Research Article

The Relationship between Changes in Serum Cortisol levels and IVF Results in Infertile Women with Mild to Moderate Depression

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Abstract: The purpose of the study was to investigate the relationship between the cortisol hormone levels of depressed infertile women with their IVF results throughout the treatment process to identify the consequences of IVF. In this prospective study, 74 women who were being treated by IVF at the Mother and Child Infertility Center of Shiraz and were affected by mild to moderate depression based on Beck inventory were randomly selected as the subjects. The sampling and follow-up were performed from April 2014 to March 2015. Cortisol levels were measured in the patients' serum at three stages: 1) before the treatment (T_1), 2) on the day of egg retrieval (T_2), and 3) and on the day of transferring the embryo to the uterus (T_3). Sampling was conducted from 9 a.m. to 10 a.m. To analyze the data, the researchers used SPSS. In findings 32.43% of the subjects got pregnant after the treatment, and 54.16% miscarried before the eighth week of their pregnancy. The researchers did not find a significant relationship between the subjects' depression scores and pregnancy outcome. In patients with a low cortisol level on the day of egg retrieval, chances of pregnancy were significantly higher (p=0.01). The relationship between the two variables of imperfect eggs and cortisol level was significant (p=0.011); higher levels of cortisol at the beginning of treatment correlated with a greater number of imperfect eggs in patients. The relationship between the number of miscarriages and cortisol levels was not significant. In conclusion, there is a significant relationship between the level of the hormone cortisol in the serum of patients undergoing IVF and their pregnancy and number of imperfect oocytes. Therefore, controlling anxiety and depression in infertile women, which results in lower levels of cortisol during their IVF treatment, can lead to better treatment outcomes.
Keywords: Cortisol, IVF, infertility

INTRODUCTION

As a treatment for infertility, IVF is a popular method with high chances of success. Depending on the mother's age, the success rate of this method varies from 13% to 35%. A wide variety of factors such as the age of the mother, physical factors, environmental factors, lifestyle, and psychological factors can affect the outcome of IVF [1]. Infertility treatments, due to the uncertainty of their results, can bring about stress, anxiety and even depression [2]. Both physically and psychologically, IVF is a stressful treatment whose outcomes are affected by a variety of known and unknown factors [2, 3]. The source of this chronic stress

is the risk of permanent infertility and loss of all hope for fertility. Moreover, the treatment process of IVF itself and the possibility of failure at any of the stages and the chances of miscarriage after positive pregnancy test results can aggravate the stress [4, 5].

It is estimated that 40.8% and 86.8% of infertile women suffer from depression and anxiety respectively [6, 7], which are reinforced by the length, complications, and stressfulness of the modern treatments for infertility [7, 8].

Various mechanisms have been suggested with regard to the effects of stress on infertility: stress can disturb the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system, thereby changing the secretion pattern of cortisol; the release of this biomarker adversely affects the stability of pregnancy, and the higher the level of stress, the more severe the disturbance [2, 5, 8, 9]. Also, many studies support the role of the sympathetic nervous system in controlling the secretion of the steroid and maturation of follicle: it has been proved that changes in the ovarian fluid caused by stress-induced hormones influence the development of follicles [2].

The hormone cortisol has immunity characteristics that can possibly affect the immunologic conditions necessary for implantation [2]. An increase in the concentration of the hormone due to stress and anxiety can delay or prevent the development of follicle and the implantation of blastocyst; it can also, through its impact on the granulosa cells, adversely affect the quality of an egg and reduce the chances of fertilization in IVF [2, 5].

Since changes in hormone levels as a result of anxiety and depression can affect the outcome of IVF in infertile women, the present study is dedicated to investigating the relationship between the cortisol hormone levels in infertile women with mild to moderate depression and their IVF results throughout and after the treatment process to identify the consequences.

MATERIAL AND METHOD
Patient

In this prospective study, the subjects were selected from the infertile women who were visiting the infertility center of Shiraz University of Medical Sciences; sampling was based on the purposive method. The sampling and follow-up were conducted from April 2014 to March 2015. The patients who had been examined by the specialists at the infertility center and judged to require IVF were asked to complete the Beck inventory. 74 infertile women with scores indicating mild to moderate depression (10-47) were selected and asked to complete the informed consent form for participation in the study. The other inclusion criteria were having general heath, not using alcoholic drinks, not being a smoker or a drug addict, not being affected by a chronic disease, and not having received any form of psychological therapy.

Measurement of hormones

Blood samples were taken from all the participants at three stages: 1) before the treatment (T_1), 2) on the day of egg retrieval (T_2), and 3) and on the day of transferring the embryo to the uterus (T_3). The samples were taken at the laboratory of the Mother and Child Hospital before the treatment process from 8 a. m. to 9 a. m. The amount of blood taken at each stage was 3 cc; the samples were kept at -30 degrees Celsius. The amount of the hormone cortisol in the serum samples was measured using the ELISA method. The laboratory results were recorded and analyzed after the completion of the subjects' treatments when the outcomes were known. In the present study, a positive IVF outcome was defined as a positive pregnancy test, presence of a gestational sac at week eight and hearing the embryo's heartbeat through vaginal sonography after the transfer of the oocyte, and continuation of pregnancy until week 12.

Statistical analysis

The collected data were analyzed using SPSS, v. 16. Independent t-test was used to examine the relationship between the participants' cortisol levels and depression scores with their pregnancy outcomes. To determine the relationship between the numbers of perfect and imperfect eggs with the subjects' cortisol levels and depression scores, the researchers used Pearson correlation coefficient. Finally, Mann-Whitney test was used to study the relationship between the number of miscarriages and the above-mentioned variables. Significance level in the present study was set at 0.05.

RESULTS

The collected data from 74 women whose blood samples had been taken and had reached the embryo transfer stage were analyzed. Table 1 shows the demographic characteristics of the participants. The participants' age varied from 21 to 41. Their average length of infertility was found to be 5.57 years.

Table 1- Quantitative presentation of the demographic characteristics of the participants (n=74)

Variable	M±SD	Minimum	Maximum
Age (years)	30.81±5.05	21	41
BMI (kg/m^2)	25.67±4.27	18.83	37.64
Length of infertility (years)	5.74	1	19

In 75.7% of the cases, infertility was of the primary type and in the rest it was of the secondary type. With regard to the cause of infertility, in 23.43% of the cases the cause was a male factor, in 67.25% of the cases it was a female factor, in 86.14% of the cases it was a combination of female and male factors, and unknown factors accounted for 21.16% of the cases. Based on the collected data, about 60% of the couples had not used any contraceptive methods from the beginning of their married lives (Table 2).

Table 2- Qualitative presentation of the demographic characteristics of the participants (n=74)

Variable		n	percentage
Type of infertility	Primary	56	75.7
	Secondary	18	24.3
Cause of infertility	Male	32	43.2
	Female	19	25.7
	Both	11	14.9
	Unknown	12	16.2
Use of contraceptive methods	Yes	30	40.54
	No	44	59.45

The average levels of the hormone cortisol in the participants on the day of egg retrieval in the unfertilized women and the fertilized women were 16.12 ± 8.56 and 11.47 ± 5.08 respectively; the difference was significant (p=0.01); a reduction in the cortisol level on this day increased the chance of fertilization and vice versa. However, the level of the hormone cortisol as measured before the treatment and on the day of embryo transfer did not differ significantly between the fertilized and unfertilized women. Also, there was not a significant relationship between the participants' depression mean scores and fertilization (p=0.67) (Table 3).

Table 3-The relationship between cortisol levels and depression scores and the fertilization of the fertilized participants (n=24) and unfertilized participants (n=50)

Variable	Fertilization status	Mean	SD	p-value
Cortisol level T_1	Fertilized	20.46	8.16	0.59
	Unfertilized	19.29	9.06	
Cortisol level T_2	Fertilized	11.47	5.08	0.01
	Unfertilized	16.12	8.56	
Cortisol level T_3	Fertilized	15.67	6.90	0.60
	Unfertilized	16.65	7.78	
Depression mean score*	Fertilized	23.95	9.16	0.67
	Unfertilized	23.04	8.24	

*depression means score at the beginning of the study
T_1: before the intervention; T_2: day of egg retrieval; T_3: day of embryo transfer

There was a significant relationship between the two variables of imperfect eggs and initial cortisol level in infertile women (p=0.011); the positive correlation coefficient showed that the relationship was direct; that is the higher the level of the hormone cortisol at stage T_1, the greater the number of imperfect eggs. Yet, in the other stages, cortisol levels were not found to correlate with the number of perfect eggs (Table 4).

Table 4-The relationship between cortisol levels and depression scores and the number of perfect and imperfect eggs

Variable	Egg	Pearson correlation coefficient	p-value
Cortisol T_1	Imperfect	0.296	0.011
	Perfect	0.121	0.30
Cortisol T_2	Imperfect	0.061	0.61
	Perfect	0.070	0.55
Cortisol T_3	Imperfect	-0.093	0.44
	Perfect	-0.026	0.83
Depression mean score*	Imperfect	-0.048	0.68
	Perfect	-0.025	0.83

* Depression means score at the beginning of the study
T_1: before the intervention; T_2: day of egg retrieval; T_3: day of embryo transfer

24 women whose pregnancy tests were positive were monitored until the eighth week; through vaginal sonography, the gestational sacs and embryos' heartbeats were checked. In only 11 cases, the embryos' heartbeats were heard and the gestational sacs were seen. In order to establish the relationship between the frequency of miscarriage and hormone cortisol level in these 24 women, the researchers used Mann-Whitney test. Based on the significance levels, cortisol levels did

not correlate with the frequency of miscarriage in any of the stages T_1, T_2, or T_3 (Table 5).

Table 5-The relationship between cortisol levels and depression scores and the frequency of miscarriage in the fertilized participants (n=24)

Variable	Miscarriage	Mean	SD	p-value
Cortisol T_1	Yes	24.68	6.30	0.134
	No	19.20	7.73	
Cortisol T_2	Yes	15.50	6.10	0.201
	No	9.95	4.29	
Cortisol T_3	Yes	15.73	4.45	0.394
	No	13.12	3.96	

T_1: before the intervention; T_2: day of egg retrieval; T_3: day of embryo transfer

DISCUSSION

24 out of 74 women who were treated by IVF got pregnant; and 13 out of the 24 pregnant women miscarried before the eighth week. The researchers did not find a significant relationship between the participants' depression scores and fertilization outcome. Patients whose cortisol levels were low on the day of egg retrieval were found to have significantly higher chances of pregnancy (p=0.01). The researchers also found a significant relationship between the two variables of imperfect eggs and cortisol level T_1 (p=0.011); the higher the participant's level of hormone cortisol in stage T_1 was, the higher the number of her imperfect eggs. The researchers did not find a significant relationship between the frequency of miscarriage and the participants' cortisol levels.

In the present study, there was not a relationship between the psychological data obtained from the questionnaire results and the treatment outcome. Similarly, Brain's study (2007) showed that the extent of anxiety and depression in patients who were being treated for infertility did not affect their treatment outcomes [10]. Hashemi came up with the same result in her study [8]. Yet, some studies, e.g. Turner *et al.;*, showed that increased anxiety and stress correlated with reduced chances of pregnancy [11]. It has also been observed that women who are under greater stress often require more embryo transfer cycles to become pregnant [12]. These differences in findings can be attributed to the differences between the instruments used by researchers to measure their subjects' psychological characteristics. Thus, it appears that self-report depression questionnaires do not provide a suitable criterion for predicting the outcome of infertility treatments and more accurate instruments are required.

The pathophysiological changes that occur in women in response to anxiety and depression-inducing conditions are very complicated, yet important. In the present study, the stress-related variable measured and analyzed was the hormone cortisol. Previous studies showed that the hormone cortisol could delay or prevent the development of ovarian follicles and embryo implantation [5]. Yuan *et al.;* discovered that a high concentration of the hormones, cortisol and norepinephrine, on the day of egg retrieval can reduce chances of pregnancy [2, 5]; this finding is consistent with the results of the present study. In the present study, the patients whose cortisol levels were low on the day of egg retrieval had significantly higher chances of pregnancy. This seems to be due to the fact that cortisol adversely affects the quality of follicles and prevents their proper development [13]. However, Smeenk *et al.;* who measured the levels of cortisol and norepinephrine in their subjects' urine samples, found that only the amount of the hormone adrenalin correlated with women's fertilization and the cortisol level did not have any effect on their chances of pregnancy [14]; this discrepancy between the results can be due to the differences between the types of the samples taken and the time when the samples were taken. In the present study, the researchers took blood samples at a certain time —right at the time of egg retrieval—which is very close to the time of its secretion.

To study the relationship between the hormone cortisol with the growth of the oocytes, the researchers compared the level of the hormone with the number of perfect and imperfect oocytes in the participants. Based on the collected data, the participants' initial levels of cortisol correlated with the number of their imperfect oocytes; the higher the participant's level of cortisol, the greater the number of her imperfect oocytes (imperfect in terms of size and growth). In other words, high levels of cortisol in infertile women adversely affected the growth of the oocytes. According to the study of Michael *et al.;* the adverse effect of the hormone cortisol on the granulosa cells reduced the quality of the oocytes and delayed their development [13-15].

All the patients who were pregnant based on the BhCG test on the 16th day after embryo transfer were monitored in weeks 8 and 12. Out of the 24 pregnant women, 13 miscarried before week 8; the sonography results of the remaining 11 participants showed that their embryos' heartbeats could be heard. In week 12, all of them were checked again through sonography and were found to be healthy and their pregnancies continued. The researchers evaluated the relationship between miscarriage in the patients and

their cortisol levels and did not find any links between cortisol levels and frequency of miscarriages; this conclusion is consistent with the results of several other studies, including Serafina [4]. Miscarriage in the first three months of pregnancy, especially the first few weeks, is often due to genetic factors and the poor quality of the oocytes as a result of women's advanced ages. Similar to the present study, in most studies the average age of the female subjects was high (about 30 and above). Therefore, it appears that miscarriage in such cases can be attributed to genetic factors rather than depression, anxiety, and the resultant hormones.

CONCLUSION

The activity of the hypothalamic–pituitary–adrenal system as a result of stress, anxiety and depression and the secretion of such hormones as cortisol, regardless of the results of depression questionnaires, can affect the rate of pregnancy and the quality of eggs in women who are undergoing IVF. The results of the study showed that increased cortisol on the day of egg retrieval (T_2) can, by adversely affecting the quality of follicles or the number of perfect oocytes, have a negative impact on the fertilization outcome of infertile women. Moreover, high levels of cortisol at the beginning of infertility treatment can adversely affect the quality of the growing eggs. Thus, it seems necessary that women who are being treated for infertility should receive interventions that will reduce their stress, anxiety and depression from the beginning of the treatment process until the very end of it.

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