Kafkas Univ Vet Fak Derg
23 (4): 579-586, 2017
DOI: 10.9775/kvfd.2017.17394

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Investigation on Diagnosis and Metabolic Profile of Ovarian Cysts in Dairy Cows

Nora MIMOUNE [1,2] Rachid KAIDI [2] Mohamed Yassine AZZOUZ [1,2,3]
Safia ZENIA [1] Mohamed Hocine BENAISSA [1,2,4] Gary ENGLAND [5]

[1] Higher National Veterinary School, Bab-Ezzouar, Algiers, ALGERIA
[2] Institute of Veterinary Sciences, LBRA, University Saad Dahleb, Blida, ALGERIA
[3] CHO, El-Harrach, Algiers, ALGERIA
[4] Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biophysical Station, Nezla, Touggourt, ALGERIA
[5] School of Veterinary Medicine and Science, University of Nottingham, Nottingham, Leicestershire, UNITED KINGDOM

Article Code: KVFD-2017-17394 Received: 07.01.2017 Accepted: 06.04.2017 Published Online: 12.04.2017

Citation of This Article

Mimoune N, Kaidi R, Azzouz MY, Zenia S, Benaissa MH, England G: Investigation on diagnosis and metabolic profile of ovarian cysts in dairy cows. *Kafkas Univ Vet Fak Derg,* 23 (4): 579-586, 2017. DOI: 10.9775/kvfd.2017.17394

Abstract

A clinical study was performed to evaluate the diagnostic methods for ovarian cysts (OC), and to determine the metabolic profiles of animals with OC in the region of Mitidja in the North of Algeria. A total of 504 non-pregnant lactating cows were used in this study. Ultrasonography was performed by EXAGO scanner and was combined with assessment of serum P4. Biochemical serum parameters were assayed by spectrophotometry and insulin and cortisol serum measurement was performed by electrochemiluminescence. The results showed an overall incidence of 11.9% of OC. The incidence of OC was higher among cows in third lactation. Holstein breed was the most affected by OC compared with other breeds (P<0.001). There were no effects of average BCS (Body Condition Scoring) and milk production on the incidence of OC (P>0.05). OC were single in 91% of cases. They were found mainly on the right ovary (66.66%). Seasonality had a significant influence on incidence rate of OC with higher incidence rates during winter and spring (71.66%); while, 28.33% of OC were detected during the summer and autumn (P<0.05). OC were associated with low serum concentrations of glucose, insulin and urea as well as high levels of cortisol. Ultrasound examination and progesterone assays were proposed as the most effective diagnostic combination to diagnose OC. In conclusion, in addition to hormonal imbalances, metabolic disorders are involved in the formation and/or persistence of OC. Therefore, the use of metabolic indicators in understanding and exploration of OC is of great interest.

Keywords: *Cow, Ovarian cyst, Metabolic profile, Ultrasonography*

Sütçü İneklerde Ovaryum Kistlerinin Tanısı ve Metabolik Profili Üzerine Bir Çalışma

Özet

Ovaryum kistlerinin tanısında kullanılan metotları değerlendirmek ve Cazayir'in kuzeyindeki Mitidja bölgesindeki ovaryum kistli hayvanların metabolik profillerini belirlemek amacıyla klinik bir çalışma gerçekleştirildi. Toplam 504 gebe olmayan laktasyondaki inekler bu çalışmada kullanıldı. EXAGO tarayıcı kullanılarak ultrasonografi uygulandı ve serum P4 verileri ile birlikte değerlendirildi. Biyokimyasal serum parametreleri spektrofotometri ile belirlenirken insülin ve kortizol serum ölçümleri elektrokimyasal görüntüleme ile gerçekleştirildi. Ovaryum kist insidansı %11.9 olarak belirlendi. Ovaryum insidansı üçüncü laktasyondaki ineklerde daha fazla oranda tespit edildi. Diğer türler ile karşılaştırıldığında Holstein ırkında ovaryum kisti daha fazla oranda gözlemlendi (P<0.001). Ovaryum kisti üzerine Vücut Kondisyon Skorunun bir etkisi gözlemlenmedi (P>0.05). Ovaryum kisti, vakaların %91'inde tek taraflı olarak tespit edildi. Kistler çoğunlukla (%66.66) sağ ovaryumda yer almaktaydı. Mevsimin ovaryum kistlerinin ortaya çıkmasında önemli bir etkiye neden olduğu, kış ile ilkbahar aylarında (%71.66) insidansın arttığı yaz ve sonbaharda ise düşük seviyede (%28.33) olduğu belirlendi (P<0.05). Ovaryum kistlerinin düşük glukoz, insülin ve üre ile yüksek kortizol serum konsantrasyonları ile ilişkili olduğu belirlendi. Ultrason incelemesi ile birlikte progesteron testi uygulamasının ovaryum kistlerinin tanısındaki en etkili kombinasyon olduğu önerildi. Sonuç olarak, hormonal düzensizliğe ilave olarak metabolik bozukluklar ovaryum kistlerinin oluşmasında ve devamlılığında rol oynamıştır. Bu nedenle, metabolik indikatörlerin kullanılması ovaryum kistlerini anlama ve incelemede büyük önem arz etmektedir.

Anahtar sözcükler: *İnek, Ovaryum kisti, Metabolik profil, Ultrasonografi*

İletişim (Correspondence)
+213 790 901606
nora.mimoune@gmail.com

INTRODUCTION

Cystic ovarian disease (COD) is one of the most common reproductive disorders in cattle, causing reproductive failure in dairy cattle [1]. Ovarian cysts have been defined as fluid filled structures > 25 mm in diameter that persist in the ovaries for at least 10 days in the absence of a corpus luteum and are classified as follicular or luteal according to the degree of luteinization and P4 (progesterone) secretion [2]. The reported incidence of ovarian follicular cysts in dairy cattle showed a range from 5.6% to 18.8% [1].

Treatment efficiency of cows with ovarian cysts (OC) requires an early diagnosis at the end of the waiting period or during the reproductive period [3], taking into consideration the fact that during the first weeks post-partum, over 60% of the OC may regress spontaneously [1]. Moreover, the choice of a therapeutic strategy must also depend on the degree of accuracy (predictive value) of the diagnosis of OC (follicular cyst [FC] or luteinized cyst [LC]) because incorrect diagnosis leads to inadequate treatment [4,5]. Indeed, it is very common that veterinary practitioners diagnose ovarian cyst based on a single visit to the affected animals without reassessing the diagnosis a few days later [5], which does not provide an accurate picture of the problem because one visit indicates only the size of OC. Moreover, without hormonal analysis, it is impossible to determine if the cyst is functional or not. Similarly, diagnosis of OC is traditionally based on the history of the animal affected and the result of rectal palpation. However, the criteria suggested in the literature to distinguish types of OC are divergent and poorly defined. For example, although nymphomania characterizes FC and anoestrus is noted in LC, it is also recognized that cows with either type of cyst may show variable behavior [4]. Therefore, it seems essential to use ultrasound or P4 analysis rather than rectal palpation [1]. According to Rauch et al.[5], positive predictive value for FC or LC diagnosed by rectal palpation is 66%, it increases to 74% for FC and 85% for LC when ultrasound examination is performed. This same value also increases by performing P4 assay [5].

The present study aimed to identify the difficulties of OC diagnosis in practice and to compare the usefulness of each diagnostic method (rectal palpation, ultrasonography and P4 assay) to distinguish OC types. Furthermore, metabolic profile and BCS of cystic cows that were over 60 days post-partum (PP) were investigated.

MATERIAL and METHODS

Animals

The study was performed on 504 nonpregnant lactating cows (Holstein, Montbeliard, Fleckvieh and crossed breeds), selected from 12 herds of the Mitidja (in the North of Algeria), aged between 4 to 12 years, housing and feeding conditions were similar for all the animals, lacking of any other disorder. The study was conducted between 3 April 2013 and 5 August 2015. Animals included in the present study were more than 60 days post-partum (60-215 days PP in range), and were presumed to have OC after the first rectal palpation of veterinary practitioners. Only cows bearing ovarian structures with a diameter larger than 25 mm, in the absence of any corpus luteum and persisting for at least 10 days were considered as having OC [2]. The complaint of breeders was the successive failures of artificial insemination (AI). Control group (n=30) were cycling dairy cows that were also more than 60 days PP but without OC. These cows expressed estrus behavior, and had corpus luteum identified a few days later by rectal palpation and ultrasound examination.

Ultrasound Examination and BCS

Ultrasonography was performed by EXAGO scanner (ECM, Noveko International Inc., Angouleme, France) and equipped with 5-7.5 MHz linear transducer. Body condition scoring was evaluated according to Edmonson et al.[6].

Biochemical and Hormonal Analyses

Blood samples from each animal were collected by jugular venipuncture on the day of ultrasound examination. Collection of all samples was performed before feeding (except for BHB, 1-4 h after feeding) [7]. After collection, blood serum was separated from coagulated blood by centrifugation (3.000 rpm/20 min) and stored at -20°C until analysis.

Blood serum parameters including glucose, total protein, triglycerides, cholesterol, urea, creatinine, aspartate aminotransferase (AST), alanineaminotransferase (ALT), gamma glutamyl transpeptidase (GGT) and β-hydroxy-butyrate (BHB) were assayed by spectrophotometry on automated clinical chemistry analyser Architect plus, ci 4100 (Architect c Systems, Abbott Diagnostics, Germany). Insulin and cortisol Serum measurement was performed on another analyzer Cobas e411 by electrochemiluminescence (Roche Diagnostics GmbH, Germany). The minimum detection limit was 0.5 nmol/L for cortisol and 0.2 µU/mL for insulin.

Ultrasound examination was combined with assessment of serum P4 which was determined on Architect plus, 4100 by competitive immunoassay using chemiluminescence technology. According to the manufacturer, the minimum detection limit was 0.1 ng/mL. A serum cut-off value of 1 ng/mL was considered in the classification of OC (FC: P4 <1 ng/mL; LC: P4 ≥1 ng/mL) [5,8,9]. Cysts with thin walls ≤3 mm, a uniformly anechogenic fluid, with a P4 concentration <1 ng/mL were considered FC. In contrast, cysts with walls ≥3 mm, showing a visible echogenic rim or echogenic spots with a P4 concentration ≥1 ng/mL were considered LC [8,9].

Statistical Analysis

Statistical analysis was performed using the STATISTICA software (Version 10, Stat Soft France, 2003). Statistical differences in the concentrations of metabolic parameters between the cycling and the cystic cows were carried out using Student's t-test. Chi-squared test were used to analyze the characteristics of cystic animals and their effects on OC incidence. Stage of lactation, BCS, mean milk yield, behavior, breed, and season were considered as covariates. A logistic regression was constructed using the different risk factors as fixed effects and herd as the random effect. The effects of each variable were measured using an odds ratio along with 95% confidence intervals. Pearson's coefficient was used to calculate correlations between different measurements. Data are expressed as percentage or mean ± standard deviation. The results were considered significant when P<0.05.

RESULTS

Incidence of Ovarian Cysts

Among the 504 animals examined, 60 cows were affected by OC (11.9%). Identification of OC type based on rectal palpation of the veterinary practitioners, ultrasound examination and P4 serum level, is presented in *Table 1*. Based on rectal palpation, veterinary practitioners identified 60% FC and 40% LC. Ultrasound examination showed different percentages of OC (FC: 51.66%; LC: 48.33%) whereas P4 analysis combined with ultrasonography revealed 55% LC and 45% FC (P<0.05) *(Fig. 1, Fig. 2)*.

General Characteristics of Cows with OC

Table 2 presents the characteristics of cystic cows including incidence of OC according to the stage of lactation, reproductive behavior and the breed of the cow. Parity was excluded as all cystic animals were multiparous. Dairy production and BCS between cycling and cystic cows was also compared in this table.

Incidence of OC was higher among cows in the third lactation, followed by those in the fourth lactation (41.66% and 36.66%, respectively). Regarding other ranks of calving (2^{nd} and 5^{th} lactation), OC were noted in 18.33% and 3.33%, respectively (P<0.001). According to the farmers and veterinary practitioners, anoestrus was the predominant sexual behavior associated with the two types of OC (93.33%). Nymphomania was noted in only four cases (6.66%) (P<0.001). Holstein-Friesian cows were more affected than Montbeliard cows (61.66% *vs* 38.33%; P<0.001). The other breeds (Fleckvieh and crossed) were not affected in the present study. The average BCS of both cystic cows and cycling cows were not significantly different (3.1 *vs* 3.08; P>0.05). Similarly, milk production did not differ between the two groups (cycling and cystic cows) (P>0.05).

Description of Ovarian Cysts

The characteristics of ovarian cysts diagnosed in this study were elucidated in *Table 3*. Ovarian cysts were single in 91% of cases. Polycystic ovaries were found in 8.33% of animals (P<0.001). In most cases, OC were diagnosed on the right ovary (66.66%). The left ovary was affected in 28.33% of the cases whereas the two ovaries were simultaneously affected in 5% of the cases (P<0.001). The majority of OC were noted during the winter season (71.66%) while 28.33% were detected during the warm season (P<0.01).

Table 4 presents the results of the multivariate analysis, which uses cow status (cystic cows versus control animals) as the dependent variable and stage of lactation, season, behavior and breed as the independent variables. The analysis shows that the odds to have OC increased if the cow was in the third or fourth lactation. Prim'Holstein cow was the most exposed to develop OC with anoestrus as the predominant behavior. Summer and winter had the most negative effect on OC development.

Table 1. *Type of ovarian cysts according to the diagnostic methods*

Type of OC	Rectal Palpation n (%)	Ultrasonography n (%)	P4 Assessment n (%)
FC	36 (60)	31 (51.66)	27 (45) **
LC	24 (40)	29 (48.33)	33 (55) *
Total	60	60	60

** P<0.05; ** P<0.01*

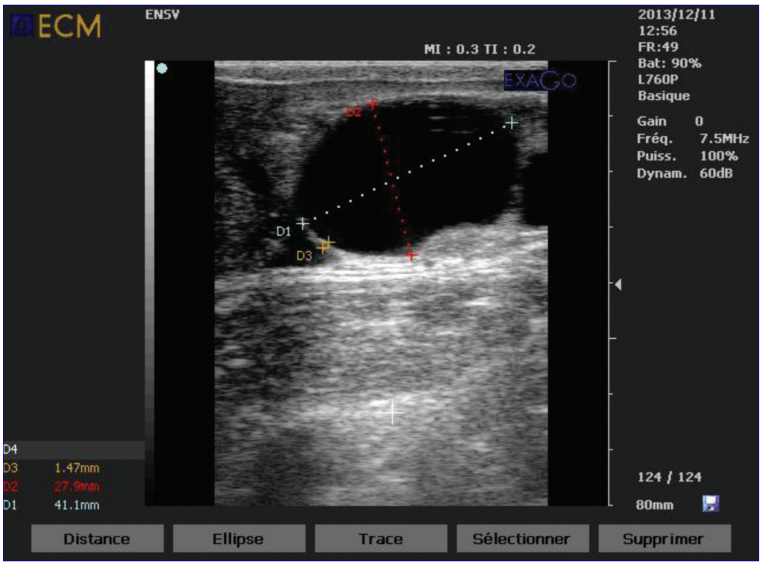

Fig 1. Follicular cyst

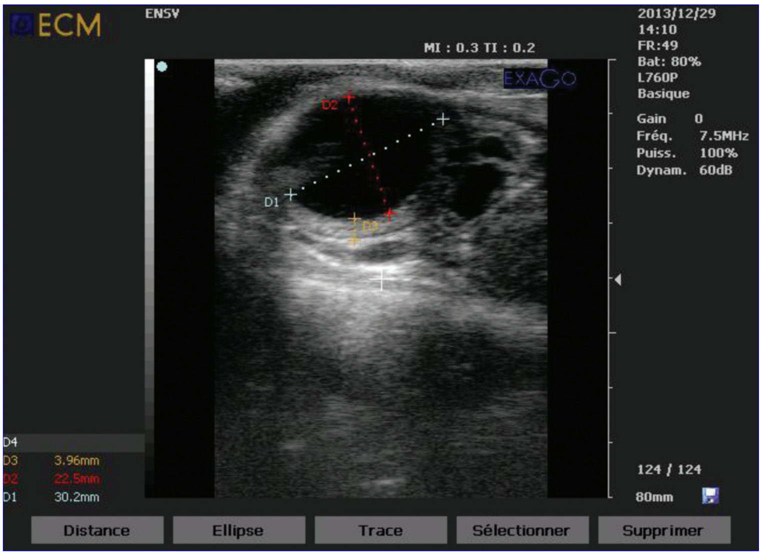

Fig 2. Luteal cyst

Table 2. *Characteristics of cows with OC*			
Characteristic (n=60)		**n**	**%**
Stage of lactation	2	11	18.33
	3	25	41.66
	4	22	36.66
	5	2	3.33***
Behavior	Nymphomania	4	6.66
	Anoestrus	56	93.33***
Breed	Prim'Holstein	37	61.66
	Montbéliard	23	38.33***
BCS	Cystic cows	3.1	
	Cyclic cows	3.08^{ns}	
Dairy production (L/day)	Cystic cows	12.88	
	Cyclic cows	12.5^{ns}	

ns: *Not significant; *** P<0.001*

Table 3. *Description of ovarian cysts*			
Ovarian Cysts (n=60)		**n**	**%**
Position	Right	40	66.66
	Left	17	28.33
	Right and left	3	5***
Number	Single	55	91.66
	Polycystic	5	8.33***
Season	Winter	23	38.33
	Spring	10	16.66
	Summer	21	35
	Autumn	6	10**

*** P<0.01; *** P<0.001*

Concentrations of Metabolic Parameters

The results about blood metabolic parameters are summarized in *Table 5*. They are expressed as mean ± standard deviation. To interpret these results, the reported and/or established values of these metabolites in previous studies were also mentioned.

Table 5 demonstrated that cystic cows had significantly low serum concentrations of glucose than cycling ones. However, the mean values were within the accepted normal range in the two groups. Insulin and urea concentrations were very low in cystic cows and were below the reference range while in cycling animals, the values were in the reference range. In contrast, cortisol concentrations were higher in cystic cows compared with cycling ones, but the values remained within the reference range. Although the mean values of BHB were in the normal range, there was a significant difference between the two groups of animals.

In general, concentrations of total protein, cholesterol, triglycerides, creatinine and the activity of liver enzymes (AST, ALT, and GGT) were all within the accepted range and the statistical test revealed no significant differences between the two groups of animals.

Correlations among blood metabolites, metabolic hormones, breed, stage of lactation, season, BCS and mean milk yield are summarized in *Table 6*. There were significant associations between glucose and insulin (P<0.01), glucose and cholesterol (P<0.05), glucose and cortisol (P<0.01).

Table 4. *Results of logistic regression model of cows with OC. Odds ratios along with 95% confidence intervals were calculated according to different parameters*

Parameter		**Odds Ratio**	**95% CI**	**P**
Stage of lactation	2	0.61	0.22 to 1.75	0.3
	3	1.9643	0.75 to 5.12	0.01
	4	1.3509	0.53 to 3.46	0.04
	5	0.1724	0.03 to 0.95	0.04
Behavior	Nymphomania	0.0714	0.02 to 0.22	<0.0001
	Anoestrus	14	4.51 to 43.5	<0.0001
Breed	Prim'Holstein	0.5850	0.22 to 1.53	0.02
	Montbéliard	1.7095	0.65 to 4.47	0.01
Season	Winter	0.93	0.38 to 2.28	0.03
	Spring	0.8	0.26 to 2.45	0.8
	Summer	1.2564	0.4888 to 3.2294	0.01
	Autumn	1	0.232 to 4.3098	0.3

Table 5. *Metabolic profile of cystic cows compared with cystic animals and reference range*

Parameter	Cystic Cows (n=60)	Cyclic Cows (n=30)	Reference Range
Glucose (mmol/L)	3.66±0.37	4.67±1.28 ***	2.1-5.56 [7,10-14]
Insulin (µU/mL)	1.65±1.03	5.02±1.3 ***	4.92-11.25 [14-16]
TP (g/L)	74.17±9.94	77.6±8.67 ns	70-94 [10,12,13]
TG (mmol/L)	0.19±0.10	0.19±0.13 ns	0.06-0.2 [7,10,11,17-22]
Chol (mmol/L)	3.61±0.88	3.22±1.43 ns	1.3-8.0 [10,13,15,19]
Cortisol (nmol/L)	51.71±15.74	30.41±13.87 ***	11.59-92.46 [9,10,17]
AST (U/L)	84.15±28.51	83.69±35.20 ns	56-176 [12,13,22]
ALT (U/L)	22.88±4.7	22.57±7.26 ns	11-40 [21,22]
GGT (U/L)	22.81±6.2	23.46±6.11 ns	17-51 [12,13]
Urea (mmol/L)	1.39±0.23	4.02±3.2 ***	3.3-6.06 [10,11,18,22]
Creatinine (µmol/L)	114.33±29.9	111.26±27.02 ns	88.4-240 [7,19,22]
BHB (mmol/L)	0.61±0.35	0.33±0.3 ***	<0.8 [7,10,16]

TP: *total protein;* ***TG:*** *triglycerides;* ***Chol:*** *cholesterol;* ***AST:*** *aspartate aminotransferase;* ***ALT:*** *alanine aminotransferase;* ***GGT:*** *gamma glutamyl transpeptidase;* ***BHB:*** *β-hydroxy butyrates,* ***ns:*** *Not significant; *** P<0.001*

Table 6. *The relationship among parameters in cows with ovarian cyst*

Parameter	Parameter																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1-glu	1																
2-insu	0.42	1															
3-TP	0.06	0.03	1														
4-TG	0.22	-0.1	0.08	1													
5-Chol	0.36	0.09	-0.58	0.42	1												
6-Cort	0.4	0.06	0.01	0.11	0.01	1											
7-AST	-0.1	-0.3	-0.02	-0.25	-0.23	-0.4	1										
8-ALT	-0.3	-0.7	-0.03	-0.3	-0.3	-0.12	0.14	1									
9-GGT	-0.02	-0.4	-0.04	-0.01	-0.1	-0.04	0.06	0.11	1								
10-Urea	-0.2	-0.36	0.23	0.04	0.02	0.05	-0.22	-0.22	-0.04	1							
11-Crea	-0.06	-0.48	0.07	-0.3	-0.3	-0.03	-0.06	-0.14	-0.14	0.12	1						
12-BHB	-0.56	-0.3	-0.47	0.01	-0.44	-0.14	-0.12	-0.09	-0.04	-0.09	-0.02	1					
13-MMP	-0.22	-0.7	0.18	-0.2	-0.32	0.07	-0.15	-0.02	-0.17	-0.11	0.03	0.1	1				
14-BCS	-0.32	0.06	0.09	-0.1	-0.11	-0.03	-0.23	-0.03	-0.32	-0.02	0.1	-0.08	-0.02	1			
15-Se	-0.4	-0.23	-0.23	-0.14	-0.09	-0.22	-0.06	-0.11	-0.07	0.01	-0.14	-0.14	-0.1	-0.02	1		
16-ST	-0.01	-0.4	-0.4	-0.3	-0.1	-0.32	-0.07	-0.14	-0.05	0.04	-0.22	-0.1	0.22	0.08	-0.09	1	
17-Br	-0.3	-0.09	-0.25	-0.4	-0.22	-0.09	-0.04	-0.12	0.01	-0.16	-0.09	0.03	0.08	-0.22	-0.001	-0.01	1

Glu: *glucose;* ***insu:*** *insuline;* ***TP:*** *total proteins;* ***TG:*** *triglycerides;* ***Crea:*** *creatinine;* ***MMP:*** *mean milk production;* ***Se:*** *season;* ***ST:*** *stage of lactation;* ***Br:*** *breed*

DISCUSSION

In the present study, the incidence of OC was 11.9% for a total of 504 animals examined. This result is very close to that determined by Brito and Palmer [23] and remains below that observed by Gümen et al.[24]. The divergence of results might therefore be explained by the difference of diagnosis methods, times and criteria of OC diagnosis, animal factors (breed, age, parity and lactation stage) other factors such as breed, age, parity, season, nutrition, breeding management, and monitoring periods.

The current study evaluated the effectiveness of three methods of OC diagnosis in dairy cows. These included: Rectal palpation by veterinary practitioners (more than 10 years of experience), ultrasound examination and measurement of serum P4. The results showed that veterinary practitioners identified 36 FC and 24 LC whereas ultrasound diagnosed 31 FC and 29 LC. In contrast, hormonal analysis combined with ultrasound examination identified 27 FC and 33 LC. Therefore, the diagnosis had become more accurate. Progesterone concentrations noted were similar to those reported in the literature [4,25]. Data supposed that 9/60 (15%) of animals received inadequate treatment by because of treatment of FC by PGF2a, which is generally used to induce luteolysis. Indeed, OC misdiagnosis leads to incorrect therapeutic choice which delays conception. The probability of a false classification of OC is larger with the structures diagnosed firstly as being FC, in agreement with Leslie and Bosu [4]. These results reiterate the importance of ultrasound examination and hormonal diagnosis in the identification of OC type.

LC was more frequent than FC. This result supports those found by Leslie and Bosu [4] and differs from those suggested by Carroll et al.[25]. Our result is reasoned by the fact that the LC are FC at a late stage and the complaint of breeders followed upon the successive failures of the artificial insemination (AI) which can exceed more than 100 days, therefore, it's plausible that FC are transformed into LC if they are not treated in the opportune period. Most cases of OC have been associated with anoestrus in accordance with previous data [4,26,27]. In fact, it is very difficult or impossible to identify types of OC based on the clinical appearance of affected animals and on one rectal palpation. In this study, all cows affected by OC were multiparous. Within these animals, the incidence was higher for those in third lactation, followed by those in the fourth lactation. Data demonstrated that the OC were associated with higher milk production [28]. Among the OC, 91.66% were single and 8.33% were multiple. A high incidence of polycystic ovary was noted by Silvia et al.[8]. The right ovary was more affected than the left ovary. This is consistent with studies conducted by different authors [29,30]. The Holstein-Frisian cow was more affected by OC followed by the Montbeliard. This fact is consistent with what has been reported in the literature [23]. The BCS did not differ between the two groups of animals (cycling and cystic). The mean values obtained were within the reference range which is 2.5-3.4 [31]. This result was in agreement with those of Vanholder et al.[32] and Yousefdoost et al.[14]. Opsomer et al.[27] reported that the BCS at calving is not associated with the risk of OC development. In the contrast, increasing milk production in early lactation is considered a predisposing factor in the formation of OC [28]. In this study, no association between milk production and OC were recorded. It is important to note that our protocol targeted cows that were over 60 days PP and therefore outside the period of risk. Ovarian cysts were common in winter and summer, respectively. This is consistent with the result of López-Gatius et al.[28]. Lack of exercise, vitamins and minerals in winter and heat stress in summer are the main factors favoring the appearance of OC [28,33]. As shown above, stage of lactation, breed and season are clearly related to OC development.

In this study, samples were taken towards the end of peak lactation, as from the reproductive period. In this period, the animals can be expected to have recovered from a negative energy balance and loss of BCS, resulting in a restoration of blood levels of different metabolites (Non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), glucose, insulin, Insulin like Growth Factor 1 (IGF-1)). Therefore, this work was carried out in order to study the relationship between metabolic profile and the persistence and/or formation of OC in dairy cows that were over 60 days PP. To interpret the biochemical parameters, analysis of liver enzymes seems essential to reveal any hepatic dysfunction that can affect the metabolism of the animal.

Cows with OC were characterized by lower glucose levels than normal cows although the values noted were within the normal reference range. These data support those noted by Braw -Tal et al.[34] and Khan et al.[35]. In the same context, Vajdi et al.[36] reported that glucose levels were significantly different between animals that become pregnant after first AI (artificial insemination) and those with failed to first AI. However, it seems that glucose may be associated with infertility when it is well below its usual values [37].

Insulin levels were significantly different between the 2 groups of animals. Insulin levels were below normal accepted reference values previously established. Our results are similar to those of Vanholder et al.[32] and Hein et al.[16]. In another study, Obese et al.[38] found that insulin concentrations were higher in animals that had an early resumption of cyclicity compared to those who had a delay. The importance of insulin in follicular growth, maturation and ovulation, and in the stimulation of steroidogenesis has been widely accepted. Insulin also stimulates expression of LH receptors in granulosa cells, and indirectly by stimulation of IGF-1 receptor [32]. Therefore, hypoinsulinemia may not only reduce the production of androgens and estradiol, but also alters the follicle's ability to acquire LH receptors, which compromises development and ovulation and promotes persistence of follicles as anovulatory structures [16]. Previous studies have revealed that insulin secretion is impaired even after the administration of glucose in cystic cows [27]. However, although the phenomenon of 'insulin resistance' is linked to the polycystic ovary syndrome associated with metabolic disorders including diabetes [39], the authors were unable to demonstrate such relationship in the case of OC in cows [27]. More recently, Hein et al.[16] observed low expression of insulin receptor (IR and IRS1) in cystic cows.

The present study found no significant difference in the concentrations of total cholesterol, total protein, triglycerides, creatinine and the activity of liver enzymes (AST, ALT, and GGT) between the groups of animals. Similar results were noted by Vajdi et al.[36], Khan et al.[35] and Ghoneim et al.[40].

Urea concentrations between cycling and cystic cows were significantly different. Cystic animals had low concentrations of urea serum. In fact, several authors have studied the consequences of low urea levels on reproductive function in dairy cows; Miettinen [37] noted that reproductive function is impaired as soon as urea concentrations are less than 2.5 mmol/L. Enjalbert [41] mentioned that reproductive disorders appear only in cases of severe and prolonged nitrogen deficiency although it is rare. Kaur and Arora [42] reported that a low urea levels may reflect a lack of ammonia in the rumen resulting from insufficient protein intake. According to these authors, low intake caused by a lack of nitrogen has long been known in ruminants and had been particularly well studied with low nitrogen fodder. Following this deficiency, microbial protein synthesis, appetite, diet digestibility and efficiency of the use of metabolizable energy decrease. It results in hypoglycemia and low insulin, inhibiting hypothalamic GnRH secretion, pulsatile secretion of LH and P4 synthesis. However, the results differ from those noted in previous research [14], which have found that cystic cows had high concentrations of urea compared to cycling cows. Result discrepancies can be explained by diet received by cows, the time of sampling relative to food intake and the physiological stage of the animal.

The average concentrations of cortisol were significantly higher in cystic cows compared to cycling animals although the values were within the accepted normal range. Our results are consistent with those of Khan et al.[35] in buffaloes but differ from Probo et al.[9] in cattle and Ghoneim et al.[40] and El -Bahr et al.[43] in camels. This divergence may be explained by the difference in breeding conditions, climatic factors, stage of lactation and food intake. For these criteria, we may also add the way of OC development (spontaneous or induced by ACTH injection). According to Dobson and Smith [44], any change in the environment (especially PP) may be at the origin of a stress which increases the cortisol concentrations. This induces a reduction in the secretion of estradiol and a rise in serum P4. Thus, the concentrations of LH and FSH are reduced by negative feedback, the preovulatory LH surge is not observed and OC develop.

Serum concentrations of BHB were significantly higher in cystic cows although its values were in the reference range. Vanholder et al.[32] and Jackson et al.[45] reported similar results. According to Probo et al.[9], low BHB may indicate a low mobilization of fat reserves. Despite the involvement of BHB in the OC pathogenesis [5], the results of the present work have demonstrated no influence of this parameter in OC maintaining.

This study demonstrated that ultrasound examination combined with assessment of serum P4 was the best method to distinguish OC type. LC was predominant and the majority of cystic cows were in the third lactation. Anoestrus was the most common behavior observed by these cows. Stage of lactation, season and breed constitute the risk factors of OC development. Clearly, other risk factors need to be considered when trying to understand the problem of OC. Metabolic disorders are involved in the formation and/or persistence of OC. This is supported by the high incidence of OC in dairy cows that had over 60 days PP. In this study, the OC were associated with low serum concentrations of glucose, insulin and urea as well as high concentrations of cortisol. Although we have tried to explain the involvement of each of these parameters in the OC pathogenesis, the mechanism exact leading to the formation of OC remains obscure. The role of nutrition in the changes of the concentrations of these metabolites is widely reported. The use of biochemical indicators in understanding and exploration of OC is of great interest.

Acknowledgements

The authors are grateful to the staff of Nuclear Research Laboratory, Pathological Anatomy Laboratory of Douera (Algiers, Algeria) for their kind assistance during all analyses and to Mr. GUEDIOURA Abd Elmoumène for his kind help.

REFERENCES

1. Ortega HH, Palomar MM, Acosta JC, Salvetti NR, Dallard BE, Lorente JA, Barbeito CG, Gimeno EJ: Insulin-like growth factor I in sera, ovarian follicles and follicular fluid of cows with spontaneous or induced cystic ovarian disease. *Res Vety Sci*, 84, 419-427, 2008. DOI: 10.1016/j.rvsc.2007.05.010

2. Vanholder T, Opsomer G, De Kruif A: Etiology and pathogenesis of cystic ovarian follicles in dairy cattle: A review. *Reprod Nutr Dev,* 46, 105-119, 2006. DOI: 10.1051/rnd:2006003

3. Hanzen CH, Bascon F, Theron L, López-Gatius F: Les kystes ovariens dans l'espèce bovine, 3. Aspects thérapeutiques. *Ann Méd Vét,* 152, 103-115, 2008.

4. Leslie KE, Bosu WTK: Plasma progesterone concentrations in dairy cows with cystic ovaries and clinical responses following treatment with fenprostalene. *Can Vet J*, 24, 352-356, 1983.

5. Rauch A, Krüger L, Miyamoto A, Bollwein H: Colour Doppler sonography of cystic ovarian follicles in cows. *J Reprod Dev*, 54, 447-453, 2008. DOI: 10.1262/jrd.20025

6. Edmonson, AJ, Lean IJ , Weaver LD, Farver T, Webster G: A body condition scoring chart for Holstein cows. *J Dairy Sci,* 72, 68-78, 1989. DOI: 10.3168/jds.S0022-0302(89)79081-0

7. Kaneko JJ, Harvey JW, Bruss ML: Clinical Biochemistry of Domestic Animals. San Diago, USA, Academic Press, 932, 1997.

8. Silvia WJ, Hatler TB, Nugent AM, Laranja da Fonseca LF: Ovarian follicular cysts in dairy cows: An abnormality in folliculogenesis. *Domest Anim Endocrinol*, 23, 167-177, 2002. DOI: 10.1016/S0739-7240(02)00154-6

9. Probo M, Comin A, Mollo A, Cairoli F, Stradaioli G, Veronesi MC: Reproductive performance of dairy cows with luteal or follicular ovarian cysts after treatment with buserelin. *Anim Reprod Sci*, 127, 135-139, 2011. DOI: 10.1016/j.anireprosci.2011.07.019

10. Leroy JLMR, Vanholder T, Delanghe JR, Opsomer G, Van Soom A, Bols PEJ, De Kruif A: Metabolite and ionic composition of follicular fluid from different sized follicles and their relationship to serum concentrations in dairy cows. *Anim Reprod Sci*, 80, 201-211, 2004. DOI: 10.1016/S0378-4320(03)00173-8

11. Alves BG, Alves KA, Lúcio AC, Martins MC, Silva TH, Braga LS, Silva TV, Viu MAO, Beletti ME, Jacomini JO, Santos RM, Gambarini ML: Ovarian activity and oocyte quality associated with the biochemical profile of serum and follicular fluid from Girolando dairy cows post-partum. *Anim Reprod Scie*, 146, 117-125, 2014. DOI: 10.1016/j.anireprosci.2014.02.019

12. Aller JF, Callejas SS, Alberio RH: Biochemical and steroid concentrations in follicular fluid and blood plasma in different follicular waves of the estrous cycle from normal and superovulated beef cows. *Anim Reprod Sci*, 142, 113-120, 2013. DOI: 10.1016/j.anireprosci.2013.09.009

13. Duffield TF: Subclinical ketosis in lactating dairy cattle. *Vet Clin North Am: Food Anim Pract*, 16, 231-253, 2000. DOI: 10.1016/S0749-0720(15)30103-1

14. Yousefdoost S, Samadi F, Moghaddam G, Hassani S, Jafari Ahangari Y: A comparison of hormonal, metabolite and mineral profiles between Holstein cows with and without ovarian cysts. *Int J Agri Sci*, 2 (12) : 1107-1115, 2012.

15. Cuvelier C, Cabaraux JF, Dufrasne I, Istasse L, Hornick JL: Transport sanguin et métabolisme hépatique des acides gras chez le ruminant. *Ann Méd Vét*, 149, 117-131, 2005.

16. Hein GJ, Panzani CG, Rodríguez FM, Salvetti NR, Díaz PU, Gareisa NC, Benítez GA, Ortega HH, Rey F: Impaired insulin signaling pathway in ovarian follicles of cows with cystic ovarian disease. *Anim Reprod Sci*, 156, 64-74, 2015. DOI: 10.1016/j.anireprosci.2015.02.010

17. Cairoli F, Comin A, Socca S, Fazzini U, De Amicis I, Battocchio M: Hormonal and metabolic profiles in post-partum ovarian cysts in dairy cows. *Vet Res Commun*, 32 (Suppl. 1): S123-S125, 2008. DOI: 10.1007/s11259-008-9099-x

18. Ferguson JD: Diet, production and reproduction in dairy cows. *Anim Feed Sci Technol*, 59, 173-184, 1996. DOI: 10.1016/0377-8401(95)00898-5

19. Brugère-Picoux J: Maladies métaboliques et biochimie Clinique de la vache laitière. *La Dépêche Technique*, 46, 30, 1995.

20. Sutton-McDowall ML, Yelland R, MacMillan KL, Robker RL, Thompson JG: A study relating the composition of follicular fluid and blood plasma from individual Holstein dairy cows to the in vitro developmental competence of pooled abattoir-derived oocytes. *Theriogenology*, 82, 95-103, 2014. DOI: 10.1016/j.theriogenology.2014.03.011

21. Yotov SA, Atanasov AS, Georgiev GB, Dineva JD, Palova NA: Investigation on some biochemical parameters and effect of hormonal treatment in anoestrous dairy cows with cystic ovarian follicle. *Asian Pacific J Reprod*, 3, 41-45, 2014. DOI: 10.1016/S2305-0500(13)60183-9

22. Sylvain B: Interprétation et valeurs usuelles des paramètres sanguins en biochimie clinique vétérinaire. *Revue Francophone Des Laboratoires* Mars, 420, 2010.

23. Brito FC, Leonardo C, Palmer W: La maladie kystique ovarienne chez les bovins. *La Médecine Vétérinaire Des Grands Animaux-Rondes Cliniques*, 04, 10, 2004.

24. Gümen A, Guenther JN, Wiltbank MC: Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. *J Dairy Sci*, 86, 3184-3194, 2003. DOI: 10.1530/rep.1.00653

25. Carroll DJ, RA Pierson, ER Hauser, RR Grummer, DK Combs: Variability of ovarian structures and plasma progesterone profiles in dairy cows with ovarian cysts. *Theriogenology*, 34, 1990. DOI: 10.1016/0093-691X(90)90528-2

26. Garverick HA: Ovarian follicular cysts in dairy cows. *J Dairy Sci*, 80, 995-1004, 1997. DOI: 10.3168/jds.S0022-0302(97)76025-9

27. Opsomer G, Wensing Th, Laevens H, Coryn M, De Kruif A: Insulin resistance: The link between metabolic disorders and cystic ovarian disease in high yielding dairy cows? *Anim Reprod Sci*, 56, 211-222, 1999. DOI: 10.1016/S0378-4320(99)00048-2

28. López-Gatius F, Santolaria P, Yániz J, Fenech M, López-Béjar M: Risk factors for postpartum ovarian cysts and their spontaneous recovery or persistence in lactating dairy cows. *Theriogenology*, 58, 1623-1632, 2002. DOI: 10.1016/S0093-691X(02)01046-4

29. Tanabe TY, Brofee RD: Treatment of cystic ovarian follicles in dairy cows with chorionic gonadotropin. *Theriogenology*, 18, 497-512, 1982. DOI: 10.1016/0093-691X(82)90183-2

30. Kaikimi AS, Chikalikar GK, Dindorkar CV: Reproductive disorders in Holstein-Friesian x Gir F_1 crossbred cows. *Indian J Anim Sci*, 53, 556-558, 1983.

31. Waltner SS, McNamara JP, Hillers JK: Relationships of body condition score to production variables in high producing Holstein dairy cattle. *J Dairy Sci*, 76, 3410-3419, 1993. DOI: 10.3168/jds.S0022-0302(93)77679-1

32. Vanholder T, Leroy JLMR, Dewulf J, Duchateau L, Coryn M, De Kruif A, Opsomer G: Hormonal and metabolic profiles of high-yielding dairy cows prior to ovarian cyst formation or first ovulation post-partum. *Reprod Domest Anim*, 40, 460-467, 2005. DOI: 10.1111/j.1439-0531.2005.00601.x

33. Baitlesov EU, Nasibov NF, Khil'kevich SN, Titova VA, Tyapugin EA: Etiology and pathogenesis of ovarian cysts in cows and possibility of normalizing ovarian function. *Russian Agri Sci*, 33, 4, 260-263, 2007. DOI: 10.3103/S1068367407040155

34. Braw-Tal R, Pen S, Roth Z: Ovarian cysts in high-yielding dairy cows. *Theriogenology*, 72, 690-698, 2009. DOI: 10.1016/j.theriogenology.2009.04.027

35. Khan FA, Das GK, Pande M, Pathak MK, Sarkar M: Biochemical and hormonal composition of follicular cysts in water buffalo *(Bubalus bubalis)*. *Anim Reprod Sci*, 124, 61-64, 2001. DOI: 10.1016/j.anireprosci.2011.02.020

36. Vajdi S, Zamiri MJ, Nazifi S, Rowghani E: The relationship between physiological blood parameters and fertility in Holstein cows. *Iran Agri Res*, 21, 173-186, 2002.

37. Miettinen PVA: Metabolic balance and reproductive performance in Finnish dairy cows. *J Vet Med Series A*, 37, 417-424, 1991. DOI: 10.1111/j.1439-0442.1990.tb00923.x

38. Obese FY, Martin GB, Blackberry MA, Ayim-Akonor M, Gomda Y: Upgrading local cattle in tropical West Africa: Metabolic hormone concentrations during the post-partum period in Sanga and Friesian-Sanga crossbred cows. *Livest Sci*, 171, 84-92, 2015. DOI: 10.1016/j.livsci.2014.11.007

39. Zhao Hongcui, Zhao Yue, Tianjie Li, Min LI, Junsheng Li, Rong Li, Ping Liu, Yang Yu, Jie Qiao: Metabolism alteration in follicular niche: The nexus among intermediary metabolism, mitochondrial function, and classic polycystic ovary syndrome. *Free Radic Biol Med*, 86, 295-307, 2015. DOI: 10.1016/j.freeradbiomed.2015.05.013

40. Ghoneim IM, Waheed MM, El-Bahr SM, Alhaider AK, Al-Eknah MM: Comparison of some biochemical and hormonal constituents of oversized follicles and preovulatory follicles in camels *(Camelus dromedarius)*. *Theriogenology*, 79, 647-652, 2013. DOI: 10.1016/j.theriogenology.2012.11.019

41. Enjalbert F: Alimentation et reproduction chez les bovins. Journées nationales des GTV: la reproduction, Tours, France. Paris: *SNGTV*, 49-55, 1998.

42. Kaur H, Arora SP: Dietary effects on ruminant livestock reproduction with particular reference to protein. *Nutr Res Rev*, 8, 121-136, 1995. DOI: 10.1079/NRR19950009

43. El-Bahr SM, IM Ghoneim, MM Waheed: Biochemical and hormonal analysis of follicular fluid and serum of female dromedary camels *(Camelus dromedarius)* with different sized ovarian follicles. *Anim Reprod Sci*, 159, 98-103, 2015. DOI: 10.1016/j.anireprosci.2015.06.002

44. Dobson H, Smith RF: What is stress, and how does it affect reproduction? *Anim Reprod Sci*, 60-61, 743-752, 2000. DOI: 10.1016/S0378-4320(00)00080-4

45. Jackson RA, Wills JR, Kendall NR, Green MJ, Murray RD, Dobson H: Energy metabolites in pre- and post-partum dairy cattle as predictors of reproductive disorders. *Vet Rec*, 168, 562, 2011. DOI: 10.1136/vr.d1565