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Full Length Research Paper

Genetic and nongenetic effects on the number of ovarian follicles and oocyte yield and quality in the bovine local (Oulmes Zaer), exotic breeds and their crosses in Morocco

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The effects of genetic and non genetic factors on the number of ovarian follicles and oocyte yield and quality in the bovine local breed (Oulmes Zaer), exotic breeds and their crosses in Morocco was investigated. In this study, females in very bad body conditions (BCS < 2) were not slaughtered and the average. The body condition scores (BCS) was 2.94 ± 0.89. Although some individual values were out of the normal ranges, mean values of total proteins, albumin, urea, β-OH and GOT remain normal and were 77.83 ± 8.74 g/l, 32.4 ± 4.41g/l, 4.43 ± 2.13 mmol/l , 0.83± 0.48 mmol/l et 45.55 ± 11.95 UI/l, respectively. The mean number of ovarian follicles per cow (2-8 mm) was high (22.98 ± 8.41) whereas the oocyte yield (and 2.60 ± 1.53) was very low. The effects of genetic group, age and BCS on the number of follicles, oocyte yield and the quality were significant.

Key words: Cows, follicular population, oocyte yield and quality.

INTRODUCTION

In vitro production technique of embryos has increased rapidly during the last decade (Nibart and Marquant-LeGuienne, 1995). In fact, oocytes collected from slaughtered females do mature successfully and are fertilized and transferred to recipient cows (Brachett et al., 1982; Coscioni et al., 2001). The number of embryos resulting from these manipulations varies according to countries, regions and laboratories. Chian (2002) and Vassena (2003) reported a blastocyst recovery average of 27 and 23%, respectively.

Previous investigations concerned mainly the biochemical and hormonal profiles of the female's reproductive biology (Kendrick et al., 1999). Subsequently, the level of nutrition has been shown to influence, in a significant way, reproductive efficiency in cattle. The nutritional influence include proteo-energetic rate, vitamins (Butler et al.,1989 ; Ferguson and Chalupa, 1989; Hurley and Doane,1989), calcium, phosphorus, magnesium, potassium, manganese, selenium and iodine necessary to maintain different reproductive processes such as follicular growth and oocyte yield and quality (Atherton, 1994) as well as age (Dielman et al., 1983).

Furthermore, it has been shown that insufficient and/or unbalanced nutrition may cause several reproductive disorders (Enjalbert, 1998) and delay onset of ovulation (Fergusson, 1996). In addition, energetic deficiency may reduce follicular growth and development and hormone

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Table 1. Distribution of cows according to breed, age and BCS.

Breed		Local (Oulmès - Zaer)			Crosses (Local x Exotic)			Exotic (Frisian, Holstein)			
Age (years)		< 3	3-5	> 5	< 3	3-5	> 5	< 3	3-5	> 5	Total
B	2	-	-	19	-	1	4	-	1	3	28
C	3	-	2	3	-	8	7	6	5	4	35
S	4	-	2	-	1	3	-	1	2	2	11
	5	-	-	-	2	2	-	1	1	-	6
Subtotal		-	4	22	3	14	11	8	9	9	80
Total		26			28			26			80

secretion: IGFI, estrogens and progesterone (Drion et al., 1996; Burns et al*.,* 1997; Kendrick et al., 1999; Reksen and Ropstad, 2002).

To monitor the nutritional status of farm animals, the body condition score (BCS) has been used as a simple and reliable tool by several investigators (Heinrichs and Ishler, 1989; Edmonson et al*.,* 1989; James et al., 1994). The objective of this study is to evaluate the effects of genetic (breed group) and non genetic (nutritional status (BCS) and age) factors on the number of follicles and oocyte yield and quality in the local and exotic breeds of cows and their crosses in Morocco.

MATERIALS AND METHODS

Animals and data collection

A total number of 80 cows of different breed and age groups and body conditions were randomly identified on the day before slaughter at the slaughterhouses in Rabat, Salé and Casablanca regions of Morocco during spring (April through June).

Cows were identified and examined to determine the breed: Local (Oulmès-Zaer), exotic (Frisian, Holstein) and their crosses (L x E) and age: below 3 years, 3 to 5 years and above 5 years. The Body Condition Score (BCS) was determined as described by Edmonson et al. (1989) (Table 1).

Metabolic profile

Blood samples were obtained from the jugular vein on the day before slaughter in 10 ml dry vacutainers for determination of total proteins, albumin, urea, β-hydroxybutyrate (β-OH) and glutamic-oxaloacetic transaminase (GOT) concentrations. Blood samples were transferred to the laboratory at 5°C and centrifuged at 2100 x g for 30 min and sera stored at –20°C in aliquots until assayed using an enzymatic colorimetric method of commercially available kits.

Ovaries

Both ovaries were collected immediately after slaughter, washed with sterile saline and then transported to the laboratory within 3 h in separate vials containing saline in isotherm at 25-30°C. The ovaries were cleaned from connective tissue and washed with a 70% alcohol solution. Apparent surface follicles: 2-8 mm (Dewit et al., 2001) were counted and aspirated into PBS using a 20-G needle (Kumar et al., 1997). Follicular fluid (with oocytes) was transferred into Petri dishes and examined at magnitude of 14. The oocytes were classified into four categories according to their morphological characteristics (De-Loos et al., 1989).

Quality 1 (Q_1): compact multilayered cumulus, homogenous cytoplasm.
Quality 2 (Q_2): compact multilayered cumulus, homogenous cytoplasm, but with a darker zone at the periphery of the oocyte.
Quality 3 (Q_3): less compact cumulus, irregular cytoplasm, with darker clusters.
Quality 4 (Q_4): expended cumulus, cumulus cells scattered in dark clamps, irregular cytoplasm with dark clusters.

Statistical analysis

The data were analyzed using the ANOVA 1 with a model containing genotype, age and BCS as main effects (SPSS 10.0 for Windows). Preliminary analyses of data were carried out to decide which effects were significant. When a factor had a significant effect, the means were then compared using Student-t test. The level of significance is considered to be 5%.

RESULTS

Genotype, age and BCS effects on the ovarian function expressed in terms of number of follicles and oocyte yield, as well as quality will be presented.

Body condition score

The overall mean BCS before slaughter was 2.94 ± 0.89 for cows of local and exotic breeds and their crosses in this experiment. Animals in bad body conditions (BCS<2) were not slaughtered (Figure 1).

Metabolic profile

The overall average mean values of total proteins, albumin urea, β-hydroxybutyrate (β-OH) and glutamic-oxaloacetic transaminase (GOT) were within the normal ranges (Table 2) and did not differ significantly among breeds.

Table 2. Average metabolic parameters for cows of local and exotic breeds and their crosses before slaughter.

Parameter	m ± ES (Min-Max)	Normal range (nr)	Individuals below nr (%)	Individuals over nr (%)
Total proteins (g/l)	77.83 ± 8.74 (54 to 100)	60-85	1 (1.25)	15 (18.75)
Albumin (g/l)	32.40 ± 4.41(23.53 to 45.91)	30.30-35.50	23 (28.75)	18 (22.50)
Urea (mmol/l)	4.43 ± 2.13 (0.60 to 8.25)	2.20-7.40	12 (15)	8 (10)
β-OH (mmol/l)	0.83 ± 0.48 (0.05 to 2.08)	0.20-1.00	4 (5)	26 (32.50)
GOT (UI/l)	45.55 ± 11.95 (26.00 to 87.20)	8-93	0	0

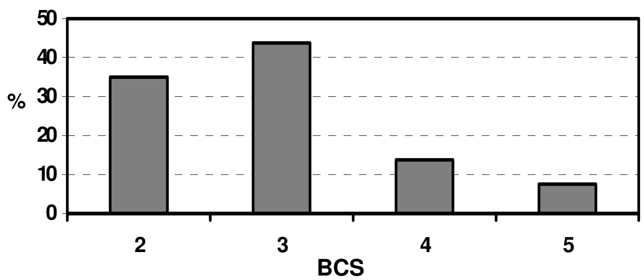

Figure 1. Distribution of the BCS for cows of local and exotic breeds and their crosses before slaughter.

Follicular and oocyte yields

All the examined ovaries showed evidence of follicular growth. A total of 1839 follicles of 2 to 8 mm in diameter were counted and 208 oocytes aspirated on the 160 ovaries collected from 80 cows. The overall mean follicular and oocyte yields were 22.98 ± 8.41 and 2.59 ± 1.53, respectively (Table 3). The analysis of variance showed that the effects of genetic group and age were significant ($P<0.05$) while the effect of BCS was very highly significant ($P<0.001$). In fact, the local breed has the lowest performance (18.96 ± 6.52 and 1.93 ± 1.57 for the number of follicles and oocyte yield, respectively). In addition, cows below three years of age grow more follicles (27.25 ± 6.91) and yield more oocytes (3.17 ± 0.83). The performances of the local x exotic crosses are not significantly different from those of the exotic. On the other hand, both the number of follicles and the oocyte yield increase significantly as the BCS increases. In fact, the lowest performances were observed when cows were in relatively bad body conditions (BCS=2), i.e. 17.30 ± 5.08 and 1.54 ± 1.10 and the highest, i.e. 37.83 ± 5.70 and 4.50 ± 0.84 when animals were in good condition (BCS=5) for follicle number and oocyte yield, respectively (Table 3).

Quality of the oocytes

Among the 208 oocytes collected from 1839 follicles, 164 (79%) were classified as good quality (Q_{1-3}) and may be selected for *in vitro* maturation. Whereas 44 oocytes (21%) were of poor quality (Q_4) and therefore can not be selected for *in vitro* maturation process. The overall mean oocyte yields were 2.03 ± 1.36 and 0.54 ± 0.79 for Q_{1-3} and Q_4, respectively (Table 3). The analysis of variance showed that the effects of genotype, age and BCS were significant ($P<0.05$) on the Q_{1-3}, but not on Q_4. Thus, exotic animals, young cows and individuals in good body condition (BCS=5) performed better. The effects of different interactions (genotype x age, genotype x BCS, age x BCS) have not been tested given the number of individuals per group (Table 1), whereas the oocyte yield seems to be correlated to the measured metabolic parameters.

DISCUSSION

The average number of follicles in this study (22.98 ± 8.41) is higher than reported in Buffalo (5.20; Kumar et al., 1997) suggesting breed and/or management differences. The current overall oocyte yield for local, local x exotic and pure exotic cows, 2.59 ± 1.53, remains below that reported earlier, 5 to 6 oocytes (Nibart and Marquant-Leguienne, 1995). Several factors, particularly, nutrition (Kendrick et al., 1999; Kruip et al., 1996), and/or method (Kumar et al., 1997) may have a significant effect on this trait. Using the direct aspiration technique of surface follicles, the average yield was 2.59 oocytes par female, which is similar to that observed in Buffalo (2.35; Kumar et al., 1997). In contrast, the punction and slicing of the ovaries yielded 3.10 and 6.25 oocytes, respectively due to the fact that superficial and deep follicles might be reached. A histological investigation is ongoing to estimate the distribution of the ovarian follicles in the Oulmès Zaer breed. The difference in oocyte yield using aspiration or punction may partially result from the negative pressure applied to the follicle at the time of collection. Infact, a negative pressure of 50 mmHg does affect the number and quality of the oocytes (Ward et al., 2000)

The fact that BCS had a significant effect on the number of follicles, oocyte yield and quality (Rhind et al., 1989; Dominguez, 1995; Kumar et al., 1997) supports the effect on nutrition on reproduction process, particularly at the ovarian level. Infact, cows in poor body conditions (BCS = 1 - 2) had less ovarian follicles during the luteal phase and tend to produce less follicles (Rhind et al., 1989) because the number of follicles that leave the ovarian reserve depends

Table 3. Effects of breed and age groups and BCS on the average number (m ± ES) of follicles and oocyte yield and quality.

Effects	n	Number of follicles	Oocyte yield	Oocyte quality	
				Q_{1-3}	Q_4
Overall mean	80	22.98 ± 8.41	2.59 ± 1.53	2.03 ± 1.36	0.54 ± 0.79
Genetic group		*	*	*	
L	26	18.96 ± 6.52^a	1.93 ± 1.57^a	1.44 ± 1.39^a	0.48 ± 0.75
L x E	28	24.71 ± 8.94^b	2.86 ± 1.38^b	2.39 ± 1.26^b	0.46 ± 0.64
E	26	25.19 ± 8.33^b	3.23 ± 1.61^b	3.54 ± 1.43^b	0.69 ± 0.79
Age (years)		*	*	***	
< 3	11	27.25 ± 6.91^a	3.17 ± 0.83^a	2.67 ± 0.89^a	0.50 ± 0.79
3 –5	27	25.93 ± 9.38^b	3.04 ± 1.53^b	2.74 ± 1.46^b	2.29 ± 0.54
> 5	42	19.74 ± 6.85^c	2.29 ± 1.74^c	1.57 ± 1.33^c	0.71 ± 0.89
BCS		***	***	***	
2	28	17.30 ± 5.08^a	1.54 ± 1.10^a	1.07 ± 0.94^a	0.47 ± 0.79
3	35	23.80 ± 7.57^b	2.91 ± 1.60^{bc}	2.39 ± 1.25^{bc}	0.67 ± 0.83
4	11	26.73 ± 5.26^c	3.27 ± 1.10^c	2.73 ± 1.10^c	0.55 ± 0.82
5	6	37.83 ± 5.70^d	4.50 ± 0.84^d	4.30 ± 1.03^d	0.17 ± 0.43

Means within a column that do not have a common superscript differ significantly
Level of significance :
L : local (Oulmès-Zaer)
L x E : local x exotic
E : exotic (Frisian, Holstein)
BCS : Body Condition Score
* : significant (P< 0.05)
** : highly significant (P< 0.01)
*** : very highly significant (P< 0.001)

upon the individuals BCS (Drion et al., 1996). On the other hand, differences may result from the body condition scoring method which might not be adapted to the local breed. Nevertheless, It has been reported that the BCS is not highly correlated to the metabolic activity (Zarco, 1993), whereas energy balance, specific hormones and metabolites remain precise indicators.

In the present study, the low oocyte yield of cows with BCS = 2 may be related to the level of energy in the diet. In addition, nutrition related metabolic and hormonal changes may affect not only follicular development and growth but also the oocyte quality (Dominguez, 1995). Thus, aspiration will yield significantly more (1.53) oocytes in cows fed a high energy diet than in those fed a lower energy diet (1.37) (Kendrick et al., 1999). Oocytes with expended cumulus and scattered cells are considered to be abnormal quality in this study. Similar oocytes have been matured, fertilized *in vitro* and developed into viable embryos (Moreno et al., 1992). This suggests that some oocytes partially loose the cumulus during the aspiration process while remaining viable.

Nutritional status of the animals as monitored by total proteins, albumin, urea, β-hydroxybutyrate and the glutamic-Oxaloacetic transaminase (GOT), showed that 78% of the cows have a normal total protein level (60 to 85 g/l) (Fontaine and Cadore, 1995), 94% with normal albumin level (30.3 to 35.5 g/l) (Kaneko, 1989). Urea and β– OH values are the normal range for the majority of cows (Table 2) (Fontaine and Cadore, 1995; Dargel, 1987). Surprisingly, all the cows (100 %) GOT values are normal suggesting hepatic integrity on these animals (Fontaine and Cadore, 1995).

The individual variations of ovarian follicles and the low oocytes yield require histological investigation of the ovaries of the local breed (Oulmès-Zaer) to evaluate the distribution of the oocytes in the ovarian cortex. In this study, aspiration technique of the oocytes using a 20 G needle used seems to be less efficient than oocyte punctioning or ovarian slicing.

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