

# Metabolic profiles of high-yielding dairy cows with ovarian cysts formation

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## Abstract:

**BACKGROUND:** Ovarian cysts are among the diseases which cause reproductive failure and economic losses in dairy herds. High yielding dairy cattle are susceptible to reproductive failure caused by ovarian cysts, as a result of their exposure to stressful conditions during lactation. **OBJECTIVES:** This study aimed to monitor metabolic profile tests in dairy cows with ovarian cysts in comparison with cycling cows. **METHODS:** Forty high-yielding Holstein dairy cows were enrolled for this study (20 cows with ovarian cysts and 20 cyclic cows). Seven weeks after parturition, 40 cows without retained placenta, including healthy ones were selected. Ovarian cysts were detected as follicular-like structures, >20 mm in diameter, persisting for at least 7 days, without corpus luteum and were monitored by ultrasound examination. Blood samples were collected from the jugular vein and delivered to the laboratory for measurement of calcium, magnesium, phosphorus, sodium, potassium, beta-hydroxy butyric acid, non esterified fatty acids (NEFA), glucose, cortisol, insulin and BUN. **RESULTS:** In this study, when serum calcium, phosphorus, sodium, potassium, magnesium and glucose of cows with ovarian cysts were compared with cows that had normal ovarian status, there was no significant difference. BUN, beta-hydroxy butyrate, NEFA and cortisol of cows with ovarian follicular cysts as compared to the cows with normal ovaries, showed a significant increase. Serum insulin values decreased significantly in ovarian follicular cystic cows than in cyclic cows. **CONCLUSIONS:** Increasing blood cortisol and decreasing blood insulin may play a major role in the formation of ovarian cyst and any detectable change in NEFA, BHBA and BUN.

## Introduction

Ovarian cysts are important disorders that lead to subfertility in dairy cattle, since they elongate the calving interval (Vanholder et al., 2006). Prolongation of the calving interval and its treatment costs results in economic

losses to the dairy farmers. Based on current knowledge and recent literature, ovarian cysts may be recognized as follicles with a diameter of at least 2 cm, which are present on one or both ovaries in the absence of any active luteal tissue and which clearly interferes with the normal ovarian cyclicity (Vanholder et al.,

2006). Macroscopically, cysts can be divided into follicular and luteal cysts, which are considered to be different forms of the same disorder (Opsomer et al., 1996). Luteal cysts are believed to be follicular cysts in later stages (Garverick, 1997). Measurement of progesterone levels in blood plasma, milk or milk fat can help in discriminating between the two types. Ultrasonography can be useful in supplying extra information. Follicular cysts have a thin wall (<3 mm) and the follicular fluid is uniformly anechogenic, while luteal cysts have a thicker wall (>3 mm), which is observable as an echogenic rim. Also, the latter often have echogenic spots and web-like structures in the follicular fluid (Jeffcoate and Ayliffe, 1995). Luteal cysts should not be confused with hollow corpora lutea, which are not pathological at all (Vanholder et al., 2006). Cystic ovarian follicles (COF) occur at different times during lactation. The incidence varies between 6 and 30% (Day, 1991a; Lopez-Diaz and Bosu, 1992; Laporte et al., 1994). The diagnosis of COF is mostly carried out during the first 60 days post partum (Day, 1991a; Vanholder et al., 2006), mainly because of the close monitoring of cow fertility during this period (Laporte et al., 1994). The self recovery percentage of these early cysts is 60–65% (Day, 1991b). Despite this high self-recovery rate, its importance in dairy cow fertility is significant (Lopez-Gatus et al., 2002). By delaying/interfering with ovarian cyclicity, COF increase the time for first insemination and the interval from parturition to conception. In addition, COF decreases the pregnancy rate after first insemination and increases the number of services per conception (Hooijer et al., 2001).

A genetic predisposition exists for COF, but the heritability is rather low, being 0.07 to 0.12. However, the rate of incidence in Dutch Holstein Friesian herds is on the increase (Vanholder et al., 2006). Genetic selection to reduce the incidence of COF can be successful, despite the low heritability (Vanholder et

al., 2006). The clinical signs that accompany ovarian cysts are variable. Anoestrus is most common, especially during the postpartum period. Irregular estrus intervals, nymphomania, relaxation of the broad pelvic ligaments and development of masculine physical traits are other signs which may be present, especially later during lactation (Vanholder et al., 2006).

The most widely accepted hypothesis describing the formation of a cyst is that LH release from the hypothalamus-pituitary is altered: the pre-ovulatory LH-surge is either absent, insufficient in magnitude or occurs at a wrong time during dominant follicle maturation, which leads to cyst formation (Lopez-Diaz and Bosu, 1992; Hamilton et al., 1995).

Hormonal or metabolic abnormalities seem to play an important role in the development of ovarian cysts in dairy cows. This could be related to the early post parturient energy balance, in which insulin-like growth factor 1 (IGF-I) can represent a nutritional and reproductive marker (Zulu et al., 2002; Cairoli et al., 2008). Therefore, the aim of the present study was to monitor metabolic profile tests in dairy cows with ovarian cysts in comparison with cycling cows.

## **Materials and Methods**

Forty high-yielding Holstein dairy cows that calved normally during spring were enrolled for this study (20 cows with ovarian cysts and 20 cyclic cows). The average 305-day milk yield of high-yielding Holstein dairy cows were 10600 kg, with 3.5% fat and 3.25% proteins. All the experiments were performed at a large dairy farm (with 1500 animals).

The average 305-day milk yield of the herd was 10800 kg milk with 3.5% fat and 3.25% protein. Cows were fed according to their requirements for maintenance and milk production. The ration consisted of high quality roughages (maize silage, grass silage and sugar beet pulp), soybean meal and concentrates.

Body condition scores (BCS) were recorded by the same experienced operator throughout the study at close up, day 1 post partum, and then once a week. Scoring was performed on a five-point scale (1 = thin, 5 = fat), with 0.25 increments as described by Edmondson et al. (1989). The occurrence of retention of foetal membranes and/or endometritis, identified as risk factors for ovarian cysts (Lopez-Gatius et al., 2002), were recorded per cow.

Cows in the control group were matched with cows in the treatment group (parity, milk production, stage of lactation and others).

Ovaries of all the cows were examined by linear array ultrasonography utilizing a 7.5 MHz rectal probe (PieMedical, Maastricht, The Netherlands) seven weeks after parturition. Twenty cows with ovarian cyst were defined as a follicular structure of 2.0 cm or more in diameter, which persisted for at least 7 days (that is, three or more scanning sessions) in the absence of a corpus luteum.

Twenty cyclic cows were detected by ultrasound examination as non-cystic control group synchronously. Ultrasound examinations were performed by the same operator throughout the study.

All blood samples were collected 3 h after morning feeding from the jugular vein in plain vacuum tubes and sent to the laboratory for measurement of calcium, magnesium, phosphorus, sodium, potassium, beta-hydroxy butyric acid, NEFA, glucose, cortisol, insulin and BUN.

The plasma cortisol levels were determined by a radioimmunoassay kit (Coat- A- Count [125I]; Diagnostic Products Cooperation, L.A., USA).

The following blood serum components were determined at the Clinical Pathology Laboratory, Department of Clinical Sciences, Veterinary Faculty, Shahrekord University, Iran: non esterified fatty acids (NEFA) were determined using an enzymatic method (NEFA C, Waco chemicals, Neuss, Germany) and also,  $\beta$ -hy-

droxybuturate (BHBA) was determined using an enzymatic method ( $\beta$ -Hydroxybuturate, LiquiColor, Stanbio laboratory, Boerne, TX, USA). Insulin concentrations in serum were analyzed using a commercially available ELISA, validated for cattle (Immuno-Biological Laboratories, Hamburg, Germany), according to the instructions of the manufacturer. Serum total calcium (S-tCa) was analyzed by colorimetric method using commercial kit (Darman Kave Company, Isfahan, Iran). Cortisol was determined by a solid-phase RIA (DPC, Los Angeles, CA, USA, presently SMSD.) according to the manufacturer's instruction. The method has earlier been evaluated for use in bovine samples (Bolanos et al., 1997) (detection limit of 14 nmol/l, intra-assay CV 2.2-6.3%). The content of inorganic phosphorus was determined photometrically using the commercial kit (Pars Azmoon Company, Tehran, Iran). The determination of magnesium levels in serum of cows was carried out using atomic absorption spectrophotometry (Model Unicam 939; Thermo Electron, Andover, USA).

The concentrations of sodium and potassium in serum were estimated using a flame photometer (Model PFP7; Jenway, Essex, UK) in 1:200 diluted samples using the procedures explained by Evans (1954).

Serum was analyzed for BUN using the procedure from Chaney and Marback (1962), with phenol nitroprusside (Sigma-Aldrich #/ A1727, St. Louis, MO) and alkaline hypochlorite solution (Sigma-Aldrich #6403) as the color reagents.

**Statistical analysis:** The data were analyzed using SPSS version 10. Paired sample t-test and  $p < 0.05$  was used to determine the statistical significance.

## Results

Mean value ( $\pm$ SE) for constituents measured in blood and BCS in the cystic and cyclic cows are shown in Table 1. Serum calcium, phos-

Table 1. Measurement of blood parameters and BCS (mean±SE) in cystic and cyclic ovarian cow. (\*) Values are significant at  $p < 0.05$ .

Item	Cystic ovarian cow	Cyclic cow
Glucose	61.33±1.87 mg/dL	59.55±2.33 mg/dL
Calcium	8.76±0.186 mg/dL	8.36±0.31 mg/dL
Magnesium	2.43±0.05 mg/dL	2.32±0.12 mg/dL
Phosphorus	4.14±0.178 mg/dL	4.04±0.17 mg/dL
Sodium	135.22±1.01 mEq/L	134.33±1.19 mEq/L
Potassium	4.5±0.06 mEq/L	4.52±0.04 mEq/L
BUN	19.66±0.57 mg/dL	16.88±0.42 mg/dL*
BHBA	0.88±0.04 mmol/L	0.55±0.03 mmol/L*
NEFA	0.71±0.04 mmol/L	0.43±0.07 mmol/L*
Cortisol	0.88±0.001 µ/dL	0.69±0.003 µ/dL*
Insulin	5.9±0.43 U/ml	8.98±0.51 U/ml*
BCS	3.91±0.02	3.33±0.08
Loss in BCS	0.83±0.03	0.66±0.08

phorus, sodium, potassium, magnesium and glucose of cows with ovarian cysts when compared with cows that had normal ovarian status, showed no significant difference ( $p < 0.05$ ). The serum levels of BUN, beta-hydroxy butyrate, NEFA and cortisol in cows with ovarian follicular cysts showed a significant increase compared to cows with normal ovaries ( $p < 0.05$ ). Also, serum insulin values decreased significantly in the ovarian follicular cyst, as compared to the cyclic cows ( $p < 0.05$ ).

## Discussion

High producing dairy cattle are in further risk for reproductive problems including ovarian cysts due to stressful condition. Cattle with ovarian cysts have higher blood cortisol ( $0.88 \pm 0.001 \mu\text{dL}$ ) levels than cyclic cattle ( $0.69 \pm 0.003 \mu\text{dL}$ ) and therefore stress works as a mediator through which endogenous cortisol is released that inhibit the preovulatory surge of LH. The present study indicates the major role of increasing blood cortisol in the early post parturient in high yielding dairy cattle. Cortisol (hydrocortisone) is a steroid hormone or glucocorticoid, produced by the zona fasciculata of the adrenal cortex (Cunningham and Klein, 2002). It is released in response to

stress, acting to restore homeostasis in high producing dairy cattle. Cortisol's primary functions are to increase blood sugar through gluconeogenesis; suppress the immune system; and aid in fat, protein and carbohydrate metabolism (Melvin, 2000; Cunningham and Klein, 2002). However, prolonged cortisol secretion results in significant physiological changes in dairy cattle such as ovarian cyst (Cairoli et al., 2008).

The inhibition of secretion of corticotropin-releasing hormone (CRH), results in feedback inhibition of adrenocorticotrophic hormone or corticotropin (ACTH) secretion (Thurston et al., 2003). Some researchers believe that this normal feedback system may become dysregulated when animals are exposed to chronic stress (Baravalle et al., 2007). This shuts down the reproductive system, resulting in an increased chance of miscarriage and in some cases, temporary infertility (Sunak et al., 2007).

In the present study, BUN concentration was higher in cattle with ovarian cysts ( $19.66 \pm 0.57 \text{ mg/dL}$ ) than in cyclic cattle ( $16.88 \pm 0.42 \text{ mg/dL}$ ) in relation to the increasing blood cortisol concentration.

The gluconeogenic action of cortisol promotes protein breakdown in muscle, skin

connective tissue and bones. Cortisol causes mobilization of amino acids from the extra hepatic tissue, mainly from the muscle. As a result, more amino acids become available in the plasma, which enter into the gluconeogenesis process of the liver and thereby, promote the formation of glucose and elevation of BUN. Cortisol also causes a moderate decrease in the rate of glucose utilization by most cells in the body (Cunningham and Klein, 2002; Kaneko et al., 2008).

In the same manner, cortisol promotes mobilization of fatty acids from muscle, it promotes the mobilization of fatty acids from adipose tissue that leads to increase in the concentration of free fatty acids in the plasma, and also increases the utilization of fatty acids for energy. Cortisol also expresses lipoprotein lipase activity in adipose tissue by transcription activation of the gene, as well as by posttranslational stabilization of the enzyme. Lipoprotein lipase is the rate limiting enzyme for triglyceride accumulation in adipose tissue (Cunningham and Klein, 2002). This action of cortisol resulted in an increase in BHBA ( $0.88 \pm 0.04$  mmol/L) and NEFA ( $0.71 \pm 0.04$  mmol/L) levels in the blood of cattle with ovarian cysts in the present study.

Insulin also acts as a liporegulatory hormone stimulating lipogenesis and inhibiting lipolysis by inhibition of hormone-sensitive lipase and stimulation of lipoprotein lipase (Coles, 1980; Cunningham and Klein, 2002). Vanholder et al. (2005) investigated the pathogenesis of cystic ovarian disease in high-yielding dairy cows postpartum. The results of their study suggested that ovarian cyst formation is associated with lower insulin levels but not with other distinct hormonal and metabolic alterations (Vanholder et al., 2005). In the present study, it was observed that the serum concentration of insulin in ovarian cystic cows was lower ( $5.9 \pm 0.43$  U/ml) than that in cyclic cows ( $8.98 \pm 0.51$  U/ml). The decrease in insulin may play a major role in ovarian cysts formation as well as

metabolic and hormonal changes in cattle. The activity of hormone-sensitive lipase increases the low concentration of insulin in the ovarian cystic cows. This action results to increase in NEFA concentration in cattle with ovarian cysts. Therefore, free fatty acids produced from the mobilization of fat are transported to the liver and oxidized to produce acetyl-CoA and NADH. Acetyl-CoA may be oxidized via the TCA cycle or metabolized to acetoacetyl CoA. Its oxidation via the TCA cycle depends upon adequate supply of oxaloacetate from the precursor propionate (Melvin, 2000; Cunningham and Klein, 2002). If propionate and consequently oxaloacetate, is deficient, oxidation of acetyl-CoA via the TCA cycle is limited and it is metabolized to acetoacetyl CoA and subsequently to acetoacetate and BHBA (Melvin, 2000; Cunningham and Klein, 2002; Kaneko et al., 2008). Leory et al. (2004) studied the concentration and composition of non-esterified fatty acids (NEFA) in the follicular fluid (FF) of high-yielding dairy cows, which were determined during the period of negative energy balance (NEB) early post partum. They suggested that NEB may hamper the fertility of high-yielding dairy cows through increased NEFA concentrations in FF, thereby, affecting the oocyte's quality.

A decrease in insulin level may be one of the major determinants of the initial increase in proteolysis during ovarian cyst formation. Consequently, resulting in the elevation of BUN in cattle with ovarian cysts.

Beam and Butler (1998) noticed that the nadir of the NEB occurred later post partum in cystic cows than in ovulatory cows. Moreover, cystic cows even mobilized less body reserves and derived a smaller percentage of their milk yield from body weight loss (Beam and Butler, 1997).

Cows that develop cysts have higher serum NEFA concentrations during the first week(s) post partum than ovulatory cows (Huszenicza et al., 1988; Zulu et al., 2002), although Beam

and Butler (1998) were unable to observe this. Interestingly, in rats, elevated NEFA concentrations for 48 h can decrease insulin secretion by the  $\beta$ -cells of the pancreatic islets in response to a glucose challenge (Mason et al., 1999). Moreover, NEFA are cytotoxic to several cell types, including bovine granulosa and theca cells (Vanholder et al., 2006). So, prolonged exposure to high NEFA concentrations during periods of NEB may hamper follicle growth and development; thereby disrupting the complex endocrine system and promoting the formation of ovarian cysts. Although, elevated serum ketone concentrations increase the risk of delayed cyclicity and cyst occurrence post partum, they do not exert any negative effects on bovine follicle cells in vitro (Huszenicza et al., 1988; Opsomer et al., 2000). Consequently, ketone concentrations in the postpartum dairy cow seems to be an indicator of the severity of the NEB, but not a mediator of the negative effects of the NEB on reproduction at the ovarian level (Vanholder et al., 2006).

In this study, serum levels of calcium, phosphorus, sodium, potassium, magnesium and glucose of the COF group as compared to control group showed no significant difference. So, it can be said that, the ration consist of any mineral requirements for maintenance and milk production in the present study. Most nutrients are homeostatically regulated; therefore, their value in profile testing for monitoring and assessment of nutritional status is limited. However, sampling cows when they are metabolically stressed, e.g., just prior to and following calving, could potentially result in identifying cows that are more prone to metabolic disease problems (Beam and Butler, 1997).

**Conclusion:** COF are one of the most frequent and important ovarian disorders in modern high yielding dairy cows, which have been the subject of most researches in recent decades. However, many aspects of the disease, and especially pathogenesis, remain unclear. In particular, the endocrine and follicular chang-

es that precede spontaneous cyst formation are still unknown, mainly due to the heterogeneity and unpredictability of the disease. The present study indicated that the pathogenesis of ovarian cysts is not related to the increase in blood NEFA, BHBA and BUN. It can be suggested that increasing the blood cortisol and decreasing blood insulin may play a major role in the formation of ovarian cyst and any detectable changes in NEFA, BHBA and BUN.

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## متابولیک پروفایل گاوهای پر تولید مبتلا به کیست‌های تخمدانی

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### چکیده

**زمینه مطالعه:** کیست‌های تخمدانی یکی از مهمترین بیماری‌هایی است که منجر به اختلالات تولید مثلی و ضررهای اقتصادی فراوان در گاوهای شیری می‌شود. گاوهای پر تولید به دلیل اینکه در طول شیردهی در معرض استرس‌های گوناگون قرار می‌گیرند بیشتر مستعد به کیست‌های تخمدانی می‌شوند. **هدف:** ارزیابی تست متابولیک پروفایل در گاوهای پر تولید مبتلا به کیست‌های تخمدانی. **روش کار:** دو گروه ۲۰ رأسی گاوهای فاقد کیست و گاوهای مبتلا به کیست تخمدانی انتخاب گردید. ۷ هفته پس از زایمان طبیعی، تخمدان گاوهایی که فاقد جفت ماندگی بودند با استفاده از دستگاه سونوگرافی بررسی گردید. گاوهایی که اندازه ساختار فولیکولی موجود در تخمدان‌هایشان بیشتر از ۲۰mm بود و حداقل ۷ روز این ساختار بدون حضور جسم زرد باقی مانده بود را به عنوان گاوهای مبتلا به کیست تخمدانی و گاوهایی که فولیکول کمتر از ۲۰mm داشتند را به عنوان گاوهای فاقد کیست‌های تخمدانی طبقه بندی شدند. سپس نمونه خون از ورید و داج آنها اخذ شد و پارامترهای معمولی که در تست متابولیک پروفایل صورت می‌گیرد (کلسیم، منیزیم، فسفر، سدیم، پتاسیم، بتا هیدروکسی بوتیریک اسید، اسیدهای چرب غیر استریفیه، انسولین، کورتیزول، گلوکز خون، نیتروژن اوره خون) اندازه‌گیری گردید. **نتایج:** میزان کلسیم، فسفر، سدیم، پتاسیم، منیزیم و گلوکز در سرم گاوهای مبتلا به کیست تخمدانی در مقایسه با گاوهایی که وضعیت تخمدانی طبیعی داشتند تفاوت معنی‌داری را نشان نداد. میزان ازت اوره ی خون، بتا هیدروکسی بوتیرات، کورتیزول و اسیدهای چرب غیر استریفیه در سرم گاوهای مبتلا به کیست فولیکولی تخمدان در مقایسه با گاوهای با تخمدان طبیعی افزایش معنی‌داری را نشان داد. میزان انسولین سرم خون در گاوهای مبتلا به کیست‌های تخمدانی بطور معنی‌داری کمتر از گاوهای سالم بود. **نتیجه‌گیری نهایی:** افزایش کورتیزول و کاهش انسولین سرم خون مهمترین نقش را در بروز کیست‌های تخمدانی داشته که متعاقباً میزان ازت اوره ی خون، بتا هیدروکسی بوتیرات و اسیدهای چرب غیر استریفیه در سرم تغییر می‌یابد.

**واژه‌های کلیدی:** گاو شیری، متابولیک پروفایل، کیست تخمدانی