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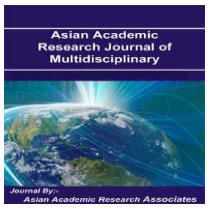


# EXPRESSION LEVEL OF FSH-R GENE IN NORMAL DOMINANT AND CYSTIC OVARIAN FOLLICLES OF IRAQI LOCAL BREED COWS

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## Abstract

The present study was carried out to investigate the possible etiology and pathology of cystic ovarian follicles by determining the expression levels of mRNA for FSH-r gene in follicular cells of dominant follicles in comparison with cystic ovarian follicles in Iraqi cows. Forty four ovaries were collected from sexually mature cows (4-6) years with unknown reproductive status, during the period extended from December 2011 to October 2012, this study were performed in two steps, firstly aspiration of follicular fluids from dominant follicles and cystic follicles and stored in (-20℃) until estradiol and progesterone assay, secondary section of the follicular wall in to two hemispheres, and stored at -70℃ to -80 ℃ to molecular study. Macroscopic examination of the ovaries revealed that the numbers of dominant follicles are (23) samples while cystic follicles were in (21) samples. There was a significant difference ($P<0.05$) in the diameter of cystic ovarian follicles (37.56 ± 0.64 mm) compared with dominant follicles (19.93 ± 0.32 mm). Results of hormonal assay showed higher estradiol-17β (865.96±10.64 ng/ml) and progesterone (84.8±1.35 ng/ml) concentrations in follicular fluids of cystic ovarian follicles, which were significantly higher ($P<0.05$) in comparison with those of dominant follicles which were (314.39±2.55 ng/ml )and (50.25±1.57 ng/ml) respectively. Molecular study, to evaluate the relative quantification of FSH-r gene in dominant and cystic follicular cells, has been done by extraction of the total RNA and assay its concentration from these cells, synthesis data of the complementary DNA (cDNA), that done by reverse transcription PCR (q-RT-PCR) technique, of target gene and compared of the gene expression in dominant and cystic follicular cells, showed down-regulation in the expression of FSH-r gene in the healthy dominant follicles, yet the expression are up-regulation in these cells may explain the synergistic activity of estrogen and FSH which may leads to the cystic follicular development on the ovary. This may be assistance to understand the etiology and pathology of this case (disease).

**Keywords:** Gene Expression, Cows , Ovarian Follicles , FSH-r gene.

## 1. Introduction

Cystic ovarian follicle (COF) a serious cause of the reproductive failure in cattle because they occur frequently and prolong the intervals from postpartum to first estrus and conception (Garverick , 1997).COF is defined as ovarian structures, larger than a pre-ovulatory follicle (>25 mm in diameter) and persists for ten or more days in the absence of a corpus luteum (Gordon, 2002; Youngquist & Threlfall ,2007).

Calder *et.al.* (2001) & Fleischer *et.al.*(2001) reported that the (20 mm) diameter as a minimum size of the follicular cyst in cow, but Hatler *et.al.*(2003) succeed that the follicles typically ovulate at 17 mm in diameter, while Vanholder *et.al.*(2006) suggested that the COF should be defined as "follicles with a diameter of at least (20 mm) that are present on one or both ovaries in the absence of any luteal tissue and that clearly interfere with normal ovarian cyclicity.

COF results from a malfunction of the neuroendocrine mechanism controlling ovulation which developed when one or more follicles fail to ovulate and subsequently do not regress but maintain their growth and steroidogenesis with absence of luteal tissue, there for this interferes with the estrous cycle (Arthur *et.al.*, 2001; Wiltbank *et.al.*,2002).

COF is a cause of temporary infertility, especially in the postpartum period. It is formation of a cyst after ovulation failure and causes ovarian dysfunctions (Hooijer *et.al.* ,2001; Silvia *et.al.*, 2002). Characterized by thin wall and accumulation of an excess amount of follicular fluid (FF) inside the follicle which contains of many components, including hormones like estradiol-17β (E2) and very small amounts of progesterone (P4). The FF also contain of proteins like glyceraldehyde-3-phosphate dehydrogenase (GABDH) with its receptors (Ball & Peters, 2004; Isobe *et.al.*, 2005; Maniwa *et.al.*,2005; Monniaux *et.al.* ,2008).

The physiology and etiology of COF are poorly understood, however there is much conjecture regarding of the biological cause of COF, like altered of the pre-ovulatory LH surge from the hypothalamus-pituitary is either absent or insufficient during dominant follicle maturation, which leads to cystic formation (Gordon,2002;Wiltbank *et.al.*,2002;Peter, 2004).

The follicular fluids contain high estradiol-17β and progesterone concentration in COF compared with the dominant follicles, yet the ratio of E2:P4 was greater than one (Silvia *et.al.*,2002;Peter, 2004) , so the steroid hormones in COF, particularly high concentrations of E2, would lead to genetic alterations, because the E2 inhibits P4 secretion.The dominant follicle will produce sufficient E2 to induce a pre-ovulatory LH-surge ,whereas the super basal concentrations of P4 blocks the pre-ovulatory LH-surge and lead to fail the new dominant follicle to trigger a LH-

surge and becomes cystic, while reduce of the P4 concentrations are likely to be associated with cyst turnover (Fortune *et.al.* ,2001; Beg & Ginther ,2006).

The FSH and LH are the necessary regulators of follicular maturation, because this two gonadotropins act by binding to and activating their specific receptors such as FSH-r and LH-r (Wiltbank *et.al.* ,2002).The FSH-r exclusively are found on granulosa cells, during prenatal follicle development (Heckert *et.al.* ,2000; Dunn & Mayo,2006) , the genetic alteration effects on functioning of many cells kinds and/or tissues. However little is known about the genetic alterations that may be involved in pathogenesis of COF ,the Gene expression comparisons may aid in understanding additional causes of COF, and will be vital to understand the entire process of ovulation failure and cyst formation.

The expression of FSH-r (number of receptor sites on the membrane) is increased by FSH activation (Nakamura *et.al.* ,1995; Minegishi *et.al.* ,2000), therefore enhance FSH action in the follicles by increasing FSH-r expression, yet the FSH-induced the granulosa cell aromatase activity and follicular growth (Yada *et.al.* ,1999).

## 2. Materials and Methods

### 2.1. Materials:-

### 2.1.1. Quantitative Reverse Transcriptase Real-Time PCR Kits:-

All kits which used in quantification of gene expression levels by qRT-PCR, and hormonal kit which used to RIA method, with their companies and countries of origin.

| No. | Kit | Company | Country |
|---|---|---|---|
| 1 | AccuZol™ Total RNA Extraction Kit<br>-Trizol 100ml | Bioneer | Korea |
| 2 | AccuPower® RocktScript RT PreMix<br>- RocketScript Reverse Transcriptase (200 u)<br>-5× Reaction Buffer (1×)<br>- RNase Inhibitor (1 u)<br>-DTT (0.25 mM)<br>-Dntp (250 µM each) | Bioneer | Korea |
| 3 | AccuPower® Greenstar™ qPCR PreMix<br>- SYBER Green fluorescence<br>- Exicycler™ 20 µL reaction<br>-8 Well strips × 12 each<br>- DEPC – D.W. 1.8 ml × 4 tubes | Bioneer | Korea |
| 4 | EZ-10RNA Mini-Preps Kits Handbook<br>-RNase-Free DNase Set | Bio basic | Canada |
| 5 | Immunotech RIA Progesterone (kit) | Beckman Coulter | France |
| 6 | Immunotech RIA Estradiol (kit) | Beckman Coulter | France |

### 2.1.2. Primers:-

Two set of primers are used in this study, first primer used for GAPDH gene as Housekeeping gene and other primer used for FSH-r gene as target gene. These primers were designed by using NCBI- Gene Bank data base and Primer 3 design online, the primers used in quantification of gene expression using quantitative (real time-PCR) techniques based SYBER Green DNA binding dye, and supported from (Bioneer, Korea) company.

The Primers with their sequences and product size.

| Primer | Sequences | Product size (Base Par) |
|---|---|---|
| GABDH- forward<br>GABDH- reveres | 5'-AGCAACAGGGTGGTGGACCT-3'<br>5'-AGTGTGGCGGAGATGGGGCA-3' | 133 |
| FSHR-forward<br>FSHR- reveres | 5'- CCACGTGATGGCGGTCAGA -3'<br>5'-GCTCCCCTGAGCCAGACGCA -3' | 360 |

### 2.2. Methods:-

### 2.2.1. Collection of the Follicles:-

The presence study depends on collection of (Evans & Fortune,1997) ovaries from sexually mature cows (4-6 years) old with unknown reproductive status slaughterhouses during December 2011 to October 2012, these ovaries transported within 1-2 hrs. in cool box (ice) to the vet. laboratory and freed from the surrounding tissue and over bursa, then each ovaries was subjected to washing in phosphate buffer saline (PBS) and one washing in ethanol 70%, examined by macroscopic exam (color, consistency, corpus luteum (CL) stage, follicular numbers and follicular size) according to (Stock & Fortune,1993; Berisha *et.al.* ,2000). Its ovaries were divided in to two groups' dominant follicles (DF) and cystic ovarian follicles (COF) were used in this study.

### A-Dominant Follicles :-

The dominant follicles (DF) group are (Yada *et.al.* ,1999) samples with diameter range 17-25 mm, contain only healthy follicles which having transparent fluids, signs of mucus production in the uterus and cervix and present of regress corpus luteum for previous estrus cycle.

### B- Cystic Ovarian Follicles:-

The cystic ovarian follicles (COF) are (Nakamura *et.al.,* 1995) samples having large un-ovulated persistent follicles on the ovary and diagnosed by the macroscopic notation such as the follicle

diameter are greater than 25 mm, absent of any corpus luteum in both the right and left ovaries and the follicular walls of the cysts were thin and translucent.

### 2.2.2. Study Design:-

These two groups DF and COF have been done in two steps which are:

#First step: Aspiration of follicular fluids (FF) from DF and COF and stored in -20℃ until estradiol 17 β (E2) and progesterone (P4) assay by used the radio-immunoassay (RIA) method.

# Second step: Section of the follicular wall in to two hemispheres and stored at -70℃ to -80 ℃ until RNA extraction and finally rt-PCR data analysis were undertaken (Relative quantification).

## 2.3. Estradiol-17β and Progesterone Assay in Follicular Fluid:-

The FF was aspirated from DF and COF by 18 gage needle attached to 10 ml size disposable syringe which was inserted into follicular cavity, and collected separately in test tubes contain anticoagulant, then centrifugation 2000 rpm for 10 minutes and stored at -20 ℃ until hormonal assay,as a described previously by(Vanholder,2005;Aad *et.al.*,2006).

## 2.4. Molecular analysis:-

### 2.4.1. Isolation of the Follicular Cells:-

Isolation of the follicular cells from the follicular frozen wall part (dominant or cyst) which are comprise from granulosa and theca cells (externa & interna) according to (Nogueira *et.al.*, 2007).

1- Weight 100 mg of frozen follicular wall and placed in a petri dish.

2- Gently scraped of internal layer using a plastic knife to isolate the follicular cells.

3- Flushed this piece with PBS to remove remaining follicular cells.

4- The solution was collected in 1.5 ml tube per dominant or cystic samples then centrifugation 12000 rpm for 10 minutes at 4℃ .

5- Re suspended the sediment in numbered 1.5 ml tubes.

6-The follicular cell mashed was stored at -70℃ to -80℃ in deep freeze system until total RNA extraction.

### 2.4.2.Total RNA Extraction:-

The total RNA was extracted from a follicular cell pellet using of the total RNA extraction reagent (Accuzol® Usere manual, BIONEER-Korea) and according to the manufacturer's instructions.

### 2.4.3. Quantification of total RNA:-

Quality control standards were applied to all RNA samples in this study, these were that the purity was 1.7-1.9, total RNA samples were adjusted at same concentrations. This is performing by nanodrop spectrophotometer machine (OPTIZEN POP. MECASYS KOREA).

### 2.4.4. Purity evaluation of total RNA:-

A- The purity of RNA determined, by reading the absorbance in nanodrop spectrophotometer, so the RNA has its absorption maximum and the ratio 260/280 is used to assess the purity of DNA and RNA, a ratio of ~2.0 is generally accepted as "pure" for RNA, therefore selected only purity samples to treat with DNase.

B- To determine of RNA integrity in two experiment by gel electrophoresis.

### 2.4.5. DNase Treatment:-

Regarding to the disadvantage of the SYBR green I , which it binds to any double-stranded DNA and produce of non-specific primer-dimers. Then treatment the extracted total RNA by DNase enzyme to remove the trace amounts of genomic DNA by using (DNase I enzyme), according to company instructions (BIOBASIC, USA) .

### 2.4.6. cDNA Synthesis:-

Reverse transcription-PCR control was performed with primers for GAPDH to check the removal of all the contaminating genomic DNA. First-strand cDNA was synthesized from 1500 ng of RNA using the cDNA synthesis kit (AccuPower® RocktScript RT PreMix), following the manufacturer's instructions.

### 2.4.7. Quantitative real-time PCR:-

According to method described by (Wang & Hardy ,2004), calculated the relative expression by q (rt-PCR) for target gene FSH-r gene in follicular cells of DF in comparison with COF, the ΔCT USING A REFERENCE GENE METHOD can be used by normalizing gene expression of target gene (FSH-r) with gene expression of housekeeping gene (GABDH) as a reference gene. This method used the difference between reference and target Ct values for each sample, the expression level of the reference gene are taken into account using following formula:

**Expression value (Fold yield) = $2^{\wedge CT\ (reference) - CT\ (target)}$**

### 2.4.7.1. Two-Step real-time PCR:-

The two-step reaction, revere to the reverse transcription amplification occur in separate tubes ( two-step rt-PCR which mean separates the reverse transcription reaction from the rt-PCR assay), two-steps protocol may be preferred when using a DNA binding dye (such as SYBR Green I) because it is easier to eliminate primer-dimmers through the manipulation of melting temperatures (Marisa & Juan ,2005).

### 2.4.7.2. Performed of q (real time-PCR):-

The q(rt-PCR) was performed by using AccuPower® Greenstar™ qPCR PreMix reagent kit (Bioneer, Korea) and Exicycler™ 96 Real-Time Quantitative Thermal Block (Bioneer, Korea), according to method described by (Chen *et.al.*, 1999).

The SYBER Green I based q(rt-PCR) premix reagent kit is designed for PCR amplification of cDNA for target gene by using FSH-r primer and housekeeping gene (GAPDH).

### 2.4.7.3. Experimental Design of q (real time- PCR):-

For quantification of FSH-r gene expression in dominant and cystic follicular cells, internal control gene as a housekeeping gene (GAPDH) was used for normalization of gene expression levels, therefore, preparing two q(rt-PCR) master mixes as the following.

I)- q(rt-PCR) master mix for FSH-r target gene (forward & revers), preparing 20 µL of total volume cDNA template for these gene:

| qPCR premix | | Volume |
|---|---|---|
| cDNA template | | 10 µL |
| Primers | FSH-r -F | 2 µL |
| | FSH-r-R | 2 µL |
| DEPC water | | 6 µL |
| Total | | 20 µL |

**II)- q (rt-PCR) master mix for GAPDH-r gene (forward & revers), preparing 20 µL of total volume to cDNA template for these gene:**

| qPCR premix | | Volume |
|---|---|---|
| cDNA template | | 10 µL |
| Primers | GAPDH-F | 2 µL |
| | GAPDH-R | 2 µL |
| DEPC water | | 6 µL |
| Total | | 20 µL |

After that, q(rt-PCR) premix were added into *AccuPower GreenStar q(rt-PCR) PreMix tube*, then rt-PCR tubes sealed by the optical adhesive film and mixed by vigorous vortexing for re-suspension of premix pellet. The tubes centrifuged at 3000 rpm for two minutes by using vortex/centrifuge, then start *Exicycler™ 96 Real-Time Quantitative*, thermal Block instrument and performed optimization for target gens to determine the performance of SYBR Green I q(rt-PCR) assay, by identifying the optimal annealing temperature for each target gene, then loaded the specific *Exicycler™ 96 Program* to relative quantification, according to kit instruction as the fowling:

### B- Performance of the Loaded the Specific Exicycler™ 96 Program to Relative Quantification, according to kit instruction:-

Both cDNA samples from dominant and cystic follicles were randomly used for PCR program which consists from initial step at 95℃ for five minutes for one cycle, to activate the Taq DNA polymerase, followed by different five cycles of denaturation at 95℃ for 20 seconds and a combined primer annealing/extension at the 65℃, 64℃ and 67℃ annealing temperature for 45 seconds for 40 cycles to FSH-r and GABDH-r.

### 2.5. Statistical analysis.

All the values are expressed as mean ± Se. data of DF and COF results were analyzed using *student t-test* and appropriate p-values of less than 0.05 were considered as statistically significant (Schiefler, 1980).

## 3. Results:-

### 3.1. Samples:-

The ovaries which collected from local cows divided according in to macroscopic notation in to two groups, the DF group (n=23) with diameter 19.93 ± 0.32 mm. and COF group (n=21) with diameter 37.56 ± 0.64 mm., yet there was significant difference ($P \leq 0.05$) in the diameter COF group in comparison with DF group, table (3.1).

### 3.2. Estradiol-17β and Progesterone Assay in Follicular Fluid:-

The sex steroidal hormones concentration level in the FF of COF had higher E2 concentrations (865.96 ±10.64 ng/ml), than did E2 concentrations in the FF of DF (314.39 ±2.55 ng/ml), table (3.1) & figure (3.1).

The P4 concentrations levels mean in COF showed higher (84.8 ±1.35 ng/ml), compared to those in DF was (50.25 ±1.57 ng/ml), table (3.1) & figure (3.2). There was a highly significant difference ($P \leq 0.05$) between two groups in E2 & P4 concentration.

Table (3.1): Differential between dominant and cystic ovarian follicles, data are presented as M± Se and *t*-test was used with ($p \leq 0.05$).

| Follicular state | n. | Follicular diameter (mm) | Estradiol-17β conc. In F.F.(ng/ml) | Progesterone conc. In F.F. (ng/ml) |
|---|---|---|---|---|
| Dominant Follicles | 23 | 19.93±0.32 | 314.39±2.55 | 50.25±1.57 E/P>1 |
| Cystic Ovarian Follicles | 21 | 37.56±0.64* | 865.96±10.64* | 84.308±1.35* E/P>1 |

(*) Significant differences.

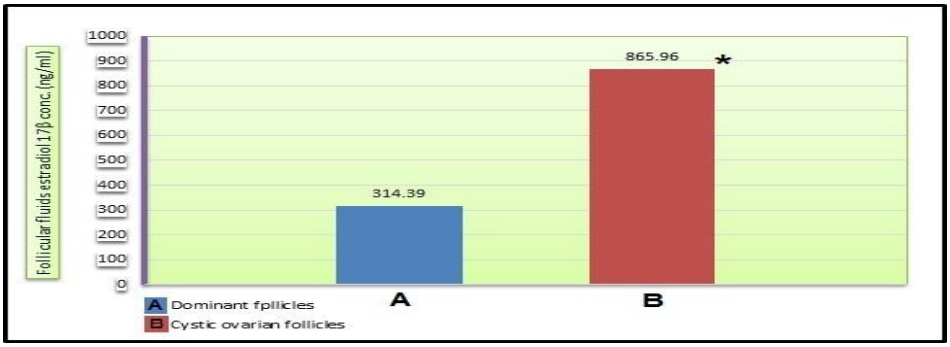



Figure (3.1): Mean of estradiol-17β concentrations (ng/ml) in follicular fluid of dominant (n=23) and cystic ovarian follicles (n=21). (*) Significant differences.

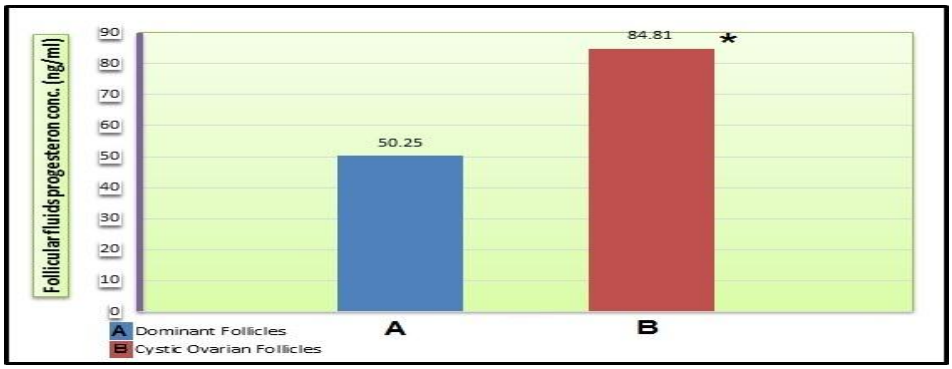



Figure (3.2): Mean of the progesterone concentrations (ng/ml) in follicular fluid of dominant (n=23) and cystic ovarian follicles (n=21). (*) Significant differences.

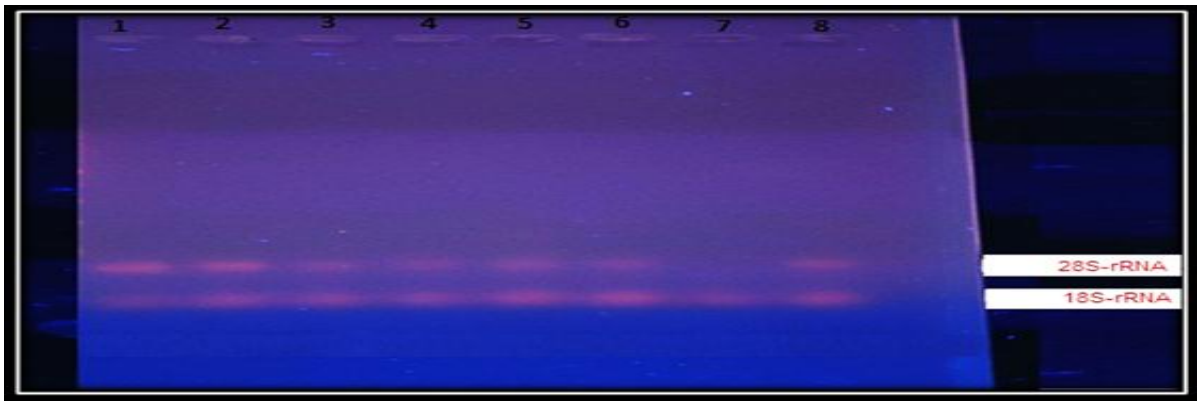
## 3.3. Molecular Analysis:-

### 3.3.1. Quantification of total RNA:-

The value of total RNA concentration was highly significant different (94.374 ±3.07 ng/µl) in follicular cells mashed of DF, while in COF are 95.64 ±2.98.

### 3.3.2. Purity evaluation of total RNA:-

The purity of total RNA samples, also assessed by using agarose gel electrophoresis of follicular cell of DF & COF, the bands were good and clear with presented two bands (18S rRNA and 28S rRNA), figure (3.3).




Figure(3.3): Agarose gel electrophoresis of RNA(28S-rRNA & 18S-rRNA) in follicular cell that obtained from secondary experimental ,dominant follicles(1,2,3,4) & cystic follicles (5, 6,7,8).

### 3.3.3. cDNA Synthesis:-

All the total RNA samples were used in cDNA synthesis step by using AccuPower® RocktScript RT PreMix kit that provided from BIONEER company, Korea in reverse transcription reaction, for converted RNA to cDNA synthesis by using rt-PCR system (Excecycler 96) ®. In temperature and time as in chapter three, this products reader by electrophoresis, then the cDNA bands were seen by U.V light, as figure (3.4).

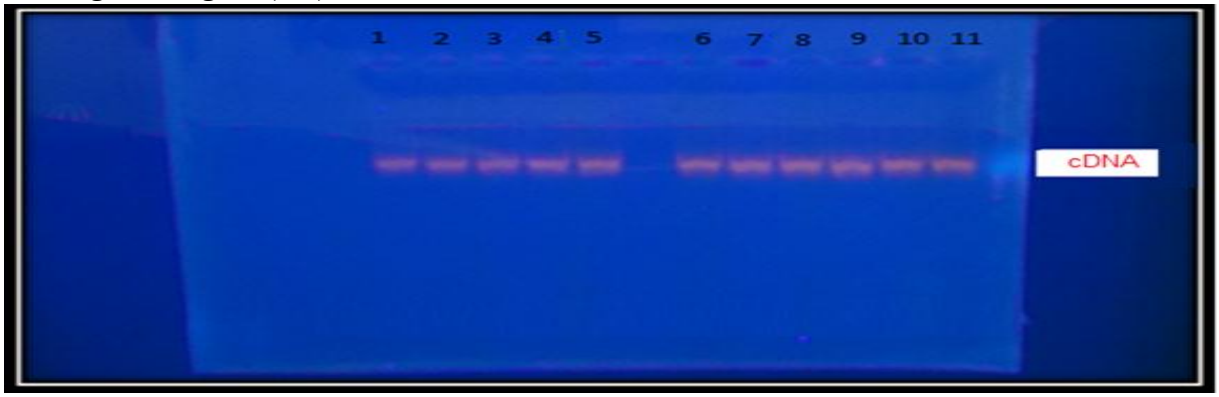



Figure (3.4): Agarose gel electrophoresis analysis of cDNA in follicular cells samples of dominant follicles (1,2,3,4,5,6) and cystic follicles (7,8,9,10,11).

### 3.4.1. Quantitative real time-PCR:-

Data analysis of SYBR green I based rt-PCR assay were divided into primer efficiency estimation and relative quantification of FSH-r gene expression level which normalized by housekeeping gene expression (GAPDH).

### 3.4.2. Relative Quantification of Target Gene Expression:-

To calculate the relative expression of target gene in follicular cells of the DF & COF, the *$2^{\Delta Ct}$ using a Reference Gene Method* used by normalizing target genes expression of FSH-r gene with expression of housekeeping or reference gene (GAPDH), table (3.2).

### A-The Ct value GABDH-r:-

The Ct value GABDH-r = 20.1680 in DF, and 20.8869 in COF, figure (3.5).

### B-Relative Quantification of FSH-r gene Expression.

FSH-r mRNA relative expression down-regulated (0.46124) in DF, and up-regulated (2.0246) in COF figure (3.6), therefore the expression ratio of FSH-r mRNA are:

**Normal expression ratio = 0.46124/0.46124=1**

**Test expression ratio = 2.0246/0.46124= 4.39 yield of this gene in follicular cells of DF (high expression or up-regulation)**

**Table (3.2): The mean of Ct values and expression value of the FSH-r gene in the follicular cells of dominant and cystic follicles.**

| Samples | Mean of CT values | | ΔCT | (2^ΔCT) Expression value |
|---|---|---|---|---|
| | **GABDH-r** | **Target gene** | | |
| Dominant Samples (n=14) | 20.1680 | 21.2844 FSH-r | -1.1164 | 0.46124 |
| Cystic Samples (n=14) | 20.8869 | 19.8691 FSH-r | 1.0177 | 2.0246 |

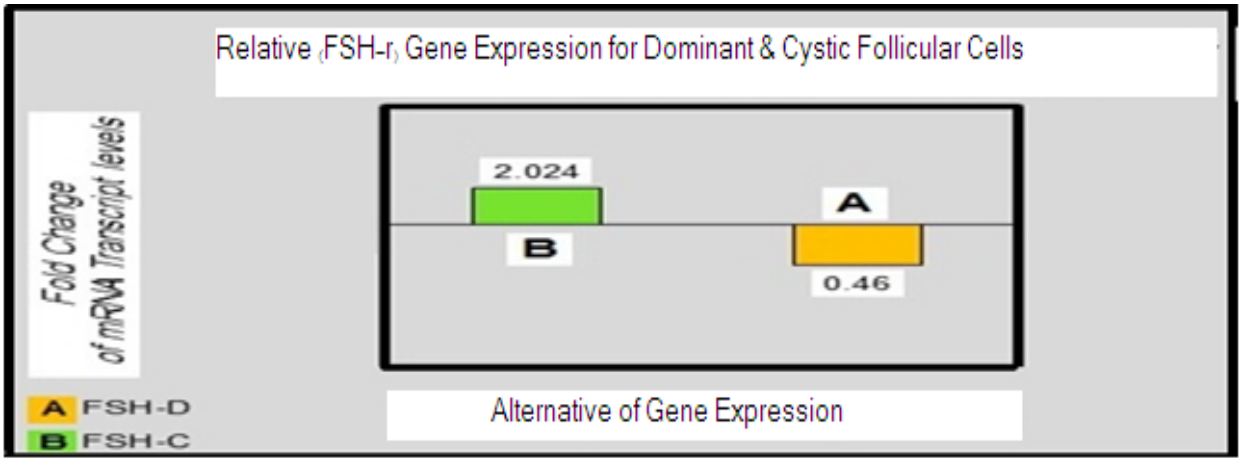



**Figure (3.5): Fold change of mRNA transcript levels of the FSH-r gene in the follicular cells of dominant and cystic follicles.**

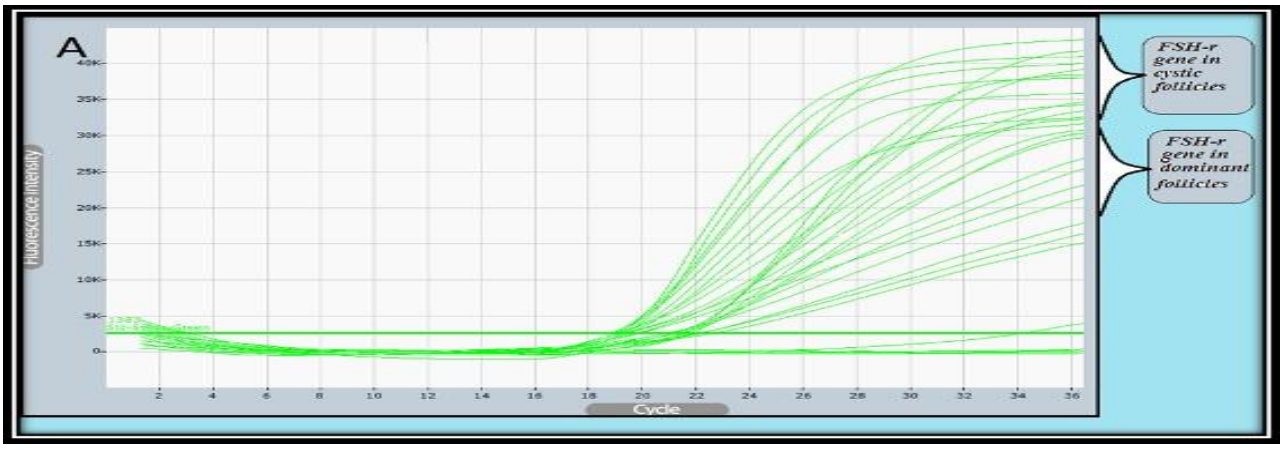



**Figure (3.6): Real Time PCR Amplification plot for FSH-r gene in dominant &cystic follicular cells by (Excecycler 96) ® system.**

## 4. Discussion:-

It's a first thesis to depict comparison analysis of gene expression of the FSH-r gene in ovaries of local Iraqi breed by using the q (rt-PCR) in order to identify new molecules useful for discrimination of bovine ovaries with and without cystic follicles.

## 4.1. Dominant and Cystic Follicular Diameter:-

This study demonstrated that the high differential ($p \leq 0.05$) significant of follicular diameter size of COF (37.56 ± 0.64 mm) compared with DF (19.93 ± 0.32 mm), table (3.1). These result findings consider characteristic of cystic follicles by the presence of a high volume of follicular fluid, and agreements with more than one like (Vanholder *et.al.*, 2006 ; Youngquist & Threlfall ,2007).

### 4.2. Estradiol & Progesterone Assay in Follicular Fluid:-

The follicular fluid of COF had higher E2 concentrations (865.96 ±10.64 ng/ml) than follicular fluid of the DF (314.39 ±2.55 ng/ml), yet the concentration of P4 was depressed (50.25 ±1.57 ng/ml) in DF, in compared with the concentration of COF (84.308 ±1.35 ng/ml), (table 3.1).

The E2/P4 ratio was greater than one that's indication to the DF came from ovaries in the follicular phase of the estrus cycle and hormonally classified as healthy (estrogen active ) according to (Mihm *et.al.,* 2006) , also Boryczko *et.al.* (1995) were classified the COF to estrogen-active cysts because this ratio greater than one (E/ P > 1) and concentration of P4 less than hundred (P < 100 ng/ml) in FF, yet this hormonal results was complete agreement with many previous studies (Calder *et.al.*,2001;Fortune *et.al.,* 2001; Beg & Ginther, 2006). The granulosa cells are the main site of production E2, therefore the high concentrations of E2 can result in genetic alterations, yet the elimination of these cells will reduce the E2 concentration (Isobe & Yoshimura ,2007), these findings support diagnosis our finding in present study.

### 4.3.1. Relative Quantification of FSH-r Gene Expression in Dominant and Cystic Follicles:-

The genomic relationship between FSH-r and LH-r , when examined together with their similar expression profiles and functions in gonadotropin signaling raise the intriguing possibility that the function of gene depends on their relative positions (Hunzicker-dunn & Mayo ,2006). In this study, used a q(rt-PCR) assay to measure relative quantification of mRNA transcript levels (gene expression) for FSH-r gene, with housekeeping gene (GABDH), for explanations pathogenesis of the COF and regarding synthesis of these receptors in the follicular cells of DF and COF.

### 4.3.2. Expression of FSH-r Gene:-

In cows with normal estrous cycles, the FSH-r is localized in granulosa cells of follicles of all diameters, and the mRNA was expressed in both theca and granulosa in DF & COF (Xu *et.al.* ,1995 ; Luo *et.al.,* 2005).

The results of the present study confirm and extend the notion that there is a relative mRNA expression for the FSH-r gene was high-regulated in follicular cells of COF alternative for mRNA expression of this gene in follicular cells of DF, table (3.2) & figure (3.5). This a high regulated result agree with previous studies (Themmen, 1991; Ginther *et.al.* ,2003; Mihm *et.al.,* 2006) whose indicating the increase of FSH-r on the granulosa cells of dominant follicle. Because of the ovarian

follicles development depends up on FSH action therefore any decline in mRNA expression for this receptors lead to disturbance in growth and function of dominant follicles, furthermore the mutation of the FSH-r gene lead to lack of FSH function which are affected ovarian follicular development, that lead to ovulation failure and infertility, but studies of Dunkel *et.al.* (1994); Xu *et.al.* (1995) and Braw-Tall & Roth (2005) observed that the level of expression of FSH-r mRNA decreased coincident with growth of the dominant non ovulatory and ovulatory follicles.

Ginther *et.al.*(2003) was explicit that the steroidal hormones concentration and FSH itself, earlier reports of both up- and down-regulation, the up-regulation refers to increase in the number of receptor site, yet the E2 up-regulates FSH-r sites, whereas the FSH stimulates granulosa cells to produce E2. This synergistic activity of estrogen and FSH allows follicular growth and development in the ovary (Themmen, 1991 ; Minegishi *et al.*, 2000).

In adult animals, LH receptor mRNA levels change dramatically during the estrous cycle, particularly after the pre-ovulatory LH surge, and after the follicle selection which is associated with an increase in mRNA for FSH-r in granulosa cells of DF (Evans & Fortune, 1997), but Kawate *et.al.* (1990) observed that the FSH-r numbers in granulosa cells of cysts are decreased when compared to normal follicles. The effects of increased estradiol production and FSH action on the granulosa cells of dominant follicles may stimulate the expression of LH receptors in granulosa cells, to respond to the pre-ovulatory LH surge (Fortune *et.al.*, 2001 & Beg & Ginther ,2006).

The growth of dominant follicles during the low FSH of ovarian follicular waves is associated with down regulation expression of the FSH-r gene. The current study used a q(rt-PCR) to identify, what gene /or genes that potentially regulate proliferation and survival in follicular cells and in expression of receptor mRNA as a key for hormone (FSH) that regulate antral follicular growth.

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