same letter in a column or row do not differ significantly (p > 0.05).

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Table 16. Effects of race and body condition score on oocyte maturation rate of cows (Arab, Kouri, and Toupouri) in Chad

Maturation	Breeds	Body condition score			Mean ± Standard deviation	p-value
		[1-2] n=12	[3] n=12	[4-5] n=12		
Rate of maturation (%)	Arab	25.00 ± 11.80^{aA}	25.08 ± 6.20^{aA}	31.25 ± 16.66^{aA}	$27.07 \pm 5.02^{\alpha}$	0.46
	Kouri	32.25 ± 6.50^{aA}	37.50 ± 6.20^{aA}	31.25 ± 7.35^{aA}	$33.33 \pm 3.19^{\alpha}$	0.06
	Toupouri	33.00 ± 9.25^{bB}	30.00 ± 9.21^{abA}	18.75 ± 11.95^{aA}	$27.08 \pm 7.78^{\alpha}$	0.02
	Mean ± SD	$29.17 \pm 6.78^{\alpha}$	$29.17 \pm 9.541^{\alpha}$	$29.17 \pm 22.44^{\alpha}$	$29.17 \pm 5.79^{\alpha}$	-
	P-value	0.03	0.25	0.36	-	-

$^{a\,b\,c}$ Values with the same letter in a row do not differ significantly (p > 0.05). $^{A\,B\,C}$ Values with the same letter in a column do not differ significantly (P>0.05). $^{\alpha\,\beta}$ Values with the same letter in a column do not differ significantly (p > 0.05).

DISCUSSION

The present study on the effects of ovarian and non-ovarian factors on the follicle population and oocyte maturation of three cattle breeds in Chad (Arab, Kouri, and Toupouri) revealed that the cows in the current study had a BCS of 3.3 ± 0.34. This value is higher than those reported by Azafack et al. (2019), which was 2.93 ± 0.64 in Cameroon. The reason can be the long duration of the dry season and the scarcity of pasture in Chad. Indeed, BCS offers a good estimate of the quantity of lipids stored, and its variations are a good indicator of the energy balance (Lefebvre et al., 2022). It also allows an indirect assessment of the animal's nutritional status (Leperre et al., 1992). In Chad, the animals do not receive food supplements. The livestock system in this area has retained a traditional character characterized by extensive herd management on natural pasture (Zampaligré et al., 2019), which could negatively affect animal performance. The animal is finally sold in poor condition to butchers or slaughterers who take it to the slaughterhouse. The present study showed that most of the cows slaughtered were young (3 to 5 years old). The decision to send young cows to the slaughterhouse could be explained by the fact that older cows (10-15 years old) were lighter due to their poor nutritional status. The proportion of pregnant cows slaughtered at the slaughterhouse during our study was 13%, lower than the 21.34% reported by Alaku and Orjiude (1991). The slaughter of pregnant cows is against state veterinary legislation and reflects the negligence of the antemortem examination, which must be applied to animals before their referrals to the slaughterhouse.

The average number of follicles per cow in this study was 24.71 ± 0.88. This value is higher than the 5.20 reported by Kumar et al. (1997) in India and 23 examined by Taneja et al. (2000) in the same country but lower than the 37.5 ± 25.2 follicles estimated by Kouamo et al. (2014) in Cameroun and 32 follicles measured by Takaji et al. (1992) for the ovaries of a cow reared in Japan. This variation in the number of follicles would be linked to parity (number of births) and the breed of the cow (Rhodes et al., 1995). The average oocyte yield per cow was 13.27 ± 0.14, lower than the results reported by Humblot et al. (2005). This difference can be related to the collection technique. Indeed, Cognié and Baril (2002) noted that 4 to 5 additional oocytes could be obtained after cutting the ovary with a razor blade (slicing technique), compared to other techniques, such as aspiration and the ovum pick-up techniques. In the present study, the overall maturation rate (32.25%) was lower than that of Margaux (2022, 67%) using the same technique. This low maturation rate could be explained by the temperature at which the ovaries were stored during transport. Indeed, keeping the ovaries at a low temperature increases the maturation rate. Bohlooli et al. (2015) reported a higher maturation rate when transporting ovaries stored at 4°C, compared to 25°C and 38°C. According to Wang et al. (2007), low temperature would reduce cellular metabolism, but at 15°C, it would reduce the apoptosis index. The preservation of the ovaries during transport is a key element in maintaining the maturation competence of oocytes (Margaux, 2022). The low rate of maturation obtained in the current work could be due to the enrichment of the culture medium by the follicular fluid of the large follicles (mature follicles). According to Choi et al. (1998), follicular fluid from mature follicles has a reduced inhibitory effect on oocyte maturation, compared to follicular fluid collected from small and medium follicles. For Takahashi (1982), certain substances, such as hormones in the follicular fluid, prevent meiosis's resumption during maturation. Choi et al. (1998) showed that bovine follicular fluid would inhibit nuclear maturation and instead increase cytoplasmic maturation as indicated by pronuclear formation. According to Sadeesh et al. (2014), slowing nuclear maturation would give the oocyte more time to synthesize, modify and store new proteins and ribonucleoproteins and thus improve its competence. A recent study found that follicular fluid could promote cytoplasmic maturation of oocytes during in vitro maturation (Armstrong, 2001). Cows between 6 and 9 years old had a higher oocyte maturation rate than cows over 10 years old. This result agrees with that of Natumanya et al. (2008) and Kouamo et al. (2014), indicating a significantly higher oocyte maturation rate in cows belonging to the 6-9-year age group.

The current study revealed that cows with average and high body condition scores had significantly higher oocyte yields than lean cows (p < 0.05). For example, an oocyte yield of 13.90 ± 0.73 was observed in cows with an average BCS (BSC = 3) and an oocyte yield of 13.62 ± 0.89 in fat cows (BSC = 4-5), compared to an oocyte yield of 10.39 ± 0.87 observed in thin cows (BSC = 1-2). The positive correlation observed between BCS and oocyte yield in this study could be explained by the fact that BSC significantly affects the number of follicles, yield, and oocyte quality. Rhind et

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al. (1989), Dominguez (1995), and Kumar et al. (1997) support the effect of nutrition on reproductive processes at the ovarian level. It should be noted that diet influences all reproductive parameters, including cyclicity, fecundity, fertility, prolificacy, and embryonic development (Celine, 2022).

CONCLUSION

Regarding the effects of ovarian and non-ovarian factors on the follicular population and oocyte maturation of three bovine breeds (Arab, Kouri, and Toupouri) in Chad, the ovaries collected from slaughtered cows are an important source of oocytes for *in vitro* production. Age, body condition score, ovarian weight, and stage of the sexual cycle of cows influence the follicular population and oocyte maturation. The culture medium with reduced follicular fluid (10%) increases the oocyte maturation rate.

DECLARATIONS

Authors' contributions

Souleyman Hachim and Ferdinand Ngoula conceived, designed the research, and reviewed the manuscript. Hervé Tchoffo and Dorice Kana Azafack collected the data, carried out data analysis, and wrote the manuscript. All authors read and approved the final manuscript.

Ethical consideration

The work was conducted based on the ethical rules of the National Institute of Science and Technology of Abeche, Chad. The authors thoroughly examined all ethical concerns surrounding plagiarism, consent to publish, misconduct, data fabrication, falsification, duplicate publishing or submission, and manuscript redundancy.

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Availability of data and materials

The datasets generated for this study are available on request to the corresponding author.

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