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Comparative Study of the Biochemical Profile of Follicular Fluid of Ewes During Estrus, Anestrous and Cystic Ovarian Diseases

[1]*Justin Kouamo,* [2]*Nadine Daliwa Almigue and* [2]*Nicolas Yanou Njintang*

[1]School of Veterinary Medicine and Sciences,
The University of Ngaoundere, P.O. Box: 454, Ngaoundere, Cameroon
[2]Faculty of Sciences, The University of Ngaoundere, P.O. Box: 454, Ngaoundere, Cameroon

Abstract: The objective of this study was to evaluate the biochemical profile of the follicular fluid of local cyclic, acyclic and cystic ewes in the Adamawa region (Cameroon) and then propose a diagnostic tool for these different groups of animals. Follicular fluid was taken from the ovaries of ewes (33 cyclic, 30 acyclic and 11 cystic, respectively) and subjected to biochemical analysis of glucose, total cholesterol, total proteins, albumin, globulins and calcium. The result revealed that the biochemical profile of cyclic ewes was characterized by normal concentrations of glucose (2.6 mmol/ l) and total cholesterol (60.7 to 66.3 mg / dl); low in total proteins (< 5.97 g/dl) and albumin (< 2.17 g/dl); normal globulins (2 to 4 g/dl) and high calcium (> 2.79 mg/ dl), with an average body condition score (BCS=3). In addition, the biochemical profile of acyclic ewes was identical to that of cyclic ewes. The biochemical profile of cystic ewes varied from that of the others by low concentrations of glucose (< 2.6 mmol/ l), total cholesterol (< 60.4 mg/ dl), globulins (< 2 g/dl) and calcium (< 2.65 mg/ dl). Thus, the evaluation of the biochemical profile of follicular fluid may be a tool for the diagnosis and treatment of acyclic and cystic ewes.

Key words: Ewes · Biochemical Profile · Follicular Fluid · Cyclicity · Ovarian Cysts · Adamawa · Cameroon

INTRODUCTION

Livestock is an essential means of increasing food resources of animal origin and the economy of a state. In Cameroon, small ruminants occupy an important place in the animal production sector. They are one of the main activities of rural households [1]. With an estimated 3 million sheep in Cameroon, the Adamawa region contributes 5.94% to the production of meat in Cameroon [2], where the main local sheep breeds are Peul, Woyla, Kirdi, Oudah and Djallonke.

Despite this diversity of breeds, the production of foodstuffs of animal origin still remains low and cannot meet the needs of the population. In terms of animal production, the sheep farming systems currently practiced in sub-Saharan Africa are characterized by a low level of productivity which can be explained mainly by genetic, food, health, climatic constraints [3] and in particular the reproductive pathologies [4]. In small ruminants, congenital and acquired pathologies of the genital tract, especially ovarian cysts in females, are the common sources of infertility in the flock and a hindrance to animal production. The slaughterhouse seems to be the best source of detection of pathologies of the female genital tract [1]. Follicular fluid is a complex, semi-viscous, yellowish extra-cellular fluid that accumulates in the antrum of the ovarian follicles as they grow.

The constituents of follicular fluid are considered to be a factor in regulating follicular development and steroidogenesis. The composition of this liquid varies considerably depending on the stage of follicular development and has variable effects on the development of oocytes. Likewise, the composition of follicular fluid in metabolites could modify quantitatively by certain ovarian pathologies such as cysts. This therefore requires the characterization of this liquid at the various stages of follicular development so that, at the appropriate stage of development, it can be used with the aim of obtaining an optimal culture medium having characteristics necessary for the development and maturation of viable oocytes [5].

Corresponding Author: Prof. Justin Kouamo, School of Veterinary Medicine and Sciences, The University of Ngaoundere, P.O. Box: 454, Ngaoundere, Cameroon. Tel: +237 675376954.

To our knowledge, several studies were carried out on the pathologies of the reproductive organs of sheep in Africa, but similar studies have not been carried out in Cameroon. So, this study aimed to find out the variation in the values of the biochemical parameters of the follicular fluid in cyclic and acyclic females and the appearance of ovarian cysts in local ewes in the Adamawa plateau, determine the prevalence of cysts, characterize the slaughtered ewes body condition; and to propose a diagnostic tool for cyclic and acyclic females and the risks of ovarian cysts from the biochemical profile of follicular fluid in slaughtered ewes.

MATERIALS AND METHODS

Study Area: The study was carried out from June to November 2019 in the Adamawa Region (Cameroon). Samples were taken at the small ruminant slaughterhouse located at the Bantai market in the city of Ngaoundere and analyzed at the private medical laboratory ADAM'S LABO Plus located in downtown Ngaoundere. Adamawa is a region of transition between North and South Cameroon. This geographical position therefore gives it a Sudano-Guinean type climate characterized by a dry season (from November to March) and a rainy season (from April to October). The average annual temperature is 23°C with a minimum of 9°C often reached in January and a maximum (31°C) reached between the months of March and April. The average annual precipitation ranges between 900 and 1500 mm.

Characterization of Slaughtered Sheep: Information was gathered from the butchers on the provenance of the animals at the slaughterhouse. Then each ewe was the subject of a general examination before slaughter to determine the main characteristics such as the origin of the animals, breed [6], body condition score (BCS) [7], weight using a Mini Mechanical Pocket Balance Scale 50kg type, precision 5g, their age as described by Salami [8] and finally their physiological status. After slaughtering and eviscerating the ewe, their uteri were examined to detect the presence or absence of the fetus. Pregnant ewes were excluded from the study.

The female reproductive organs once harvested were classified into three groups: group 1 consisting of cyclic females (N=33) having ovaries (N=66) with a corpus hemorrhagicum (CH), a large corpus luteum (CL) and >5mm follicle(s) in diameter or a regressing CL with follicle(s) >6mm in diameter; group 2 formed of acyclic females (N= 30) with ovaries (N= 60) without a CL or CH or the pressure of a regressed CL without <5mm in diameter follicle(s) and the group of cystic females (N= 11) characterized by ovaries (N= 22) having follicles of size > 10mm with the absence of a corpus luteum as demonstrated by the post-mortem examination [9, 10].

Study of Biochemical Variations in Follicular Fluid from the Ovaries: After cleaning and washing the ovaries with saline (0.9% NaCl), the follicular contents were aspirated using a sterile syringe fitted with an 18G needle. Then the follicular fluid was placed in dry 5ml tubes. In laboratory, the follicular fluid was centrifuged at a speed of 3, 000 rpm for 10 minutes then the supernatant was collected and stored at -20°C in Eppendorf tubes, until the time of analyzes.

The concentrations of glucose, total cholesterol, total proteins, albumin and calcium of follicular fluid were measured by colorimetric assay using a semi-automatic spectrophotometer brand EMP-168 Biochemical analyzer. The glucose (GOD-PAP, LS kits), total cholesterol (SIGMA Diagnostics, LS kits), total proteins (SIGMA Diagnostics, Hospitex Diagnostics), albumin (Hospitex Diagnostics) and calcium (CALCIUM AS Inmesco, Germany) were carried out. The serum globulins concentrations were deduced from those of total proteins and albumin according to the formula: Globulins = total proteins – albumin [11].

Statistical Analysis: The data collected were saved in Microsoft Excel 2010 and the statistical analysis was performed by Statgraphic plus software version 18. The Shapiro-Wilk test was used for the test of normality of the variables. One-Way analysis of variance (ANOVA) was performed on normal quantitative variables. For non-normal quantitative variables, the Fisher, Duncan and Kruskal-Wallis LSD tests were used to compare the means; as well as the chi-square for the comparison of the qualitative variables. All data were represented as a mean ±standard error of mean with a confidence limit of 95%.

RESULTS

Characteristics of Slaughtered Ewes: A total of 74 ewes were examined and classified into cyclic (33, 44.59%), acyclic (30, 40.54%) and cystic (11, 14.87%). The percentages of the luteinized follicular cyst and the follicular cysts were 9.45% and 5.42%. The sheep examined came mainly from the department of Vina. The Djallonke breed represented mainly (66.2%; N= 49).

Table 1: Proportion of cyclic, acyclic and cystic ewes according to origin, breed, age and BCS

				Health statuses						
				Cyclic		Acyclic		Cystic		
Parameters		N	F	N	%	N	%	N	%	P-value (X^2)
Origin	Mayo-Rey	23	31	24.24	8	33.33	10	45.45	5	0.045
	Mbé	6	8	12.12	4	6.67	2	0	0	
	Vina	31	42	36.36	12	46.67	14	45.45	5	
	Yoko	14	19	27.27	9	13.33	4	9.1	1	
Breed	Djallonke	49	66.2	62.5	20	64.5	20	81.81	9	0.735
	Kirdi	6	8.1	6.3	2	9.7	3	9.09	1	
	Oudah	17	23.0	28.1	9	22.6	7	9.09	1	
	Woyla	2	2.7	3.1	1	3.2	1	0	0	
Age /year	[1-2] and	25	34	30.3	10	40	12	27.3	3	0.189
	[2-4]	49	66	69.7	23	60	18	72.7	8	
BCS	Thin (1-2)	25	33.78	38.7	12	15.6	5	72.7	8	0.040
	Average (3)	45	60.81	54.8	17	78.1	25	27.3	3	
	Fat (4-5)	4	5.41	6.5	2	6.3	2	0	0	

Number (N), Frequency (F), P-value < than 0.05 are synonymous with a dependency relationship between the parameter measured and the state of health. X^2 = Chi-square

Table 2: Mean ±SEM of the biochemical parameters overall status

			Concentrations of the follicular liquid (%)			
Biochemical parameters	M±SEM	(Min-Max)	Low	Normal	High	References values*
Glucose (mmol/l)	2.43±0.8	2.39-7.6	40 (55.1)	28 (37.8)	6 (8.1)	2.6-4.54 [12]
T. Cholesterol (mg/dl)	53.32±1.8	15-72	39 (52.7)	30 (40.5)	5 (6.8)	60.4-66.93 [13]
T. Proteins (g/dl)	5.52±0.93	0.18-6.87	45 (60.8)	26 (35.1)	3 (4.1)	5.97-6.17 [14]
Albumin (g/dl)	1.8±0.65	0.08-2.43	13 (17.6)	55 (74.3)	6 (8.1)	2.17-2.65 [11]
Globulins (g/dl)	3.67±1.08	0.05-5.99	31 (41.9)	38 (51.4)	5 (6.7)	2-4 [11]
Calcium (mg/dl)	6.06±0.67	1.45-11.8	15 (20.3)	50 (67.5)	9 (12.2)	2.65-2.79 [15]

T. (Total), M (Mean), SEM (Standard error of mean), Min (Minimum), Max (Maximum). n=74

Most of the slaughtered ewe (60.85%) had an average BCS of 3 (*N*= 45). Most of the ewes examined were between 3 and 4 years of age (66%; *N*= 49). The origin and BCS have a significant association (P < 0.05) with the state of health of the different groups of animals selected for the study; on the other hand, the breed and age of the ewes examined are not significantly (P > 0.05) associated with the state of health of the animals examined (Table 1).

Overall Status of Biochemical Parameters of Follicular Fluid: Average values of glucose, cholesterol, total proteins were lower than physiological values; that of albumin were within the reference range. Only the globulins and calcium values were higher than the physiological values (Table 2). Most ewes have a low concentration of glucose (n=40; 55.1%), cholesterol (n=39; 52.7%), total proteins (n=45; 60.8%); normal albumin (n=55; 74.3%), globulins (n=38; 51.4%) and calcium (n=50; 67.5%) concentrations (Table 2).

The mean BCS does not vary significantly between cyclic, acyclic and cystic sheep. The majority of ewes examined have an average BCS of 3from 5 point scale. The total females in the study, the concentrations of biochemical parameters varied between different groups of animals. The concentration of glucose, cholesterol, and calcium decreased (P<0.01) in ewes with cystic ovaries (Table 3). Moreover, acyclic ewes had lower biochemical parameters than the cyclic ones. Similarly, the concentrations of globulins and total proteins did not vary significantly between acyclic and cystic animals. In contrast, albumin showed no significant difference between cyclic and acyclic ewes (P<0.05).

Diagnostic Tool for Cyclic, Acyclic and Cystic Ewes: Cyclic and acyclic females have the same biochemical profile and differ from that of cystic females by concentrations of glucose, total cholesterol, globulins and calcium in follicular fluid (Table 4).

Table 3: Mean ±SEM of the biochemical parameters in cyclic, acyclic or cystic ovaries in ewes

Variable	Cyclic	Acyclic	Cystic	P-value
BCS	2.93±0.6[a]	2.56±0.5[a]	2.66±0.48[a]	0.10
Glucose (mmol/l)	2.61±0.8[c]	2.4±0.44[b]	0.3±0.1[a]	0.00
T. Cholesterol (mg/dl)	63.3±10.3[c]	60.4±8[b]	25.4±8.5[a]	0.00
T. Proteins (g/dl)	5.5±0.9[c]	3.7±0.9[b]	2.7±1.3[a]	0.00
Albumin (g/dl)	1.8±0.6[b]	1.6±0.7[b]	1.1±0.6[a]	0.02
Globulins (g/dl)	3.6±1[b]	2.1±0.9[a]	1.6±0.9[a]	0.00
Calcium (mg/dl)	6±3.8[b]	4.5±2.7[ab]	1.52±1.3[a]	0.04

T. (Total), Means with different superscripts (a, b, c) differ significantly at P< 5%

Table 4: Biochemical profile of follicular fluid in cyclic, acyclic and cystic ewes

		Reproductive statuses		
Parameters	Min-Max	Cyclic	Acyclic	Cystic
Glucose	2.9-7.6	Normal (2.6 mmol/l)	Normal (2.6 mmol/l)	Low (< 2.6 mmol/l)
T. Cholesterol	15-120	Normal (60.7 - 66.3 mg/dl)	Normal (60.7 mg/dl)	Low (< 60.4 mg/dl)
T. Proteins	1.8-6.87	Low (< 5.97 g/dl)	Low (< 5.97 g/dl)	Low (< 5.97 g/dl)
Albumin	0.08-2.53	Low (< 2.17 g/dl)	Low (< 2.17 g/dl)	Low (< 2.17 g/dl)
Globulins	0.05-5.99	Normal (2-4 g/dl)	Normal (2-4 g/dl)	Low (< 2 g/dl)
Calcium	1.45-11.8	High (> 2.79 mg/dl)	High (> 2.79 mg/dl)	Low (< 2.65 mg/dl)
BCS/5	2-5	Average (3)	Average (3)	Average (3)

DISCUSSION

The number of cyclic (44.59%; *N*= 33) and acyclic (40.54%; *N*= 30) females was greater than that reported by Naafia, *et al*. [11] (*N*= 10) respectively for each group of animals. The proportion of ovarian cysts (14.87%) was lower than that obtained by Kouamo *et al*. [16] in cattle that could be referred to the high milk production in cattle (16.13%). Indeed, the period favorable to the appearance of ovarian cysts corresponds to the time of a significant energy deficit followed by a mobilization of fatty reserves. This would explain the high proportion of ovarian cysts in ewes with thin (1-2), sometimes average (3) BCS. The biochemical profiles of cyclic and acyclic sheep are identical, which makes it possible to deduce that these two groups of animals present the same diagnostic tool: Concentration of glucose, cholesterol and normal globulins; low total protein and albumin; high calcium and average BCS (3). This biochemical profile corresponds to the work of Naafia, *et al*. [11]. The biochemical profile of cystic sheep differs from that of the latter by the low concentrations of glucose, total cholesterol, globulins and calcium.

The glucose concentration observed in cyclic (2.61±0.8 mmol/l) and acyclic (2.4±0.44 mmol/l) ewes was normal; this result is similar to the work of Razzaque *et al*. [17] who observed that, in cows and buffaloes, the level of glucose in follicular fluid increases with the size of the follicles. This means that glucose metabolism is less intense in the large follicles of cyclic ewes compared to the small follicles of acyclic ewes. An increase in the volume of follicular fluid and an increase in the permeability of the follicle barrier during follicular growth could be attributed to higher glucose levels in the large follicles of cyclic ewes [18]. Naafia, *et al*. [11] and Mimoune, *et al.* [19] report three factors responsible for low glucose concentration, namely the failure of neoglucogenesis or glycogenolysis, increased absorption of peripheral glucose and a high rate of endogenous insulin. A drop in glucose causes depression of the hypothalamic function which secretes a small quantity of GnRH, which leads to atrophy of the ovaries. In addition, it is responsible for a weak progesteronemia due to an under activity of the corpus luteum promoting embryonic mortality [20]. Embryonic mortality or non-fertilization leads to the regression of the corpus luteum and the continuation of the next follicular wave, the dominant follicle of which will ovulate with the formation of a new corpus luteum. It will not last long because of the low glucose level, which is responsible for the death of the embryo. An energy deficiency alters the ability of the follicles to produce enough estradiol to ensure ovulation, hence the occurrence of ovarian cysts [21]. Funston, *et al*. [22] showed that a “glucoprivation” induced by a peripheral injection of 2-deoxy-glucose (2DG), leads to a sudden fall in the secretion of LH and FSH in the sheep, despite an increase in the rate of peripheral insulin and causes the appearance of ovarian cysts. A negative energy balance is a factor of infertility responsible for several pathological disorders of the ovary.

The concentration of total cholesterol in the follicular fluid of cyclic and acyclic ewe was normal. This result is similar to that of Bordoloi *et al*. [23] who observed normal cholesterol levels in goats with increased follicle size. The increase in steroid synthesis leads to an increase in cholesterol in the follicular fluid. The total cholesterol level in cystic ewes was below the physiological value. This result corroborates that of Rameez and Sukla [24]. Greater the energy deficit, lower the cholesterol level, and greater the risk of developing ovarian cysts. For Nibart [25], a low level of total cholesterol was associated with a high production of non-viable or dead embryos, having an impact on the occurrence of cysts. In addition, cholesterol is the precursor of the synthesis of steroid hormones which play a role in fertility, fecundity and whose imbalance could cause the occurrence of ovarian cysts.

The concentration of total proteins and albumin in follicular fluid in cyclic, acyclic and cystic ewes was below physiological values; globulins were normal in cyclic and acyclic ewes and weak in cystic ewes. This indicates that the concentration of total protein may not have a specific effect on the follicular development process and does not affect the reproductive state of the animal. These results are similar with previous reports from Arshad, *et al*. [15] who observed that a change in the concentration of total proteins in the follicular fluid does not influence the stages of the estrus cycle and the development of follicles. Mimoune, *et al*. [19] and Chandrahar, *et al.* [26] reported insignificant differences in the concentrations of total proteins, albumin and globulins in cystic cows and concluded that the cause of this condition stems from other factors. A low concentration of globulins marks a possible infection against which the body deploys using a large part of its energy reserves; which affects the functioning of the hypothalamic-pituitary axis that causes ovarian cysts. On the other hand, excess protein causes more problems than its deficiency. Albumin may be needed to bind certain chemicals as well as minerals inside the follicular fluid for various physiological functions, including the growth and maturation of follicles. The globulins in the follicular fluid may be necessary to protect the follicle from external environments. Andersen, *et al.* [27] reported that albumin content was inversely related to follicular size.

Cyclic and acyclic ewes have high calcium concentrations; cystic ewes have low calcium levels. These results are similar to those reported by Bindari, *et al*. [28]. The sudden increase in the concentration of calcium in the follicular fluid would probably be due to the degradation of poor quality oocytes, with release of calcium contained in the granules [29]. Minerals, especially calcium, depend on feed intake in quantity and quality. The main source is plants ingested on pasture. They are essential and involved in many biological and reproductive processes [30]. El-shahata, *et al*. [31] reported that calcium plays a key role in improving the number and size of pre-ovulatory follicles as well as the rate of ovulation. Calcium plays a role in the regulation of "Gap junction" between the cells of the cumulus; it is responsible for a disruption of cell cohesion, which contributes to the process of ovulation. The absence of calcium, which plays a major role in synaptic transmission through its entry into the axonal termination, would influence the functioning of the hypothalamic-pituitary axis. This disrupts the functioning of the reproductive organs and promotes the appearance of ovarian cysts. Stankiewicz, *et al.* [32] reported low concentrations of calcium in the blood and follicular fluid of females with ovarian cysts, unlike normal ones. The oocyte prepares for maturation by storing calcium in the follicular fluid in cortical granules.

CONCLUSION

The biochemical profile of cyclic and acyclic ewes are identical. The biochemical profile of cystic ewes differs by the low glucose, total cholesterol, globulins and calcium levels. Glucose, total cholesterol and calcium are useful markers for differentiating the three groups of animals (cyclic, acyclic and cystic). Indeed, the follicular fluid constitutes an environment in which the oocyte-cumulus complex develops and the granulosa cells differentiate. Thus the control of the implication of this liquid in the infertility of the ewe is part of the necessary global approach of the flock, by the practitioner in order to identify the disorders responsible for infertility in sheep farming.

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