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**A Comparative Study of the Levels of Heat Shock Protein 70,
Some Biochemical and Hormonal in the Blood of Cows Suffering
from Follicular Cystic Ovarian and Endometritis**

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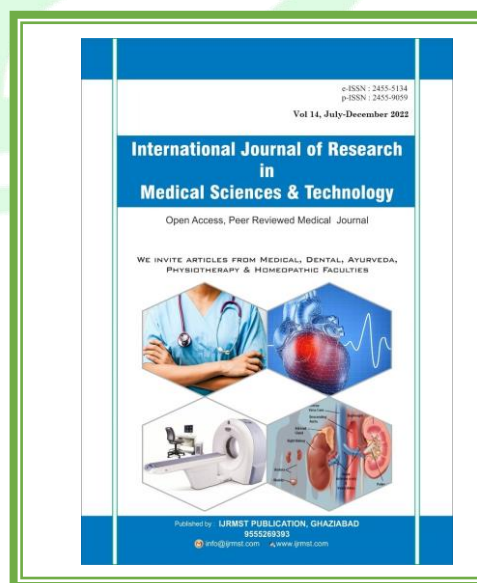
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ABSTRACT

Twenty- four reproductive systems of Frisian cross breed cows, aged between (3-5years) were obtained from Basrah abattoir during the period of (2nd March 2020 - 30th June, 2020). They were divided into 3 groups of cows with ovarian follicular cystic (n=8), cows with endometritis (n = 8), and healthy cows (n=8) depending on clinical signs. The main goal of this paper compare and report the different values of heat shock protein 70 (HSP70), some biochemical and hormonal levels in those cows in the native Basrah climate. Samples of blood were collected from all cows subjected to this study and serum was harvested from each individual sample by centrifugation. Heat shock protein 70 (Hsp70), total protein (TP), albumin(Alb), globulin, total cholesterol (Cho), triglyceride (TG), low density lipo-protein(LDL), aspartate aminotransferase (AST), cortisol, testosterone, thyroid stimulation hormone (TSH), thyroxin (T4) and triiodothyronine hormones(T3) were measured in serum samples. Results revealed a significant increase ($p \leq 0.05$) in the level of Hsp70, and thyroid stimulation hormones (TSH) of cows with two pathological cases (endometritis and follicular cysts) compared with those healthy cow. Significant decrease ($p \leq 0.05$) in the measurement of total protein, albumin, globulin, thyroxin (T4), triiodothyronine (T3) hormones, cholesterol, triglyceride, and low density lipoprotein (LDL) in cows having those two genital diseases compared with normal cows. The results also were revealed a significant increase ($p \leq 0.05$) in the level of testosterone hormone in cows with follicular cystic ovarian case compared with endometritis case and control ones. Finally, significant increase ($p \leq 0.05$) in cortisol hormone level and aspartate aminotransferase (AST) activity in cows with endometritis case compared with the control one and follicular cystic ovarian cases.

Keywords: *cystic ovaries, endometritis, Hsp70, hormones, Cows.*

INTRODUCTION

Several types of reproductive disorder are found to be the chief causes, of infertility in cattle and other animals which may alter their health and decrease their production which can cause high deficiencies in both meat and milk production all over the world. Endometritis and ovarian follicular

cyst are considered the most causative cases of fertility in dairy cows breed (Mulligan & Doherty, 2008; Williams, 2013) respectively. This is mainly due to endocrine disturbance more likely after abnormal delivery or dystocia (Gorden, 1996; Gautam et al., 2009). Several researchers found that hormonal

disturbances and abnormal metabolic cases are appeared to be the main causes to develop postpartum endometritis (Magnus & Lali, 2009), and ovarian follicular cyst especially in high milk producing cows (Cairolì et al., 2008). Ovarian follicular cysts are formed when there is one or more unrupture ovarian follicles, ova is not produced and there is follicular regression but the follicle continue to grow and secrete estrogen which may lead to continuous estrus behavior without ovulation which can be called nymphomania (Vanholder et al., 2006). Same authors classify cyst in the ovary as ovarian follicular cysts to more than 17 (Vanholder et al., 2006). Others considered that the cysts must be 2.5 cm in diameter or more when present in the ovary for longer period of time with no corpus luteum formation (Youngquist & Threlfall, 2007), this state is definitely disrupting the normal ovarian cycle.

Previously, López-Gaitus et al., (2002); Sheldon et al., (2006) declared that the post parturient problems can be a main factor for cystic ovarian formation and endometritis, referring widely that problems may be difficulty in delivery due to twining or because of placenta retention, metritis and certain metabolic disorders such as ketosis or it may be due to many

above conditions at the same time. Cows which had the above mentioned past parturition problems were process two times probability to have cystic ovaries than cows without those post parturient problems, formation of late ovarian cyst is considered of less risky than earlier cyst formation, also researchers found that high milk producer cows have greater ability to develop cystic ovaries especially older cows which have higher ability to develop chronic follicular cystic formation than newly lactating cows. Simensen et al., (2010) described other factors can be associated with ovarian cyst development in dairy cattle, such as nutrition, feeding management and housing type. Cow with low immunity against microbial infection, so establishment of endometritis, pathogenic bacteria can easily be propagated (Sheldon et al., 2009). The clinical endometritis is the most common periparturient disease, accounting for approximately 15% of the total disease prevalence (Ribeiro et al., 2013), and diagnosed by the presence of vaginal discharge (purulent or mucopurulent), 21 days or more after parturition, and it is not manifested by any systemic sign (LeBlanc et al., 2002).

MATERIALS & METHODS

Twenty-four complete cows reproductive tract were gathered during the period of 2nd March 2020 -30th June 2020 from Frisian cross breed cows (Janubi ×Frisian), aged between (3-5years) at Basrah abattoir. Eight of those systems were of cows found to have endometritis, the others eight reproductive tracts were gathered from cows having follicular cystic ovaries and the last eight of genital tracts were collected from cows not suffering from any of above cases. They were brought by a cool box contained ice and physiological saline. Those reproductive tracts were brought to the Physiological laboratory of College of Veterinary Medicine at Basrah University. Information's was recorded after clear gross examinations and isolation of specimens having endometritis symptoms and kept a part from samples having follicular cystic ovaries and a part also from normal samples, each sample was given number and blood sample of the same animal was collected from jugular vein by vacuum tube of (5ml) size before slaughtering. Serum of each sample was harvested by centrifugation of blood samples at (4000rpm) for (10) minutes. The collected serum samples were kept in Eppendorf tubes and labeled with same number of blood samples and kept at -20 °C till analysis.

SERUM BIOCHEMICAL ANALYSIS

The serum level of bovine heat shock protein70 (Hsp70) was determined by using an enzyme –linked immunosorbent assay (ELISA) reagent kit manufactured by Shanghi Korain Biotech company.

Analysis of serum aspartate aminotransferase (AST) Activity was done by (Bergmeyer, 1977).

The concentration of serum total protein (TP) values in blood serum was determined according to Biuret method (Goa, 1953), while serum albumin (Alb) values were estimated by (Doumas *et al.*,1971), and serum globulin values were done by (Maff, 1984) .

Serum total cholesterol (Cho) values were analyzed as per modified method of (McGowan, 1983). Serum triglycerides (TG) values were analyzed by reagent kit was developed by (Allain *et al.*, 1974). The serum low density lipoprotein (LDL) values were estimated per (Kanchana & Jeyanthi, 2010).

HORMONAL ASSAY

Cortisol hormone concentration measurement was performed by using cortisol enzyme immunoassay test kit catalog number: MonobindInc. akeforest, CA 92630, USA. product code 3625-300.

Testosterone hormone concentration measurement by using testosterone hormone enzyme immunoassay test kit catalog number: MonobindInc. Lakeforest, CA 29630, USA. product code 3725-300 Using ELISA reader, and ELISA washer.

Thyroxin hormone(T4), triiodothyronine(T3) and thyroid stimulation hormones (TSH) concentrations were estimated in private laboratories Bayan group for advance laboratories diagnosis at Basrah Governorate.

STATISTICAL ANALYSIS

Data was analyzed by using SPSS (Statistical Program for Social Sciences) program version 22.0 and presented as mean \pm standard deviation (Abo-Allam, 2003)

One way ANOVA was used to compare between mean of more variable.

RESULTS AND DISCUSSION

Data listed in (table 1) show clear significant differences in serum heat shock protein levels of both cows suffering from follicular cystic ovarian and endometritis when compared with healthy cows, but high significant ($p \leq 0.05$) recorded in cow suffering from follicular ovarian cyst than endometritis and healthy ones. This result agreed with results of study by (Wu *et al.*, 2019) clarified that the high significant level of Hsp70 in ovarian cyst than the level of

Hsp70 in endometritis cows may be related to increase testosterone level and inflammatory factors (Narayansingh *et al.*, 2004). Rostamtabar *et al.*, (2021) indicated that Hsp70 may be played an important role in the pathogenesis of PCOS. Heat shock proteins (HSPs) are a group of highly conserved protein molecules which can be produced by all cells under physiological and pathological conditions including high temperature, hypoxia, virus infection or stress (Tsan & Gao, 2004). Heat shock protein 70 (HSP70) is the most conserved protein, which has a variety of biological functions including molecular chaperone, regulation of immune response, anti-apoptosis and improvement of cell tolerance to stressors (Mayer, 2013). The elevated level of Hsp70 is associated with the combination of Infrared Radiation, oxidative stress and low-grade chronic inflammation in PCOS individual (Gao *et al.*, 2013), but un inconsistent with result of study by (Kohno *et al.*, 2007) which explained that testosterone can induce the inhibition of heat shock protein 72 expression or heat shock protein factor (HSF1) activation, which may directly or indirectly inhibit HSF1 activation then leading to the reduced expression of HSP70. While, Kristensen *et al.*, (2004) recorded high significant level of Hsp70 in cows, which may be produced under non stress. The expression level of Hsp70 in cattle is

playing very important role in an increased autoimmunity (Mishra *et al.*, 2011).

According to the data presented in (table 1) the both pathological cases showed significant decrease ($p \leq 0.05$) in serum total protein, albumin, and globulin concentrations than the control ones. This study was in agreement with those found by (Allam *et al.*, 2019) in cystic ovaries cows, and with previous result was recorded in cow with retained placenta (Semacan & Sevinc, 2005). Other study justified this deficiency may be related with decrease of certain amino acids needed for the biosynthesis of gonadal hormones and gonadotropins and consequence disturbances of reproductive hormone leading to repeat breeding (Pariza *et al.*, 2013). Some reports showed that there linkages between inflammation, altered gene expression in liver and adipose tissues, subclinical endometritis and reproductive performance (Akbar *et al.*, 2014). Serum albumin is a negative acute phase protein which decreases during acute inflammations (Krause *et al.*, 2014). Other results observed that subclinical endometritis of cows had a higher average percentage of PMN cell count in the uterine lumen, which was associated with a reduced hepatic synthesis of albumin.

AST serum activity is a very good indicator of hepatic diseases (Sevinç *et al.*, 2002). The significant increase of AST in endometritis cases is a good signal to liver

dysfunction in this pathological cases when compared with follicular cystic ovaries and healthy control cows which may be due to the accumulation of adipose materials in the liver cells, this result is like with that found by (Sevinç *et al.*, 2001) who documented that the abnormal liver function and increased serum liver enzyme have high correlation with fatty liver. Also, according to what noted by (Sweeney *et al.*, 1988) that endometritis may be resulted after infectious diseases which causes cause damage and necrosis of the liver of various degrees of hepatic dysfunction which led to increase in liberation of AST in blood. Cows with endometritis and PCOS exhibit high somatic cell count, indicating that some bacterial components are transferred between organs (Purba *et al.*, 2021).

Statistically significant differences of cholesterol, triglyceride, and LDL levels in serum cows suffering from endometritis, follicular cystic ovaries compared to healthy cows, those significant agreed with those finding of other authors (Semacan & Sevinç, 2005; Sevinç *et al.*, 2002) respectively, who recorded a decline in serum cholesterol level in diseased cows compared with the healthy cows manifested with loss an appetite. The low level of cholesterol in both diseased cows could result from several mechanisms including increase conversion of cholesterol to steroid or might be due to decrease in

serum glucose levels (Kore *et al.*, 2013). Similar result was recorded by (Thillard *et al.*, 2003) who documented decrease level of cholesterol in postpartum dairy cows correlation with the increase in calving-conception interval. Other authors mentioned

that low level of LDL in cows with retained placenta could result from several mechanisms including a decrease in the conversion of VLDL to LDL and may be due to enhanced in the LDL catabolism.

Table (1) shows serum levels of Hsp70, Aspartate aminotransferase, Total protein, Albumin, Globulin, Cholesterol, Triglyceride, Low density lipoprotein and High density lipoprotein of cows infected with clinical endometritis, follicular cystic ovaries and healthy cows (Mean \pm SD).

Parameters	Cystic ovaries	Endometritis	Control
HSP70(ng/dl)	36.71 \pm 0.4 a	35.85 \pm 0.8 a	20.83 \pm 1.2 b
TP(g/dl)	6.3 \pm 0.3 b	6.4 \pm 0.5 b	8.2 \pm 0.8 a
Alb(g/dl)	3.1 \pm 0.1 b	3.1 \pm 0.3 b	4.5 \pm 0.4 a
Globulin(g/dl)	3.4 \pm 0.4 b	3.3 \pm 0.4 b	4.8 \pm 0.2 a
Cho(mg/dl)	96 \pm 0.5 b	98 \pm 1.7 b	183 \pm 1.3 a
TG(mg/dl)	33 \pm 1.2 b	35 \pm 0.3 b	45 \pm 2.1 a
LDL(mg/dl)	23 \pm 1.1 b	19 \pm 1.6 b	37 \pm 0.8 a
AST(U/I)	116 \pm 1.2 b	134 \pm 3.1 a	93 \pm 1.9 c

Values express as (Mean \pm SD), n=24 cows, with different superscripts (a, b and c)in the column differ significantly ($p \leq 0.05$).

Referring to the data presented in (table 2) follicular cystic ovarian cows showed significant decrease in serum cortisol level as compared to endometritis and healthy ones, this result agreed with what found by (Cairolì *et al.*, 2008; Ghoneim *et al.*, 2013), in dairy cow and female camels respectively, and also agreed with results found by (Hazeldine *et al.*, 2010) who stated that the cortisol is inhibited by steroid DHEA which is regarded as anti- glucocorticoid which is a competitive inhibitors of cortisol, but these results are not consistent with what reported by Khan *et al.*, (2011) in buffaloes. Other researchers mentioned that cystic ovaries has an active role of cortisol which is able to inhibit LH secretion from pituitary gland, which has an influence on estrogen synthesis (Kawate *et al.*, 1993). A high level of serum cortisol in endometritis cows compared the others cows may be due to increase uterine bacterial infection. Similar results was recorded by Shalaby, (1997) who found an enhanced serum cortisol concentration in endometritis cows compared with healthy cows, but in case of testosterone it is on the contrary to that found for cortisol because the recorded testosterone concentration is found to be decreased significantly in

blood serum of cows suffering from endometritis than the cows suffering from cystic ovaries and this result coincided with that found by Kesler *et al.*, (1979) who recorded that the increased testosterone in cystic ovary cows leads to masculinization and most cystic ovary cows remain in anestrus as long as the condition persist. Serum level assays of TSH hormone showed high levels in both pathological cases than the healthy ones, while serum levels of T3 and T4 hormones showed low levels in both pathological cases than the healthy ones, this is probably due to the insulin resistant and physiological stress (Holtorf, 2014). Other researchers pointed out that the reason for a decrease in the level of these hormones in blood serum, which could be related to a lack of synthesis in the thyroid gland follicle cell because of the low iodine concentration level which has an effect on thyroid gland discharge, which led to an influence on ovarian size (Bartolome *et al.*, 2005; Djul'ger & Nezhdanov, 2006). The increase in the ovarian size may be related to hypofunction of thyroid gland and this may have a relationship to the elevated the testosterone and TSH hormones which are correlated to the hypothyroidism (Ghosh *et al.*, 1993). On other hand, Wilson &

Gorewit, (1980) indicated that the decline in serum level assay of T3 and T4 in endometritis cases may be related to enhance in the number of their receptors in the mammary glands.

Table (2) shows serum levels of Cortisol, Testosterone, Thyroxin, Triiodothyronine, Thyroid stimulation hormones, in cows infected with clinical endometritis, follicular cystic ovaries and healthy cows (Mean ±SD).

Parameters	Cystic ovaries	Endometritis	Control
<i>Cortisol</i> (nmol/l)	9.43±2.81 c	16.08±2.27 a	13.66±1. b
<i>Testosterone</i> (ng/ml)	1.97±0.04 a	1.06±0.15 b	0.83±0.11 c
<i>TSH</i> (ng/ml)	2.5±0.12 a	2.4±0.10 a	0.88±0.16 c
<i>T3</i> (ng/ml)	0.40±0.06 b	0.42±0.02 b	0.96±0.09 a
<i>T4</i> (ng/ml)	2.2±0.02 b	2.9±0.02 b	4.3±0.1 a

Values express as (Mean ±SD), n=24 cows, with different superscripts (a, b and c) in the column differ significantly (p≤0.05).

CONCLUSION

In conclusion, both disease cases are considered an influencing factors on fertility and lives of animals because they have significant influence on all blood biochemical parameters, and hormones compared with controls one. We attitude the two cases caused dysfunction and disturbance in most organs especially in endocrine system.

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