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# Study Association between *DRD2* Gene Expression and Infertility among Some of Iraqi Females

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**Abstract**: Infertility is commonest endocrine disease occurring in women of reproductive age. The aim of study to determine the D2 dopamine receptors gene expression and clarify altered concentrations of dopamine in infertile women and controls. Also, to assess their role in infertility and their correlation with measured biochemical parameters .This study was carried out in the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies - University of Baghdad through the period from January 2023-April 2023, The patients were taken from the Kamal Al-Samarrai Infertility Treatment Hospital in Baghdad the study was included 100 individuals (50 controls and 50 infertile females). Biochemical examinations were done include serum concentrations of dopamine (DA), luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin were measured by enzyme-linked immuno sorbent assay (ELISA) and enzyme linked fluorescent assay (ELFA) techniques. The RNA was extracted from whole fresh blood the concentration and purity of RNA Based on nanodrop device was performed, then converted to cDNA to measure the level of gene expression for *DRD2* by using Real Time PCR technique then converted to cDNA to measure the level of gene expression for *DRD2* by using Real Time PCR technique. In this study, the results showed a significant decreases in dopamine level in infertile Women in comparison to controls group ($P< 0.01$). In addition The analysis of hormones level (LH), (FSH) and (PRL) infertile female showed a highly significant increase in PRL and LH compared to controls group ($P\leq0.01$). The results of the fold expression of *DRD2* was showed down-regulated in patient, whereas it was up-regulated in control group. In conclusion gene expression of *DRD2* gene and Dopamine can be utilized as biomarkers for early diagnosis of female infertility in Iraqi femals as one of the laboratory diagnostic methods.

**Key words**: D2 dopamine receptor, infertility, Dopamine ,*DRD2* gene expression.

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## Introduction

Infertility is defined as the failure to achieve pregnancy after 12 months of regular unprotected sexual intercourse. Approximately 85% of infertile couples have an identifiable cause. The most common causes of infertility are ovulatory dysfunction, male factor infertility, and tubal disease. The remaining 15% of infertile couples have “unexplained infertility.” Lifestyle and environmental factors, such as smoking and obesity, can adversely affect fertility. Ovulatory disorders account for approximately 25% of infertility diagnoses; 70% of women with anovulation have polycystic ovary syndrome. Infertility can also be a

marker of an underlying chronic disease associated with infertility, in addition to genetic causes and genetic developmental disorders, which play important role in infertility females (1,2). Dopamine is known to be involved in several essential brain functions, such as locomotors, behavior, cognition, motivation and neuroendocrine secretion, with its actions mediated via dopamine receptors. In particular the dopamine D2 receptors has been implicated in reward mechanisms in the brain. Dysfunction of the D2 dopamine receptors lead to aberrant substance seeking behavior (3).

Dopamine regulates a variety of physiological and behavioral processes including reproduction which, in vertebrates, is controlled via the hypothalamic-pituitary–gonadal (HPG) axis. Hypothalamic release of gonadotropin-releasing hormone (GnRH1) previously called luteinizing hormone-releasing hormone, stimulates pituitary gonadotropic cells to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the bloodstream. These gonadotropic hormones directly alter reproductive potential by driving the synthesis of gonadal steroid hormones including testosterone, estrogen and progestin (4) furthermore, Dopamine binds to DRD2 in the pituitary lactotrophs and decreases the level of intracellular cyclic adenosine monophosphate, which in turn inhibits prolactin secretion (5). So this study aimed to determine the D2 dopamine receptors gene expression and clarify altered concentrations of dopamine in infertile women and controls, also to assess their role in infertility

**Materials and methods**

A Sample Collection. This observational study consisted of 50 infertile females as patients which have primary and secondary infertility and 50 as controls .Blood samples (5ml) were collected using EDTA tubes , (250) µl of blood from each EDTA tube was added to 750µl of Triazol in Eppendorf tube kept in a deep freezer (-20 ºC) used for molecular analysis and the rest of the samples were centrifuged; then the serum was collected and kept in (4 ºC) in a refrigerator for (ELISA and AIA Biochemical tests) included. Hormonal examinations were done. Serum concentrations of Dopamine, Prolactin, LH and FSH, *TransZol* Up Plus RNA Kit Reagent according to the manufacturer's instructions. RNA purity and concentration were determined using a spectrophotometer (Nanodrop). then synthesis the cDNA form mRNA: The expression levels of the *DRD2* gene were estimated by the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) method, a sensitive technique for the quantifying of steady-state mRNA levels. To confirm the expression of the target gene, a quantitative real-time qRT-PCR SYBR Green assay was used. The endogenous control gene GAPDH's mRNA levels were amplified and utilized to normalize the DRD2 gene's mRNA levels.

**RNA concentration and purity assessment**

The 2000c Nano drop One C (Thermo Fisher Scientific, USA) was used to evaluate the concentration and purity of extracted RNA in order to determine the quality of samples for subsequent analysis in RT-qPCR. The samples ranged in RNA concentration from 73-147 ng/µl, while the absorbance of the samples was measured at two distinct wavelengths to determine RNA purity (260 and 280nm). The presence of an A260/A280

ratio of around 2.0 suggested that the RNA sample was pure.

**Synthesis the cDNA form mRNA**

First strand cDNA synthesis, reaction component Using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit, total RNA was reverse-transcribed to complementary DNA (cDNA). According to the manufacturer's instructions, the operation was performed in a reaction volume of 20 µl. Four microliter of total RNA had to be reversely transcribed (Table 1) shown The study's designed primers.

**Quantitative Real Time PCR (qRT–PCR)**

The expression levels of the *DRD2* gene were estimated by the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) method, a sensitive technique for the quantifying of steady-state mRNA levels. To confirm the expression of the target gene, a quantitative real-time qRT-PCR SYBR Green assay was used. Alpha DNA Ltd. (Canada) designed and synthesized primer sequences for the *DRD2* gene, then lyophilized and stored at -20°C (Table 1).

**Table (1): The components of quantitative real-time PCR were employed in the *GAPDH and DRD2*gene expression experiments.**

| Components | 20 µl rxn |
|---|---|
| ***2xTransStart*® Top Green qPCR Super Mix** | 10 |
| **Nuclease free water** | 4 |
| **Forward Primer (10 µM)** | 1 |
| **Reverse Primer (10 µM)** | 1 |
| **cDNA** | 4 |

The endogenous control gene *GAPDH*'s mRNA levels were amplified and utilized to normalize the *DRD2* gene's mRNA levels. (Table2) shows the primer sequences for *GAPDH* and *DRD2* genes. The cycling protocol was programmed for the following optimized cycles and according to the thermal profile shown in (Table 3).

**Table (2): The study's designed primers.**

| Primer | Sequence (5′→3′ direction) | primer size bp | Product size bp | Ta °C |
|---|---|---|---|---|
| ***DRD2 (Gene Expression)*** | | | | |
| **Forward** | CTGCAGACCACCACCAACTA | 20 | 154 | 58 |
| **Reverse** | TGACGTCCAGAGTGACGAAG | 20 | | |
| ***GAPDH*- Glyceraldehyde 3-phosphate dehydrogenase** | | | | |
| **Forward** | GAAATCCCATCACCATCTTCCAGG | 24 | 160 | 58 |
| **Reverse** | GAGCCCCAGCCTTCTCCATG | 20 | | |

**Table (3): The thermal profile of *GAPDH and DRD2* genes expression**.

| Step | Temperature (ºC) | Time (sec.) | Cycles |
|---|---|---|---|
| **Enzyme activation** | 94 | 10 | 1 |
| **Denaturation** | 94 | 5 | 40 |
| **Annealing** | 58 | 15 | |
| **Extension** | 72 | 20 | |
| **Dissociation** | 55 ºC-95 ºC | | 1 |

Statistical analysis was carried out using SPSS version 23.Categorical variables were presented as frequencies and percentage . Chi-square test and fisher exact test were used to compare between percentages (frequencies) in this study. The odds ratios (ORs) and 95% confidence intervals (95%CIs) were used to evaluate the potential associations between gene expression dopaminergic genes and the risk of infertility *P* value for all tests was considered significant if ≤ 0.05.

## Results and discussion

### Serum dopamine

Infertile females compared with apparently healthy women showed significant decreasing in Dopamine level(13.4966±2.53960versus22.3652±15.62011; P≤0.01) (table 4) Generally, the results of the present study agree with Wasilewski (6) who found alters dopamine metabolism may cause menstrual disturbances as well as increasing the risk of miscarriage by decreased dopamine levels, which may lead to increased prolactin concentration followed by hyperprolactinemia, which cause ovulation disturbances, changes in the luteal phase as well as amenorrhea or oligomenorrhea .also, dopamine may slow down the pulsation of gonadotropin-releasing hormone, causing an increase in luteinizing hormone levels. Furthermore, decreased DA concentrations along with reduced dopamine 2 receptors. They attributed low DA concentrations to the increase in LH concentrations in infertile females. inhibitory neurotransmitters in hypothalamic-pituitary centre. This, hypothesise, is responsible for the depression and anxiety-like mood disorders commonly seen in PCOS women (7).

**Table (4): Comparison between patients and control groups in Dopamine(pg/ml).**

| Groups | Mean(pg/ml) | Std. Deviation | Std. Error of Mean | p-value |
|---|---|---|---|---|
| **Infertile Patients** | 13.4966 | 2.53960 | .35561 | 0.001** |
| **Control** | 22.3652 | 15.62011 | 3.59669 | |
| **Total** | 16.3345 | 10.87201 | 1.25539 | |

### Serum luteinizing hormone (LH)

There were significantly elevated increase in serum prolactin concentrations and LH in infertile females compared to healthy women showed significant increase in PRL (22.226 ±8.7353 vs. 12.446 ±2.26290; P≤0.01), and significant increase in LH (6.2466±3.819400 vs. 3.9680 ±.92327; P≤0.01) (Table 5).

**Table (5): Comparison between patients and control groups in LH (mlU/ml).**

| Groups | Mean | Std. Deviation | Std. Error of Mean | p-value |
|---|---|---|---|---|
| **Infertile Patients** | 6.2466 | 3.81940 | 0.54015 | 0.0001** |
| **Control** | 3.9680 | 0.92327 | 0.13057 | |

These results were agree with Al-Juaifari (8) who was found infertile females showed significant increase in LH level compared to healthy control females, especially in PCOS females .as result of the heterogeneity of infertility, there are most likely multiple underlying pathophysiologic

mechanisms. Which cause alteration in gonadotropin-releasing hormone secretion results in increased luteinizing hormone (LH) secretion. Also, high levels of LH not only has an effect on oocyte maturity and human reproduction, but also on lower fertility and higher miscarriage prevalence (9).

**Serum prolactin**

There were significantly elevated increase in serum prolactin concentrations in infertile females compared to healthy women in PRL (22.226 ±8.7353 versus. 12.446 ±2.26290; P≤0.01), (Table 6).

**Table (6): Comparison between patients and control groups in PRL(ng/ml).**

| Groups | Mean(ng/ml) | Std. Deviation | Std. Error of Mean | p-value |
|---|---|---|---|---|
| **Infertile Patients** | 22.226 | 8.7353 | 1.2354 | 0.0001** |
| **Control** | 12.446 | 2.2629 | 0.3200 | |

Hyperprolactinemia in infertility women might contribute to obesity, hyper insulinemia, and gonadal dysfunctionin in infertile females furthermore, hyperprolactinemia might be linked with increased risk of metabolic syndrome and probably become a metabolic risk. In women, it frequently leads to gonadal dysfunction including ovulatory disorder, menstrual galactorrhoea and infertility (10,11)

**Serum follicle stimulating hormone (FSH)**

There was non-significant decreas in the FSH level showed in the infertile females compared to healthy control females (5.1442 ±3.28634 versus in control 5.3180±.99297) (p>0.05) (Table 7).

**Table (7): Comparison between patients and control groups in FSH (mlU/ml).**

| Groups | Mean | Std. Deviation | Std. Error of Mean | p-value |
|---|---|---|---|---|
| **Patients** | 5.1442 | 3.28634 | .4647600 | 0.7 |
| **Control** | 5.3180 | 0.99297 | 0.14043 | |
| **Total** | 5.2311 | 2.41684 | .24168 | |

The low level of FSH and significantly higher LH hormones levels leading to increased LH/FSH ratio in infertile females associated with less response for progesterone and defect in the frequency of FSH, this leads to Elevated baseline LH/FSH ratio in women which was associated with poor ovulatory response(12,13)

Furthermore, Al Faisal and Al-Deresawi (14) were reported that results obtained from hormonal analysis showed significant lower levels FSH in infertile women. A high levels of luteinizing hormone (LH) and low levels of the follicular-stimulating hormone (FSH), so follicles in these individuals are prevented from producing a mature egg which was consider amain causes of infertility.

**Fold expression of *DRD2* gene**

In the present study, quantitative RT-PCR assay analyzed the mRNA expression of *DRD2* and compared its expression between infertile women *versus* apparently healthy control groups. The calculation of gene expression fold change was made using relative quantification (15).

The Fold of gene expression of infertile females was (0.6791) and for apparently healthy control was (1.00)

(table 8) When calculating the gene expression was significantly lower in women with infertility than apparently healthy control (Figures 1,2) show the amplification plots and dissociation curves for *DRD2* and *GAPDH* Genes.

**Table (8): Comparison between patients and control groups in Gene expression.**

| groups | Means Ct of DRD2 | Means Ct of GAPDH | ΔCt (Means Ct of DRD2) | $2^{-\Delta Ct}$ | experimental group/ Control group | Fold of gene expression |
|---|---|---|---|---|---|---|
| **Patients** | 27.8373 | 14.7944 | 13.0433 | 0.000118 | 0.000118/0.000191 | 0.6791 |
| **Control** | 27.1066 | 14.7593 | 12.3473 | 0.000191 | 0.000191/0.000191 | 1.00 |

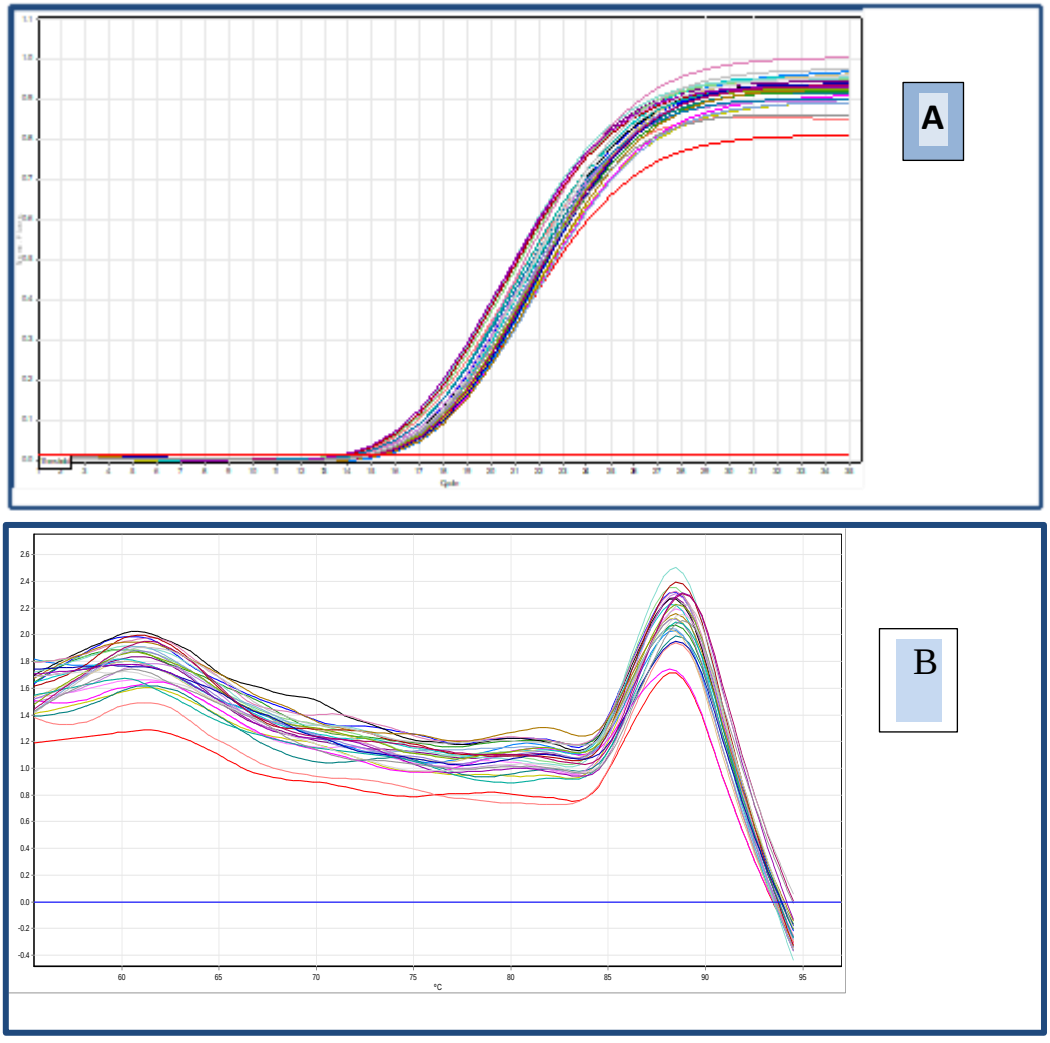


**Figure (1): A-*GAPDH* gene amplification was plotted using qPCR samples that covered all research groups. B- *GAPDH* gene dissociation curves using qPCR samples that covered all research groups.**

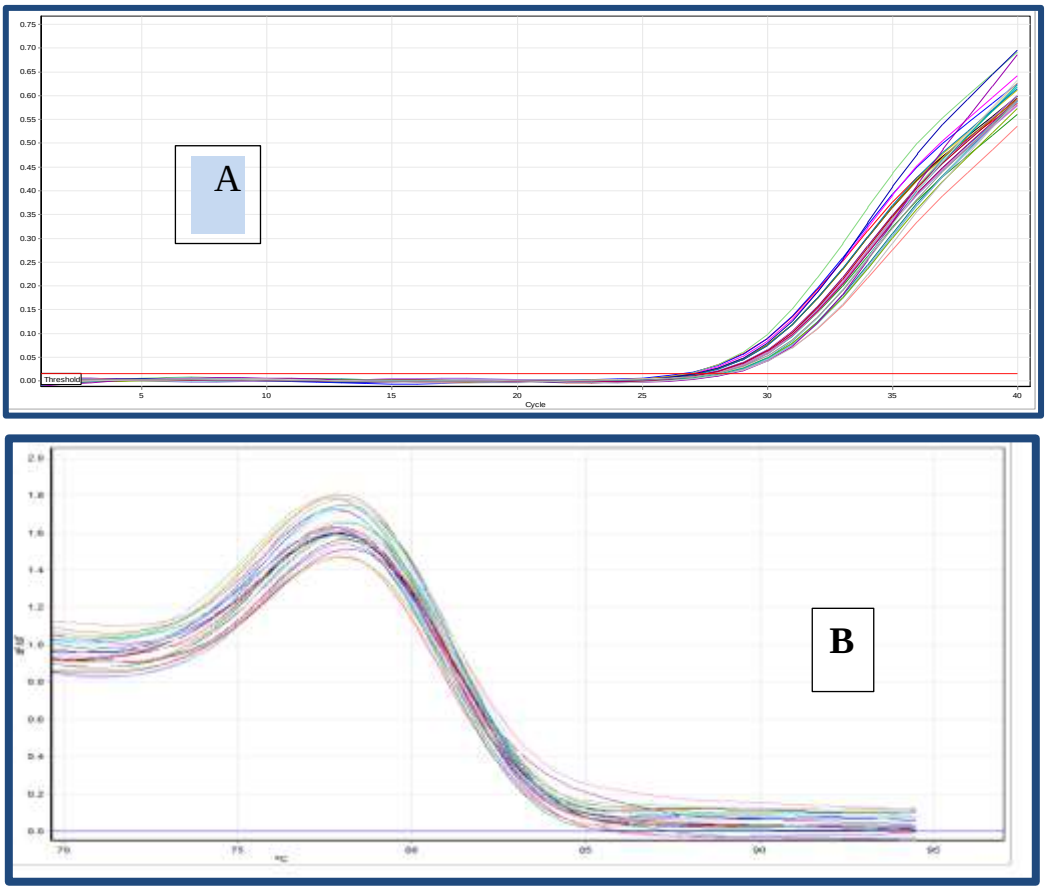


**Figure (2): A- *DRD2* gene amplification was plotted using qPCR samples that covered all research groups.B- *DRD2* gene dissociation curves using qPCR samples that covered all research groups.**

Significantly decreased in expression of *D2R*, which may result into hyper secretion of prolactin in infertile females especial in PCOS condition. which, Supporting our data, many studies suggest the role of reduced dopaminergic tone increased LH releasein in infertile females Additionally, treatment with bromocriptine,a D2 receptor agonist, can restore normal menstrual cycle and ovulation in PCOS women (6,16). Furthermore, the Decreased of *Drd2* expression and increased vascularization in the theca layer of antral and luteinized follicles in infertile females observed. A lower dopamine production and reduced efficacy of cabergoline in inhibiting Vascular endothelial growth factor (VEGF) secretion Decreased dopaminergic tone as well as deregulated Drd2 signaling explain higher VEGF and vascularization leading to increased ovarian hyper stimulation syndrome risk (17),as well as Yarmolinskaya *et al* (18) reported *DRD2* expression decrease in endometriosis areas and there was negative correlation of *DRD2* with PRL level and dopaminergic regulation decrease leading to local PRL level elevation and VEGF in infertile females. on the other hand, the elevated DA concentrations increased free radical's production which in turn reduced adenosine triphosphate values and cell viability and were associated with impaired oocyte maturation and fertilization, poor embryo quality, and decline in pregnancy rates (10).

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