



Assessment of Cortisol Hormonal Level and Relation with Age in Infertile Women With ICSI Outcome

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ABSTRACT

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In human-assisted reproduction both partners need to be evaluated during counseling because causes of infertility are due to male factors and female factors. Some body hormones play an important role in fertile women and cortisol is one of these hormones which is essential for normal ovulation, cortisol usually increases during the follicular phase of the menstrual cycle, play a role in the early stages of follicular development by their interaction with aromatase activity and granulosa cell functions. To investigate the correlation of cortisol hormonal level in serum and in follicular fluid that relationship with age in women under Intracytoplasmic sperm injection outcome. Its randomized prospective clinical trial study performance conducting in the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University, Baghdad-Iraq, during the period from November 2021 to June 2022. A total of Sixty-three Infertile Iraqi women, who planned to enter intracytoplasmic sperm injection program. The blood sample and follicular fluid sample obtained from all infertile women enter intracytoplasmic sperm injection program to measure cortisol hormonal level at specific days of this study. There was an increase in cortisol level in women aged between (30-40) years, So there was a significant finding between cortisol level and this age group and there was an increase in cortisol level in women aged < 30 years at the same day and no significant difference in cortisol level in women age more than 40. Significant differences in cortisol level appear in group of women age between (30-40) years, also the same result appears in group of women age more than > 40 years and no significant differences in cortisol level in women aged < 30. No significant differences in cortisol level in all age women group (30-40) years, <30 years, and the women age > 40 years.

KEYWORD

Uterine Scoring System for Reproduction (USSR), Pregnancy rate, Endometrial thickness, Endometrial blood flow, Pulsatility index

1. Introduction

Infertility is the inability to have children. It is a condition in which a couple is unable to effectively conceive a pregnancy following a period of irregular and unprotected sexual intercourse (Ishiura, S and Yoshida, T.,2019 [1]). There are two types of infertility: Primary infertility is when a couple has never been able to conceive. Secondary infertility: where a women have one or more pregnancies in the past but is having difficulty conceiving again (Olooto et al.,2012 [2]). Fertility falls as women age. Female fertility peaks between the ages of 18 and 24 years after having conceived, carried the pregnancy to term, or miscarriage (Agboola,2004 [3]). While it begins to fall at the age of 27, it lowers at a slightly faster rate after that .Follicle-stimulating hormone (FSH): This hormone is key to the regular menstrual cycle and helps induce the maturation of eggs in the ovaries. Women who have ovarian function loss have high

levels of FSH as the body tries to compensate for the lack of function (Gonçalves et al., 2010 [4]).

Luteinizing Hormone (LH): is responsible for the release of the mature egg during ovulation. Most ovulation prediction kits use this hormone as it increases before ovulation (De Souza et al., 2010 [5]). Anti-Mullerian Hormone (AMH) is produced by the ovarian follicles that contain immature oocytes; the levels of this hormone are used to predict the number of oocytes that are remaining in the ovary. Reduced AMH often can point to infertility (Park HJ et al., 2015 [6]). Estrogen is a hormone that supports the regulation and development of primary and secondary sex characteristics and the reproductive system in females. Estrogen dominance is sometimes found responsible for the difficulty in conception (Fisch JD et al.,2008 [7]). Progesterone is a hormone that is vital to maintaining a pregnancy. It helps the uterine lining to thicken to help grow an embryo. Low progesterone levels in a

woman can lead to miscarriages. Cortisol is a glucocorticoid with many different functions; the free hormone hypothesis appears to fit the observed effects of cortisol well. Although the ovaries lack the enzymes necessary for cortisol manufacture, they do contain glucocorticoid receptors and are impacted by cortisol. Ovarian follicles control the biological action of the hormone cortisol **(Yding Andersen et al. 2002 [8])**, Causes of Hormonal Imbalance That Can Lead to Infertility

The most common cause of hormonal imbalances that can cause infertility or difficulty in getting pregnant is Anovulation; this is a condition where a woman does not ovulate—polycystic ovarian syndrome. In PCOS, the woman has a hormonal disorder that causes the oocyte to stay immature and turns them into cysts. This can often cause difficulty in getting pregnant and can also cause infertility **(Jones, MR et al., 2016 [9]; El Hayek et al., 2016 [10]; Barbieri RL, 2017 [11]; Alzaidi, Z et al., 2021**

[12]) Hyperprolactinemia, in this condition, the woman has an excess of prolactin in their body. This causes them to have irregular periods and infertility **(Vilar L et al. 2018 [13])**

Hypercortisolism which occurs when the body is subjected repeatedly to excessive cortisol levels. There are two types of ACTH-dependent and ACTH-independent causes of Cushing syndrome **(Raff H. and Carroll T.,2015 [14])**

Hypocortisolism, the most common cause of Autoimmune adrenalitis, is the major cause of primary adrenal insufficiency, often known as Addison disease **(Michels A. and Michels N.,2015 [15])**. Other potential reasons include malignancy, infection, or adrenal hemorrhage

2. Materials and Methods

Full history was obtained from infertile couples. Infertile women were Completely examined, including general and gynecological examination, and body mass index

(BMI) was determined for each participant women following measurement of women's height and weight. Antagonist ovarian stimulation protocol was applied to all enrolled infertile women.

Blood Sample :

At Cycle Day 2, take 2ml blood sample was aspirated to measure baseline hormonal evaluation at early follicular phase LH, AMH, FSH, and Progesterone for hormone assay, which was performed by MiniVidas device .

At Day of Oocyte Retrieval take, 5 ml of blood were aspirated into a clean gel glass tube using a disposable syringe to measure cortisol and progesterone, which were carried out by ELIZA for cortisol assay and minividas for progesterone.

At the Day of Embryo Transfer, take 5ml of blood were aspirated into a clean gel glass tube using a disposable syringe to measure cortisol, which were carried out by ELIZA for cortisol assay. Follicular Fluid Sample: take 5ml of follicular fluid were aspirated into a

clean plastic tube using a disposable syringe to measure cortisol and progesterone, which were carried out by ELIZA for cortisol assay and minividas for progesterone. Using mini VIDAS equipment (VIDAS) and the enzyme-linked fluorescence assay (ELFA) method, hormone analysis was carried out. The kits were purchased from Bio Merieux and labelled VIDAS®E2, VIDAS®FSH, VIDAS®LH, VIDAS®Prog., and VIDAS®AMH method as in the study by .[15] The Principle of ELISA Method: An enzyme-linked immunosorbent assay (ELISA) is a biochemical test that detects the presence of either antigen, such as proteins, peptides, and hormones, or antibodies in a given sample using antibodies and an enzyme-mediated color change (Ellen Anckaert et al.,2002 [16])

4. Results

1- Estimation of Difference hormone levels in Blood Serum at Cycle Day 2

Including FSH, LH, Progesterone, AMH and Its Relationship with the Age, the Age that Have been Divided in this Study to A, B, C

Age and Progesterone hormone level: < 30years women, the progesterone is increased in this group, that have mean and stander deviation was (3.60 ± 11.72) , (31-40) years women, progesterone level was no significant with this group which have mean and stander deviation was (3.18 ± 7.94) , > 40 years women, progesterone level was significant with this group that have mean and stander deviation was $(1.2844 \pm .55615)$

The p-value of progesterone with all these age groups was significant ($p=0.04$), as in the result show in table (1.1).

Age and (AMH): < 30 years women AMH are high level in this group that have mean and stander deviation (of 2.7000 ± 1.37556), (31-40) years women, AMH level was no significant which, have mean and stander deviation was (3.1746 ± 7.93938) , > 40

years women, AMH level was significant which have mean and stander deviation was $(1.28 \pm .556)$

-The p-value of AMH with all these age groups was significant ($p= 0.04$), as in the result show in Table (1.1).

Age and (FSH) And (LH): < 30 years women, that have mean and stander deviation was (FSH 6.12 ± 1.80 , LH 5.81 ± 2.66), (31-40) years women, that have mean and stander deviation was (FSH 6.23 ± 1.96 , LH 5.29 ± 1.89), >40 women that have mean and stander deviation was (FSH 7.10 ± 2.98 , LH 6.73 ± 2.01)

-The p-value of all these age groups was no significant ($p= 0.18, 0.36$), respectively, as in the result show in Table (1.1).

2- Estimation of Cortisol, E2 Level in Serum and Cortisol in Follicular Fluid Its Relationship with the Age, the Age that Have Been Divided in this Study into A, B, C

Age and Cortisol in Serum: In (< 30) years women, cortisol level was significant with. This group that have

mean and stander deviation (of 6.40 ± 4.44) while (30-40) years women, cortisol is high concentration with these group that have mean and stander deviation (10.52 ± 12.47). But, for women > 40 years women, cortisol level was no significant and have a mean and stander deviation (of 8.33 ± 4.84). The p-value of all these age groups was significant ($p = 0.037$), as in the result show in table (1.1).

Age and Cortisol in Follicular Fluid: (< 30) years women, cortisol level in follicular fluid was no significant with this group that have mean and stander deviation (16.55 ± 9.53), while (30-40) years women cortisol level in follicular fluid was increased with this group have mean and stander deviation (18.30 ± 12.87), also > 40 years women, cortisol level in follicular fluid was significant with this group and have mean and stander deviation (13.00 ± 8.62). The p-value of all these age groups was significant ($p = -0.04$), as in the result show in Table (1.1).

Age and E2 hormonal levels include: < 30 years women, the Estrogen (E2) level was high in this group and have a mean stander deviation was (1268.31 ± 1265.71), while (31-40) years women's E2 level was significant differences, that have mean, stander deviation was (1063.86 ± 599.89) and > 40 years women, E2 level was significant differences that have mean, stander deviation was (792.86 ± 487.68).

-The p-value of E2 with all these age groups was significant differences ($p = 0.02$) as in the result show in Table (1.1).

3- Estimation of Cortisol Level in Serum at Embryo Transfer and Its Relationship with the Age, the Age that Have Been Divided in this Study to A, B, C

Age and Cortisol in Serum at Embryo Transfer: < 30 years, women which have mean and stander deviation (of 12.55 ± 8.36), (30-40) years, women which have mean and stander deviation (of 13.78 ± 9.03) and > 40 years, women

which have mean and stander deviation
(10.50±6.66)

The p-value of all these age groups was
no significant (p=-0.078) as in the
result show in Table (1.1)

Table (1): Assessment of hormonal Level at Day 2, Day of Oocyte Retrieval and Embryo Transfer, According to Different Groups of Age

	Hormones	< 30	31 – 40	> 40	P value
Mid Cycle	E2 (pg/ML)	1268.31±1265.71 A	1063.86±599.89 B	792.86±487.68 C	0.02 Sig
Cycle Day 2	Progesterone (ng/ML)	3.60±11.72 A	3.18±7.94 A	1.28±.556 B	0.04 Sig
	AMH (ng/ML)	2.70±1.38 A	2.66±2.85 A	1.28±1.02 B	0.04 Sig
	FSH (miu/ML)	6.12±1.80 A	6.23±1.96 A	7.10±2.98 A	0.18 No sig
	LH (miu/ML)	5.81±2.66 A	5.29±1.89 A	6.73±2.01 A	0.36 No sig
Day of Oocyte Retrieval	Cortisol (µg/dL) in serum	6.40±4.44 B	10.52±12.47 A	8.33±4.84 AB	0.03 Sig
	Cortisol (µg/dL) in follicular fluid	16.55±9.53 AB	18.30±12.87 A	13.00±8.62 B	0.04 Sig
	Progesterone (ng/ML) in serum	9.66±4.86	3.17±7.94 B	6.25±4.06 B	0.04 Sig
Day of Embryo Transfer	Cortisol (µg/dL) in Serum	12.55±8.36 A	13.78±9.03 A	10.50±6.66 A	0.07 No sig
Significant at the 0.05 level (2-tailed).					

5. Discussion

Cortisol in Serum and Age

In this study, the estimated of cortisol in serum was done at the day of oocyte retrieval and embryo transfer; the result found there was a significant correlation between serum cortisol and age.

According to the table, explain that's older women (31-40) years having higher cortisol concentrations than younger women <30 years. This result agreement with a similar study done by (Gan, SD. and Patel KR 2013 [17]). Also, many other authors proof the relationship between women's HPA and cortisol levels. While Eleanor (2020) disagreement with the result of a recent study, suggested that the level of cortisol was lower in the > 40 women.

Cortisol in Follicular Fluid and Age

According to table show that there was a significant increase in cortisol level in follicular fluid at the day of oocyte retrieval with age groups ranging

between (31 – 40) years and >40 years, While there was a non-significant change in cortisol level in group aging <30. However, all these results correlated with age was in agreement with (Semman et al., 1994 [18]; Nicolson et al., 1997 [19]). These authors observed an increase in cortisol concentrations in the follicular fluid of older women than young women. (Deuschle, M. et al., 1997 [20]) Shows a weak inverse correlation of follicular fluid cortisol level with patient women's age—these finding disagreement with the result of the current study.

Conclusion

There was a significant increase in cortisol hormonal levels in serum and in follicular fluid in infertile women within the ages 30-40 years and more than 40 years. There was a significant increase in cortisol hormonal levels in blood serum at the day of embryo

transfer in infertile women that entered the ICSI program.

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Author Contribution

Noor Mahmood performed the study, and Rana A. Al Saadi; Essra M. Al-Esawee; Farah A. Mohammed; supervised the work.

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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