Characterization of ovarian follicular and cystic fluids in cows

Mimoune Nora[1, 2,*], Kaidi Rachid[2, 3], Guedioura Abdelmoumène[2, 4], Benaissa Mohamed Hocine[5], Azzouz Mohamed Yassine[2]

Abstract

The study was aimed at evaluating the hormonal, histological and metabolic changes associated with ovarian cyst (OC) formation in cows. For this purpose, , the fluid was aspirated from 195 ovarian cysts and 120 ovarian large follicles collected at a local abattoir in order to assess their hormonal concentrations and metabolic changes. Pieces of the cyst wall tissue were subjected to histologic evaluation. Data showed that the cysts' fluid was characterized by the lower concentrations of glucose, cholesterol, total protein and higher urea levels than those of the large follicles (P<0.001). However, insulin, creatinine, total bilirubin, GGT, AST, ALT and alkaline phosphatase concentrations were not significantly different between the both fluids. Large follicles and follicular cysts' fluids showed higher concentrations of estradiol than the luteal cysts. Conversely, higher levels of progesterone were observed in the luteal cysts' fluid. Follicular cysts and large follicles mostly had estradiol-to-progesterone (E/P) ratio > 1, whereas the luteal cysts all had E/P ratio < 1. It can be speculated that the abnormal levels of some biochemical and hormonal parameters may lead to follicle dysfunction resulting in cyst formation.

Keywords: ovary, cyst, biochemistry, hormone, cow

[1] High National Veterinary School (HNVS), Bab-Ezzouar, Algiers, Algeria
[2] Institute of Veterinary Sciences, LBRA, Saad Dahleb University, Blida, Algeria
[3] School of Veterinary Medicine and Science, University of Nottingham, Nottingham, Leicestershire, United Kingdom
[4] Houari Boumediene University, Bab-Ezzouar, Algiers, Algeria
[5] Scientific and Technical Research Center for Arid Areas, Biophysical Station, Touggourt, Algeria
***Corresponding author:** nora.mimoune@gmail.com

Introduction

Ovarian cysts (OC) are one of the major factors affecting the fertility of dairy cattle due to their negative effects on reproductive performances, causing great economic losses to the dairy industry (Baravalle et al., 2015). Ovarian cysts are defined as large follicular structures (≥ 25 mm) in diameter that are present for 10 days in the absence of a corpus luteum (Murayama et al., 2015). In the last decades, several studies on OC have been focusing on the clinical characteristics (Peter, 2004), etiology and pathogenesis (Vanholder et al., 2006), and diagnosis and treatment (Probo et al., 2011[b]). Most of these studies suggested that the development of ovarian cysts was associated with an endocrine imbalance in the hypothalamo-hypophyseal-gonadal axis (Braw-Tal et al., 2009). This imbalance, due to a multifactorial origin, involves genetic, phenotypic and environmental factors (Peter, 2004). However, the pathogenesis and mechanism of cyst formation are still not fully understood (Vanholder et al., 2006).

The presence of ovarian follicular fluid in several animal species shows its potential importance in ovarian physiology. The fluid composition reflects changes in granulosa and theca interna secretory function, and changes in plasma components as a result of physiological or pathological mechanisms susceptible to interfere with the ovarian functions notably steroidogenesis, follicle development and corpus luteum formation. Biochemical metabolites concentration in follicular fluid of the bovine ovary fluctuates considerably with the stage of estrous cycle, follicle size and follicle status (Kor et al., 2013). Therefore, the follicular fluid composition may serve as a useful marker for the pathogenesis of OC. The objective of the current study was to identify the biochemical and hormonal changes associated with OC in cows susceptible to be involved in the OC development and /or persistence.

Material and Methods

Collection of ovaries, tissue sampling and classification of follicles.

Ovaries with (n=195) or without (n=120) cysts were collected from different breeds of nonpregnant cows (Holstein Frisian, Montbeliard, and crossed breeds) at a local abattoir in Algiers and transferred to the laboratory on ice within 30 to 40 min from slaughter. The ovaries were collected only from the genital tracts with no gross morphological lesions.

The diameters of follicles were first measured using calipers, and then their fluid was individually aspirated and stored at −20 °C. The ovaries were cut into pieces, fixed in 10% buffered formalin for 6 h at 4 °C and washed in phosphate-buffered saline (PBS) in other to be used for histological examination. For comparison purposes,

pieces of the ovarian tissue with follicles as well as with cysts were collected and processed at the same time. Only follicles that appeared macroscopically and microscopically healthy (i.e., well vascularized and with a transparent follicular wall and fluid) and whose diameter was > 10 mm were used and classified as large follicles, as previously described (Leroy et al., 2004). Ovarian cysts were diagnosed when the follicles were bigger than 25 mm in diameter in the absence of a functional CL in either the right or left ovary (Murayama et al., 2015).

Histologic study

This study was conducted at the Laboratory of Pathological Anatomy of the National High School of Veterinary Medicine in Algeria. Pieces of cystic or follicular wall were dehydrated in an ascending series of ethanol concentrations, cleared in xylene, embedded in paraffin and cut into sections 5 µm thick. Sections were mounted and stained with hematoxylin-eosin (H&E).

Biochemical and hormonal analyses

Follicular and cystic fluids samples were assayed for glucose, cholesterol, total protein, triglycerides, total bilirubin, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT) and alkaline phosphatase via spectrophotometric methods using a clinical chemistry autoanalyser, Architect plus, ci 4100 (Architect c Systems, Abott Diagnostics, Germany). The intra- and inter-assay coefficients of variation for all analyses were below 5%.

Concentration of progesterone and estradiol 17-β were assessed by RIA (Radioimmunoassay) using commercial kits, Immunotech (A Beckman Coulter Company, France). The assay sensitivity was < 6 pg/mL for estradiol 17-β and 0.05 ng/mL for progesterone. The inter- and intra-assay coefficients of variation were: ≤ 12.1% and ≤ 11.2% (E2); ≤ 6.5% and ≤ 7.2% (P4), respectively. Histologic study and cystic fluid cut-off value of 100 ng/ml were considered in the classification of OC (Follicular cyst (FC): P4 ≤ 100 ng/mL; Luteal cyst (LC): P4 ≥ 100 ng/mL) (Braw-Tal et al., 2009).

Insulin and cortisol measurements were performed on Cobas e411 by electrochemiluminescence using a human commercial kit applied to cattle (Roche Diagnostics GmbH, Germany)(Díaz et al., 2015). The minimum detection limit was 0.5 nmol/l for cortisol and 0.2 µU/ml for insulin.

All measurements were carried out according to the manufacturer's instructions.

Statistical analyses

Statistical analysis was conducted using the STATISTICA software (Version 10, Stat Soft France, 2003). Statistical differences in the concentrations of biochemical and hormonal parameters between ovarian cysts and large follicles were compared using Student's t-test. Hormonal constituents were compared by analysis of variance followed by Tukey post hoc pairwise testing. Statistical significance was accepted at $P < 0.05$. Results were expressed as mean ± standard deviation (SD).

Results

Histologic study

Histological examination of OC was performed to describe the ovarian cystic walls and identify the different types of OC.

The photomicrographs taken of the slides prepared from the 195 OC collected samples, according to the studies of Braw-Tal *et al.* (2009), show the following observed changes: Granulosa cells of 115 OC had multiple layers, the basement membrane was present but interrupted in some places, and the cells of the theca interna were hypertrophied, losing their characteristic arrangement parallel to the basement membrane as seen

Table 1. Average concentrations (± SD) of different metabolic parameters in follicular and cystic fluids of cows

	Large follicle (n=78)	Ovarian cyst (n=78)	Reference range
Glucose (g/l)	0.44 ± 0.13	0.21 ± 0.13 ***	**0.4 - 0.7** (Leroy et al., 2004)
Insulin (µUI/ml)	0.8 ± 0.37	0.77 ± 0.41 ns	**0 – 5** (Kaneko et al., 1997)
Total protein (g/l)	71.19 ± 5.2	62.1 ± 5.81 ***	**64 – 87** (Maniwa et al., 2005)
TG (mmol/l)	0.15 ± 0.03	0.14 ± 0.04 ns	**0.14 – 0.25** (Leroy et al., 2004)
AST (U/l)	127.78 ± 23.5	123.79 ± 28.89 ns	**78 - 154** (Aller et al., 2013)
ALT (U/l)	46.28 ± 19.44	44.98 ± 30.75 ns	**11 – 85.50** (Tabatabaei et al., 2011)
GGT (U/l)	18.92 ± 4.32	20.09 ± 3.52 ns	**17 – 24.1** (Aller et al., 2013)
TBR (µmol/l)	3.44 ± 1.35	3.34 ± 1.41 ns	**0.17 – 8.55** (Iwata et al., 2006)
ALP (U/l)	129.96 ± 36.79	132.77 ± 39.04 ns	**109 – 177.33 (**Acar et al., 2013)
Cortisol (nmol/l)	27.35 ± 9.32	13.05 ± 7.04 ***	**33 – 55** (Spicer et Chamberlain, 1998)
Chol (mmol/l)	1.8 ± 5.19	0.62 ± 0.18 *	**1.04 – 1.93** (Leroy et al., 2004)
Urea (mmol/l)	4.1 ± 1.2	6.25 ± 0.6 ***	**4.13 – 6.05** (Leroy et al., 2004)
Creatinine (µmol/l)	117.74 ± 105.56	116.24 ns	**103 – 155 (**Acar et al., 2013)

TG: triglycerides, TBR: total bilirubin, ALP: Alkaline phosphatase, Chol: total cholesterol, AST: Aspartate Amino Transferase, ALT: Alanine Amino Transferase, GGT: Gamma Glutamyl Transpeptidase. P values: ns: not significant; ***: highly significant; *: significant.

in the large follicles. These cysts were classified as young cysts or follicular cysts (FC). In the 80 OC remaining, the granulosa cells were arranged in only one to two layers. The theca interna showed patches of luteal-like tissue, and the basement membrane was absent. These structures represented the luteal cysts (LC) (Figure 1).

Metabolic parameters concentrations in follicular cysts

The mean follicular fluid concentrations of the different biochemical constituents measured in the follicular cysts and large follicles are shown in Table 1. In order to interprete the results, the reported and/or established values of these metabolites in previous studies are also mentioned.

Follicular cysts had higher urea concentrations and lower glucose, total protein, total cholesterol and cortisol concentrations when compared to those of the large follicles. The difference, however, in insulin, creatinine, total bilirubin, GGT, AST, ALT, and alkaline phosphatase concentrations between the two groups was not significant (P>0.05).

Steroid hormonal concentrations in follicular cysts

The results of hormonal profile analyses in follicular fluid are shown in Table 2.

The fluid from the large follicles and follicular cysts showed higher concentrations of estradiol than that from the luteal cysts. Conversely, higher levels of progesterone were observed in cystic fluid from the luteal cysts than those from the follicular cysts and large follicles. Apart from 21 cysts and 33 large follicles, most of the follicular cysts and large follicles had estradiol-to-progesterone (E/P) ratio >1. The luteal cysts, however, all had E/P ratio < 1.

Table 2. Average concentrations (±SD) of steroidal hormones in fluid from large follicles and ovarian cysts

Ovarian structure	Estradiol 17 β (pg/ml)	Progesterone (ng/ml)
Large follicle (n=120)	10937.63 ± 87.5[a]	74.4 ± 8.3[a]
FC (n=115)	10690.66 ± 76.69[a]	89.32 ± 9.14[a]
LC (n=80)	105.32 ± 16.22[b]	139.26 ± 13.8[b]
P	< 0.001	< 0.001

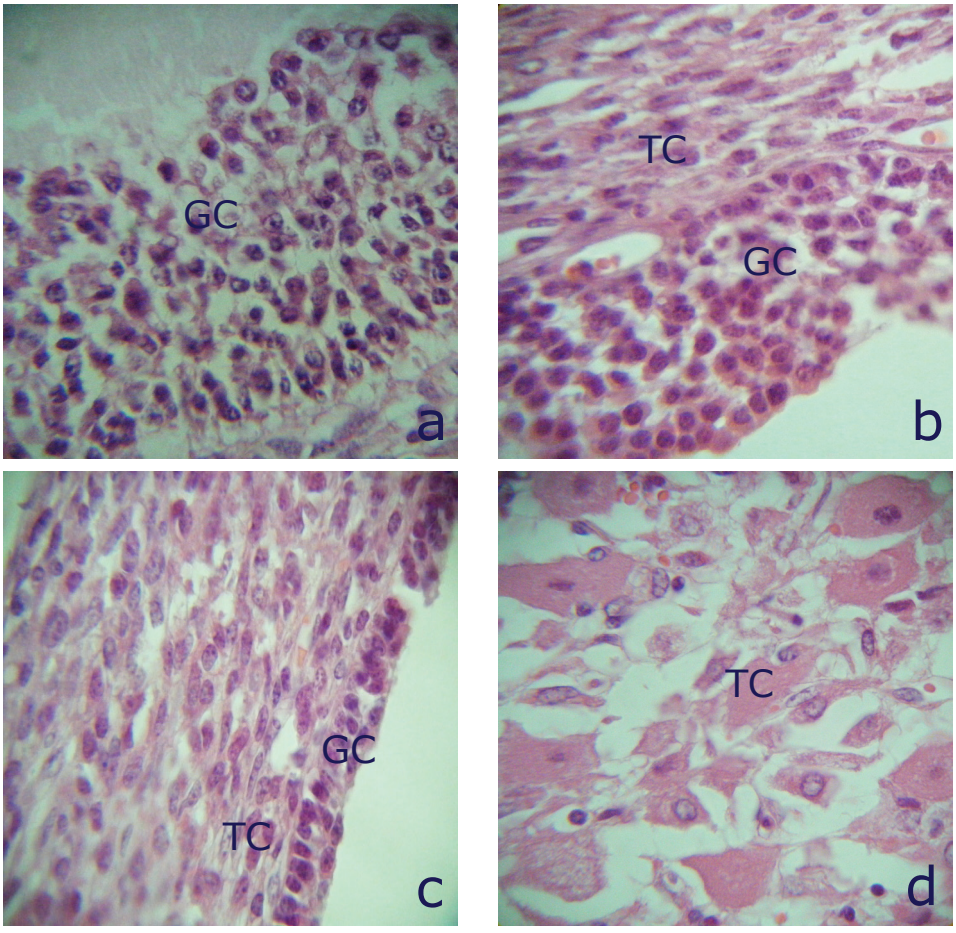

Fig. 1 Ovarian structures histology

Healthy follicle with several granulosa cell layers, (b) and (c) follicular cysts characterized by partial and gradual loss of granulosa cells and swollen theca interna cells, (d) luteal cyst showing patches of luteal-like tissue. GC: granulosa cells, TC: theca cells. Scale bar = 10 µm

Discussion and conclusions

The most accepted hypothesis for the formation of OC is the refractory status of the hypothalamus to the estrogen positive feedback, which causes ovulation to fail leading to the development of cysts. Indeed, several studies have shown that high levels of progesterone may be responsible for the occurrence of OC (Silvia et al., 2002). In the present study, FC fluid had, in some cases, estrogen/progesterone ratio <1, indicating functionally estrogen-inactive follicles (Khan et al, 2011[b]), which is in accordance with the histological study demonstrating the partial and gradual loss of granulosa cells, and indicating the start of the luteinization process. At an advanced stage (LC), granulosa cells progressively disappeared, estrogen levels decreased, while progesterone concentrations increased; this is also in agreement with the results of Braw-Tal et al. (2009) and Khan et al. (2011[a], 2011[b]). A possible explanation of the noticed progesterone abnormal increase may be the reduction of the aromatase activity, which is responsible for the conversion of progesterone to estrogen (Khan et al, 2011[a]).

Glucose, a dominant element of carbohydrates in follicular fluid, is the main source of energy for the ovary that can be metabolized through anaerobic pathway, leading to the formation of lactate (Leroy et al., 2004). Although the exact functional role of glucose in the follicular wall is not well understood, studies confirm its importance in the development, ovulation, formation and maintenance of the corpus luteum (Nishimoto et al., 2009). In the present study, glucose concentrations in the large follicles were significantly higher when compared to those of OC. Data were consistent with the previous studies in cattle (Braw-Tal et al., 2009), whereas in camels, Ghoneim et al. (2013) did not record similar results. The divergence may be inherent to the species, the nutritional status of the animal and especially the degree of post-mortem autolytic changes (Leroy et al., 2004). The low level of glucose in the cystic follicular fluid may reveal a metabolic stress that characterizes the postpartum period especially in high producing dairy cows, leading to the formation of OC rather than follicular atresia (although no pre-slaughter information was available for these animals) (Tabatabaei et al., 2011). The theca cell swelling observed in the histological examination is the most common expression of the cellular lesion. Such lesion can occur when the cells are unable to maintain normal homeostasis, mostly under the severe stress condition (Braw-Tal et al., 2009). Furthermore, hypoglycemia leads to the depression of the hypothalamic functions causing deterioration of ovarian activity, which prevents the release of gonadotropins (Rufai et al., 2013). Leroy et al. (2004) reported that glucose was strongly involved in the release of LH, reflecting its role as an important metabolic indicator in controlling GnRH activity.

Insulin and IGF-1 (Insulin- like Growth Factor 1) are involved in the regulation of ovarian function. While IGF-1 is important for follicular development, insulin, together with increasing estradiol , stimulates the final maturation of the dominant follicle leading to LH release and ovulation (Braw-tal et al., 2009). These hormones can also increase sensitivity to gonadotropins LH and FSH. Low concentrations of insulin and IGF1 noted in dairy cows probably prevent postpartum resumption of cyclicity leading to OC formation (Obese et al., 2015). Similar insulin levels in follicular and cystic fluid recorded in the present work suggest the active role of IGF-1 in this process, in accordance with the results of Hein et al. (2015) who found similar data and in previous studies, low levels of IGF-1 in cystic cows (Hein et al., 2015). However, some studies reported high levels of IGF-1 in OC (Probo et al, 2011[a]); while others have reported low levels of insulin and IGF1 in cystic fluid in dairy cows (Braw-Tal et al., 2009) and in buffaloes (Khan et al., 2011[a]).

Proteins are present in all living cells where they are intimately associated with the various phases of activity that constitutes the life of a cell. Imbalance in proteins can affect animal metabolism and energy status (Rufai et al., 2013). Follicular fluid contains a variety of proteins that play an important role in the growth, maturation and ovulation of the follicle (Sun et al., 2011). The level of these proteins is relatively uniform throughout all stages of the follicular development (Leroy et al., 2004; Rufai et al., 2013). In the present study, total protein concentrations in the ovarian cystic fluid were significantly lower than those of follicular fluid, although remaining generally within the reference range (Leroy et al., 2004; Tabatabaei et al., 2011; Acar et al., 2013). Data are consistent with those of Maniwa et al. (2005) in cattle, and differ from those of Khan et al. (2011[a]) in buffaloes. The difference can be attributed to the factors mentioned earlier. Since follicular fluid proteins originate from blood and secretions synthesized by the follicle, modification in protein levels in cystic fluid may reflect a change in the synthesis capacities, the metabolism or the structure of follicular walls, which may have a role in the pathogenesis of OC (Maniwa et al., 2005; Sun et al., 2011). These authors didn't report only the difference in total protein content but also the quality of these proteins. In cows, Maniwa et al. (2005) identified 08 additional proteins in the cystic fluid, while in sows, Sun et al. (2011) noted different levels of two other proteins. All these proteins are involved in the pathogenesis of OC (Maniwa et al., 2005; Sun et al., 2011).

Triglycerides are the storage form of lipids, and their hydrolysis yields a molecule of glycerol and three fatty acid molecules. Therefore, they could be an alternative energy source for follicular development (Leroy et al., 2004; Tabatabaei et al., 2011). Various studies have noted low levels of triglycerides in the antrum of the preovulatory follicles when compared to those of the small follicles, reflecting their rapid and continuous use by the former (Leroy et al., 2004). In the current study, similar TG concentrations were noted in follicular and cystic fluids, which is in accordance with data of Ali et al. (2010) in camels. The average concentrations mentioned were

similar to those reported in different studies of dairy cows (Leroy et al., 2004; Iwata et al., 2006; Tabatabaei et al., 2011), and remained below those reported by Acar et al. (2013) in buffaloes. The divergence may be related to the difference in breeding, age, nutrition and lipid mobilization rate of animals. Furthermore, buffaloes contain more serum triglycerides and cholesterol compared to cows and camels (Acar et al., 2013).

Cholesterol is the precursor of all steroid hormones including progesterone and estrogen in females (Kor et al., 2013). In follicular fluid, cholesterol binds with high density lipoprotein since only this fraction can pass through the blood-follicle barrier (Leroy et al., 2004). In the present study, low levels of cholesterol in the cystic fluid can be explained either by its steroid biotransformation (Kor et al., 2013) or by the influence of the low levels of glucose. In this context, Rabiee and Lean (2000) found that uptake of glucose and cholesterol by the ovary was highly correlated in sheep and cattle. Both of these metabolites are essential to ovarian function and may provide a mechanism, whereby negative energy balance can influence ovarian metabolism (Westwood et al., 2002). In addition, low levels of glucose and cholesterol in postpartum dairy cows are associated with the increase in calving-conception interval (Thillard et al., 2003). In vitro studies also demonstrated a blood cholesterol regulatory role in steroid production by the ovarian tissue. Despite all that has been reported, the mechanisms by which cholesterol may influence the fertility of dairy cattle are unclear (Westwood et al., 2002). In sheep, Rufai et al. (2013) found similar results between 02 groups of animals, cyclic and noncyclic. In camels, Ghoneim et al. (2013) reported no significant difference between concentrations of cholesterol in follicular and cystic fluid.

AST, ALT and GGT are serum enzymes present in the liver, involved in the degradation and transport of amino acids. They are sensitive indicators of liver cell injury (Tabatabaei et al., 2011). Measuring the liver enzymes activity (especially GGT) is required to interprete the values of biochemical parameters because the liver dysfunction can alter the concentrations of metabolic markers. In the current study, the concentrations of AST, ALT and GGT were unaffected by the follicle status, and the recorded values were within the reference ranges reported in different studies reflecting the integrity of liver function, confirmed also by normal concentrations of total bilirubin.

Alkaline phosphatase is a lysosomal enzyme, which is involved in the active transport of phosphates across the cell membrane and protein synthesis. It is a constituent of ovarian follicular fluid of humans, cattle, camels and pigs (Ghoneim et al., 2013). Alkaline phosphatase (ALP) is associated with prominent atresia (Wise, 1987), and is inversely correlated to the follicle size. The positive correlation between the activity of ALP and progesterone levels may indicate that ALP is a useful metabolic indicator of follicular atresia (Wise, 1987). The current study proved that the concentrations of alkaline phosphatase were similar in the follicular and cystic fluids, in agreement with data on camels (Ghoneim et al., 2013) and buffaloes (Khan et al., 2011[a]).

Ovarian cysts can be induced by exogenous administration of ACTH, assuming the association between stress and OC formation in cows, probably due to the disruptive action of the higher levels of cortisol on hormone synthesis (Khan et al., 2011a). In the present study, cortisol levels in cystic fluid were low compared to those in follicular fluid, which is in agreement with the previous studies carried out from the bovine plasma (Probo et al., 2011[a]), and the follicular fluid and the serum in camels (Ghoneim et al., 2013). In another study, however, high cortisol levels were found in cystic fluid of buffaloes (Khan et al., 2011[a]). Although stress can be one of the mechanisms involved in the pathogenesis of OC, a significant role of cortisol in the formation and/or persistence of OC was not proved or supported through the results obtained.

It is well recognized that urea is a very important indicator of the nitrogen nutritional status (Shehab El-Deen et al., 2010). In the current study, urea levels in cystic fluid exceed significantly those in follicular fluid, in agreement with the previous study (Yousefdoost et al., 2012). Due to the strong correlation between the blood and follicular fluid, elevated urea concentrations in plasma are reflected in the follicular fluid (Leroy et al., 2004). These high levels may be due to high crude protein diets (17-19%), which usually constitute the basic ration of high producing dairy cows (Shehab El-Deen et al., 2010). Nitrogen compounds excess is aggravated later by the energy deficiency explained by the lack of carbon substrates and energy for microbial protein synthesis (Gonzalez and Rocha, 1998). Low levels of glucose detected in the cystic fluid supported the result. In fact, the reports about the effect of high levels of urea on fertility are contradictory, although all authors agree that the possible adverse effect must act at the level of the oocyte (Leroy et al., 2004). Gonzalez and Rocha (1998) noted a very high uremia in cows that have more than 120 days postpartum. Thereafter, Jackson et al. (2011) reported that an excess of urea seemed to affect the ovary and uterus. Besides, cows with elevated urea and NEFA in the postpartum period are twice more likely to develop OC. In addition, Butler (2001) revealed that high amounts of urea resulted in increase in PGFα secretion from the endometrium, and decrease of LH binding to its ovarian receptors, which, in turn, results in low levels of P4 causing poor fertility.

The average concentrations of creatinine in follicular and cystic fluid did not differ significantly, and were consistent with the published data (Iwata et al., 2006; Tabatabaei et al., 2011; Acar et al., 2013).

In summary, some parameters of hormonal and metabolic profiles in cystic fluid differ from the control group. In this study, cystic fluid was characterized by low levels of glucose, total protein and cholesterol, and high concentrations of urea. Thus, we can speculate that the

ovarian cysts are not defined as a single disorder, but an association of several disorders around the same pathology.

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.

References

1. Acar D.B., Birdane M.K., Dogan N., Gurler H.(2013): Effect of the stage of estrous cycle on follicular population, oocyte yield and quality, and biochemical composition of serum and follicular fluid in Anatolian water buffalo. Anim Reprod Sci, 137: 8-14.
2. Ali A., Tharwat M., Al-Sobayil F.A.(2010): Hormonal, biochemical, and hematological profiles in female camels (Camelus dromedarius) affected with reproductive disorders. Anim Reprod Sci, 118: 372–6.
3. Aller J.F., Callejas S.S., Alberio R.H.(2013): 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.
4. Baravalle M.E., Stassi A.F., Vel_azquez M.M.L., Belotti E.M., Rodrıguez F.M., OrtegaH.H., Salvetti N.R.(2015): Altered Expression of Pro-inflammatory Cytokines in Ovarian Follicles of Cows with Cystic Ovarian Disease. J Comp Path, 153:116-130.
5. Braw-Tal R., Pen S., Roth Z.(2009): Ovarian cysts in high-yielding dairy cows. Theriogenology, 72:690–698.
6. Butler W.R.(2001): Nutritional effects on resumption of ovarian cyclicity and conception rate in postpartum dairy cows. In: Fertility in the high-producing dairy cow. BSAS Occasional Publication, 1:133–145.
7. Ghoneim I.M., Waheed M.M., El-Bahr S.M., Alhaider A.K., Al-Eknah M.M.(2013): Comparison of some biochemical and hormonal constituents of oversized follicles and preovulatory follicles in camels (Camelus dromedarius). Theriogenology, 79: 647–652.
8. Gonzalez F.H.D., Rocha D.(1998):Metabolic Profile Variations and Reproduction Performance in Holstein Cows of Different Milk Yields in Southern Brazil, Arq. Fac. Vet. UFRGS, Porto Alegre, 26 (1).
9. Hein G.J., Panzani C.G., Rodríguez F.M., Salvetti N.R., Díaz P.U., Gareisa N.C., Benítez G.A., Ortega H.H., Rey F.(2015): Impaired insulin signaling pathway in ovarian follicles of cows with cystic ovarian disease.Anim Reprod Sci, 156: 64–74.
10. Iwata H., Inoue J., Kimura K., Kuge T., Kuwayama T., Monji Y.(2006):Comparison between the characteristics of follicular fluid and the developmental competence of bovine oocytes. Anim Reprod Sci, 91: 215–223.
11. Jackson R.A., Wills J.R., Kendall N.R., Green M.J., Murray R.D., Dobson H.(2011): Energy metabolites in pre- and postpartum dairy cattle as predictors of reproductive disorders, Vet Rec, 168:562.
12. Kaneko J.J., Harvey J.W., Bruss M.L.(1997): Clinical biochemistry of domestic animals, 5th Edition. Academic Press, London, p.932.
13. Khan F.A., Das G.K., Pande M., Pathak M.K. and Sarkar M.(2011[a]): Biochemical and hormonal composition of follicular cysts in water buffalo (Bubalus bubalis). Anim Reprod Sci, 124: 61–64.
14. Khan F.A., Nabi S.U., Pande M., Das G.K., Sarkar M.(2011[b]):Bilateral follicular cysts in a water buffalo. Trop Anim Health Prod, 43:539–541.
15. Kor N.M., Khanghah K.M., Veisi A.(2013): Follicular Fluid Concentrations of Biochemical Metabolites and Trace Minerals in Relation to Ovarian Follicle Size in Dairy Cows. Annu Res Rev Biol, 3(4): 397-404.
16. Leroy J.L.M.R., Vanholder T., Delanghe J.R., Opsomer G., Van Soom A., Bols P.E.J., De Kruif A.(2004): 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.
17. Maniwa J., Izumi, S., Isobe, N., Terada, T.(2005) :Studies on substantially increased proteins in follicular fluid of bovine ovarian follicular cysts using 2-D PAGE and MALDI-TOF MS. Reprod Biol Endocrinol, 3:23.
18. Murayama C., Eiki Y., Akio M., Takashi S.(2015): Effect in dedicator of cytokinesis 6 (DOCK6) on steroid production in theca cells of follicular cysts. Biochem Biophys Res Commun, 462:415-419.
19. Nishimoto H., Hammano S., Hill G.A., Miyamoto A., Tetsuka M.(2009): Classification of Bovine Follicles Based on the Concentration of Steroids, Glucose and Lactate in Follicular Fluid and the Status of Accompanying Follicles. J Reprod Dev, 55: 219-224.
20. Obese F.Y., Martin G.B., Blackberry M.A., Ayim-Akonor M., Gomda Y. (2015): 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.
21. Peter A.T.(2004): An update on cystic ovarian degeneration in cattle. Reprod Domest Anim, 39: 1–7.
22. Probo M., Comin A., Cairoli F., Faustini M., Kindahl H., De Amicis I., Veronesi M.C.(2011[a]):Selected Metabolic and Hormonal Profiles during Maintenance of Spontaneous Ovarian Cysts in Dairy Cows, Reprod Dom Anim, 46, 448–454.
23. Probo M., Comin A., Mollo A., Cairoli F., Stradaioli G., Veronesi M.C.(2011[b]):Reproductive performance of dairy cows with luteal or follicular ovarian cysts after treatment with buserelin.Anim Reprod Sci,127: 135-139.
24. Rabiee A.R., Lean I.J.(2000): Uptake of glucose and cholesterol by the ovary of sheep and cattle and the influence of arterial LH concentrations.Anim Reprod Sci, 64: 199-209.
25. Rufai N., Razzaque W.A.A., Shah A.(2013): Biochemical Parameters of Follicular Fluid in Cyclic and Acyclic Sheep. VETSCAN, 121:7 (2).
26. Shehab-El-Deen M.A.M.M., Leroy J.L.M.R., Fadel M.S., Saleh S.Y.A., Maes D., Van Soom A.(2010): Biochemical changes in the follicular fluid of the dominant follicle of high producing dairy cows exposed to heat stress early post-partum. Anim Reprod Sci, 117: 189–200.
27. Silvia W.J., Hatler T.B., Nugent A.M., Laranja da Fonseca L.F.(2002): Ovarian follicular cysts in dairy cows: An abnormality in folliculogenesis. Domest Anim Endocrinol, 23: 167–177.

28. Spicer L.J.,Chamberlain C.S.(1998): Influence of Cortisol on Insulin- and Insulin-Like Growth Factor 1 (IGF-1)-Induced Steroid Production and on IGF-1 Receptors in Cultured Bovine Granulosa Cells and Thecal Cells. Endocrine,9 (2):153–161.
29. Sun Y.L., Ping Z.G., Li G.J., Sun Y.F., Yi K.L., Chen L., Li X.Y., Wang X.L. and Zhou X.(2011): Comparative Proteomic Analysis of Follicular Fluids from Normal and Cystic Follicles in Sows. Reprod Dom Anim.46: 889–895.
30. Tabatabaei S., Mamoei M., Aghaei A.(2011) :Dynamics of ovarian follicular fluid in cattle. Comp Clin Pathol, 20: 591-595.
31. Thillard E., Humblot P., Faye B.(2003) : Impact des déséquilibres énergétiques post-partum sur la fécondité des vaches laitières à la Réunion. In : Dixièmes rencontres autour des recherches sur les ruminants. INRA, Institut de l'élevage. Paris : Institut de l'élevage, pp. 127-130.
32. Vanholder T., Opsomer G., De Kruif A.(2006): Aetiology and pathogenesis of cystic ovarian folliclesin dairy cattle: a review. Reprod Nutr Dev, 46:105–119.
33. Westwood C.T., Lean I.J., Garvin J.K.(2002): Factors Influencing Fertility of Holstein Dairy Cows: A Multivariate Description. J Dairy Sci, 85(12):3225-37.
34. Wise T.(1987): Biochemical Analysis of Bovine Follicular Fluid: Albumin, Total Protein, Lysosomal Enzymes, Ions, Steroids and Ascorbic Acid Content in Relation to Follicular Size, Rank, Atresia Classification and Day of Estrous Cycle. J Anim Sci, 64:1153-1169.
35. Yousefdoost S., Samadi F., Moghaddam G., Hassani S., Jafari A.Y. (2012): A comparison of hormonal, metabolite and mineral profiles between Holstein cows with and without ovarian cysts.Int J Agr Sci,2(12): 1107-1115.

Karakteristika ovarijalnih folikula i cistične tečnosti kod krava

Cilj studije jeste procjena hormonalnih, histoloških i metaboličkih promjena povezanih sa nastankom ovarijalnih cisti krava (OC). U tu svrhu je iz 195 ovarijalnih cisti i 120 velikih ovarijalnih folikula prikupljenih u lokalnoj klaonici aspirirana tečnost kako bi se u njima analizirale koncentracije hormona i metaboličke promjene. Djelići tkiva zida cisti su histološki pregledani. Podaci su pokazali da tečnost iz cisti sadrži niske koncentracije glukoze, holesterola, ukupnih proteina, a povišene koncentracije uree u odnosu na tečnost iz velikih folikula ($P<0.001$). Međutim, koncentracije inzulina, kreatinina, ukupnog bilirubina, GGT, AST, ALT i alkalne fosfataze se nisu signifikantno razlikovale između dvije tečnosti.

Tečnost iz velikih folikula i folikularnih cisti je pokazala povišene koncentracije estradiola u odnosu na lutealne ciste. Obrnuto, povišene koncentracije progesterona su zabilježene u tečnosti iz lutealnih cisti. Omjer estradiol-progesteron (E/P) u folikularnim cistama i velikim folikulima je uglavnom bio >1, dok je u svim lutealnim cistama E/P omjer bio <1. Može se spekulirati kako patološke koncentracije nekih biohemijskih i hormonalnih parametara mogu biti uzrokom folikularne disfunkcije i rezultirati nastankom cisti.

Ključne riječi: ovarij, cista, biohemija, hormon, krava