# The association of serum and follicular fluid cortisol levels with reproductive hormones of infertile women undergoing intracytoplasmic sperm trails

**Noor Mahmood Ahmed [1], Rana A-R Al. Saadi [1], Essraa M. Al-Essawe [2]**

[1]Department of Applied Embryology, High Institute of Infertility Diagnoses and Assisted Reproductive Technologies, Al-Nahrain University, Iraq

[2]Department of Physiology, High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, AL-Nahrain University, Iraq

## Abstract

**Background:** In human-assisted reproduction, both partners need to be evaluated during counseling because some of the causes of infertility are due to male and female factors.

**Aim:** to investigate the relationship between cortisol levels and reproductive hormones in serum and follicular fluid in women selected for the Intracytoplasmic Sperm Injection outcome

**Material and method:** On cycle day two, each female provided a blood sample for hormonal analysis of FSH, LH, AMH, and progesterone. To induce multi-follicular growth and increase the number of oocytes retrieved, the flexible antagonist ovarian stimulation protocol was used. Cortisol and progesterone levels were measured in follicular fluid and blood samples taken during oocyte retrieval. Blood samples were also taken on the day of embryo transfer to estimate cortisol hormone levels.

**Result:** The progesterone hormone at cycle day 2 has a significant correlation with cortisol in follicular fluid on day of oocyte retrieval. Also, AMH has a significant correlation with cortisol on the day of embryo transfer. However, FSH is high significant with LH hormone. There was cortisol in serum that had a significant correlation with progesterone at oocyte retrieval.

**Conclusion**: According to the findings of this study, serum cortisol levels on the day of embryo transfer were linked to serum AMH levels. Furthermore, follicular fluid cortisol measures rather than serum cortisol could provide a better parameter for assessing ICSI outcome.

**Keyword:** Cortisol; ICSI; Follicular fluid; blood serum; hormones

**DOI Number: 10.14704/nq.2022.20.9.NQ44409** **Neuro Quantology 2022; 20(9):3588-3593**

## Introduction:

Infertility occurs when a couple is unable to produce offspring after one year of regular and unprotected sexual activity (Ishiura *et al.,* 2019). The culprit could be a woman, a man, or both. The hypothalamic-pituitary-adrenal (HPA) axis is thought to be the psychological pathway by which stress influences reproductive functioning. This system is considered central to the human stress response, resulting in the secretion of the stress hormone cortisol, which is generally released in higher doses under stressful conditions (Dickerson S., Kemeny M.E.,2004). Cortisol is a glucocorticoid hormone that regulates a variety of processes, including metabolism, blood pressure, and immune response, as well as being a reliable biological indicator of a range of negative health effects ( Marieb E.N., Hoehn K.,2007) ( Marieb E.N., Hoehn K.,2007). Stress, according to a growing body of evidence, may have a negative impact on IVF treatment outcomes by activating the HPA. Cortisol levels have an effect on clinical pregnancy, oocyte number, oocyte fertilization, oocyte cleavage, and miscarriage rates in patients undergoing IVF cycles. Cortisol levels typically rise during the menstrual cycle's follicular phase. It is thought that glucocorticoids interfere with aromatase activity and granulose cell activity in sample for hormonal examination of FSH, LH, AMH, and 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 (Ellen Anckaert, *et al*.,2002). After that, all of them underwent a controlled ovarian stimulation protocol and frequent monitoring by ultrasound, with serum E2 for all cases. The flexible antagonist protocol was used as an ovulation induction protocol. On the day of oocyte retrieval, blood samples and follicular fluid were obtained to measure cortisol, progesterone On the day of embryo transfer, blood samples were collected to determine cortisol levels. An enzyme-linked immunosorbent assay (ELISA) was used (Gan SD. et al., 2013).

**Result:**

A positive correlation was observed between (E2) and AMH with correlation coefficient (CC=0.255; p = 0.045). Also a high significant negative correlation was found between E2 and FSH (CC=0.367; p= 0.003) while, the progesterone level (P4) has a significant positive correlation with cortisol level in follicular fluid that (CC = 0.33; p = 0.01). The AMH was significantly correlated with cortisol at embryo transfer day (CC = 0.26; p = 0.043) Furthermore FSH had high significant with LH hormone (CC = 0.370; p =0.004).

Cortisol in serum was high significant correlate with cortisol in follicular fluid (CC = 0.43; p = 0.001), cortisol in serum was high significant with cortisol level at day of embryo transfer (CC = 0.58; p = 0.000). The cortisol levels in serum have significant correlation with progesterone (CC = 0.43; p =0.041).

Cortisol in serum at embryo transfer was high significant correlate with cortisol in embryo transfer (CC = 0.353; p = 0.005).

the early stages of follicular development (Fateh*, et al.,*1989). Follicular fluid (FF) is an important microenvironment for ovarian germ and somatic cell maturation and growth. The composition of follicular fluid is related to cell differentiation, gamete quality, and follicular wall rupture (Baka and Malamitsi-Puchner, 2006; El, 2013 and Kim et al., 2016). Follicular fluid accumulates during folliculogenesis, which begins in the ovary cortex with the recruitment of primordial follicles and ends in ovulation or atresia (Basini *et al.,* 2008 and la Cour Poulsen *et al.,* 2019). In an updated international glossary on reproductive treatment and infertility, assisted reproductive technology (ART) is defined as any procedure involving the *in vitro* treatment of human oocytes, spermatozoa, or embryos for reproductive purposes. Outside the body spermatozoa and oocytes combine, returning the embryos to the uterus. Intracytoplasmic Sperm Injection (ICSI) is an *in vitro* fertilization method (IVF) that involves injecting a single spermatozoon into the cytoplasm of an oocyte, bypassing the oolemma and zona pellucida (Simopoulou et al., 2016). Some of the causes of infertility are male factor (severely damaged seminal fluid characteristics), advanced maternal age, extraction of low quality or few oocytes, previously unsuccessful fertilization with standard insemination cycles, and unexplained infertility. The current study aimed to determine the relationship between cortisol levels in serum and follicular fluid with the other of reproductive hormones during the ICSI cycle.

**Subjects, Material and Method:**

This prospective clinical trial investigation was conducted at the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University, Baghdad, Iraq, during the period from November 2021 to June 2022. The ICSI program was intended for 63 infertile Iraqi women suffering from primary or secondary infertility. On cycle day 2, all patients who have intended to do an ICSI cycle are given a complete history and a blood

**Table1: correlation between reproductive hormonal levels at different stage of ICSI cycle and serum and follicular cortisol**

| | | Progesterone ng/mL (CD 2) | AM H ng/ML (CD 2) | FSH Miu/mL (day 2) | LH Miu/mL (CD 2) | Cortisol mg/dL in serum at oocyte retrieval | Cortisol mg/dL in follicular fluid at oocyte retrieval | Cortisol mg/dL At embryo transfer | Progesterone In serum at oocyte retrieval |
|---|---|---|---|---|---|---|---|---|---|
| E2 (pg/mL) (Mide cycle) | Pearson Correlation | -0.003 | 0.255* | -0.367** | 0-.023 | -0.167 | -0.172 | -0.134 | -0.056 |
| | Sig. (2-tailed) | 0.981 | 0.045 | 0.003 | 0.859 | 0.206 | 0.179 | 0.304 | 0.794 |
| Progesterone ng/mL (cycle day 2) | Pearson Correlation | 1 | 0-.040 | 0.136 | -0.233 | 0.240 | 0.331 | 0.254 | 0.182 |
| | Sig. (2-tailed) | | 0.764 | 0.310 | 0.084 | 0.078 | 0.011 | 0.057 | 0.417 |
| AMH ng/ML (cycle day 2) | Pearson Correlation | | 1 | -0.223 | 0.069 | 0.215 | -0.193 | 0.262* | 0.159 |
| | Sig. (2-tailed) | | | 0.082 | 0.599 | 0.106 | 0.134 | 0.043 | 0.458 |
| FSH (miu /mL) (cycle day2) | Pearson Correlation | | | 1 | 0.370** | 0.245 | 0.167 | 0.150 | 0.123 |
| | Sig. (2-tailed) | | | | 0.004 | 0.064 | 0.194 | 0.252 | 0.568 |
| LH miu /mL (cycle day 2) | Pearson Correlation | | | | 1 | -0.221 | -0.146 | -0.107 | 0.170 |
| | Sig. (2-tailed) | | | | | 0.102 | 0.265 | 0.425 | 0.438 |
| Cortisol(mg/dL) in serum day of oocyte retrieval | Pearson Correlation | | | | | 1 | 0.432** | 0.575** | 0.429* |
| | Sig. (2-tailed) | | | | | | 0.001 | 0.000 | 0.041 |
| Cortisol(mg/dL) in follicular fluid day of oocyte retrieval | Pearson Correlation | | | | | | | 1 | 0.334 |
| | | | | | | | | | 0.119 |
| Cortisol(mg/dL) in serum at embryo transfer | Sig. (2-tailed) | | | | | | 1 | 0.353** | 0-.104 |
| | Sig. (2-tailed) | | | | | | | 0.005 | 0.629 |

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

link between FSH and LH at cycle day2. These findings are consistent with (Rehman et al., 2014), who investigated how the FSH-LH ratio affects IVF success. The importance of the FSH-LH ratio as a predictor of outcomes after ICSI procedures was identified and verified. "Normal" and "high" responders are typically defined as having LH and FSH concentrations within normal ranges and a low FSH/LH ratio, respectively. However, there was non-significant correlation between LH hormone and cortisol , these finding agreement with (Macfarlane , *et al.,*2000 and Breen , et al., 2005).they concluded cortisol reduce LH pulse through decreased estradiol production, which is anticipated to decrease negative feedback and enable an increase in the amplitude of LH pulses. The significant findings for cortisol in serum and follicular fluid at day oocyte retrieval have been reported to be the same ( Bider .Shine.,1998 and Yong et al., 2000). They also observed a relationship between cortisol levels in follicular fluid and serum. The cortisol concentration of serum is substantially identical to that of follicular fluid, suggesting that cortisol bound to CBG entered the follicular fluid from the circulation. Adrenaline accumulates in the follicular fluid of stimulated follicles. There was no change in total cortisol levels between "good" and "poor" responder patients after IVF treatment with ovarian stimulation, according to this study. Cortisol levels in blood and follicular fluid were found to be significantly higher on the day of embryo transfer. These findings are consistent with the findings of Frederiksen et al. (2015), who concluded that extreme anxiety caused women to experience more physical symptoms (abdominal distention, breast engorgement, nausea, faintness, diarrhoea, sleep disruption) and psychological discomfort on the day of embryo transfer, which is more stressful than the day of oocyte retrieval.

## Conclusion:

The findings of the current study indicate that follicular fluid cortisol levels produced by circulation circumstances, as well as the duration of intrafollicular cortisol interaction, may have a role in ICSI outcome. More investigations are required in this regard.

## Discussion:

At cycle day 2, there was a significant correlation between E2 and AMH, these finding was consistent with those of (Grynberg *et al.,*2012). E2 would stimulate AMH via acting on the estrogen-receptor (ER) α. These findings conflict with research conducted by (Cook CL. *et al.,* 2002 and Couse *et al.,* 2005). Their finding revealed a highly significant adverse correlation between AMH and estradiol concentrations in pre-ovulatory follicular fluid from small antral follicles. The comparatively modest quantities of AMH and E2's inhibitory effect on AMH via ER do not appear to have any influence on the massive levels of estradiol present in preovulatory follicular fluid. ER.β is the primary receptor in granulosa cells from developing follicles. The current investigation found a significant relationship between FSH and $E_2$, and that increased induced a rise in FSH. These findings are consistent with those of (Cook *et al.* (2002). who revealed that FSH stimulate secretion of $E_2$. However (Corrine K.Welt, 2003) found a negative correlation between FSH and E2 via its action on the hypothalamus, and their findings contradicted the current study. In a correspond with a result by (Nielsen, S.E et al., 2013 and Nielsen, S.E. , et al., 2014), follicular fluid progesterone and cortisol levels have been found to be positively correlated at the day of oocyte retrieval. Two separate mechanisms could be interpret these finding . First, because progesterone is a precursor in the biosynthesis of cortisol, more cortisol may be created when women have higher levels of circulating P. (i.e., during the luteal phase of the menstrual cycle). Furthermore, progesterone binds to corticosteroid binding globulin in a competitive way, reducing the amount of cortisol that can bind to the protein while likely increasing the amount of available cortisol.

On the day of embryo transfer, there was a significant association between AMH and cortisol levels. This is consistent with the findings of Kudesia et al. (2018), who found a positive relationship between chronic stress and serum AMH. While this contradicts the findings of (Pigny P. et al., 2006; and Carlsson I.B. et al., 2006), they discovered a substantial correlation between psychological stress and lower AMH levels in infertile women. The current study's findings indicate a substantial

*Clinical Chemistry and Laboratory Medicine* VOL.40, N(8): pp824-31 DOI:10.1515/CCLM.2002.143

Fateh, M., Ben-Rafael, Z., Benadiva, C.A., Mastroianni, L., Jr and Flickinger, G.L. ,(1989), Cortisol levels in human follicular fluid. *J.Fertil. Steril.,* vol 51,pp. 538-541.

Francisco: Benjamin Cummings.

Frederiksen, Y., Farver Vestergaard, I., Skøvgard, NG., Ingerslev, HJ. and Zachariae ,R.,(2015) Efficacy of psychosocial interventions for psychological and pregnancy outcomes in infertile women and men a systematic review and meta-analysis. *BMJ. Open*. Vol .5,N.(1), p,6592-6592.

Grynberg, M., Pierre, A., Rey, R., Leclerc, A., Arouche, N., Hesters, L., Catteau Jonard, S.,Frydman, R., Picard, JY.and Fanchin, R., (2012) Differential regulation of ovarian anti-Mullerian hormone (AMH) by estradiol through alpha- and beta-estrogen receptors. *J. Clin Endocrinol Metab* ,VOL.97 ,PP.1649–1657.

Ishiura, S. *et al.* (2019) New vaccine therapy for Alzheimer ' s disease , VOL.6,N. (1), pp. 1–6. doi: 10.24659/gsr.6.1.

Kim, K. *et al.,(*2016) Associations between follicular fluid high density lipoprotein particle components and embryo quality among in vitro fertilization patients, *J. of Assisted Reproduction and Genetics*. Journal of Assisted Reproduction and Genetics).

Klonoff-Cohen, H., Chu, E., Natarajan, L., Sieber, W.,(2001) A prospective study of stress among women undergoing in vitro fertilization or gamete intrafallopian transfer*, J.Fertil Steril*. Vol.76,N.(4), p: 675-687.

Kudesia, R., Lipton, RB., Neal-Perry, GS., (2018) impact of stress on ovarian reserve and function*.J. Fertil Steril* ,VOL.110 ,N.(4), PP.149 - 150.

la Cour Poulsen, L. *et al.,* (2019) Progressive changes in human follicular fluid composition over the course of ovulation: quantitative proteomic analyses ,Elsevier, *Molecular and cellular endocrinology*. VOL 495, pp. 110522.

Macfarlane, MS., Breen, KM., Sakurai, H., Adams, BM., Adams ,TE.,(2000) Effect of duration of infusion of stress-like concentrations of cortisol

**Reference:**

Baka, S. and Malamitsi-Puchner, A., (2006) Novel follicular fluid factors influencing oocyte developmental potential in IVF A review, *Reproductive BioMedicine Online*. Reproductive Healthcare Ltd, Duck End Farm Dry Drayton, Cambridge CB23 8DB, UK, VOL12 ,N(4), pp. 500–506. doi: 10.1016/S1472-6483(10)62005-6.

Basini, G. *et al.,* (2008) Reactive oxygen species and anti-oxidant defences in swine follicular fluids, *J. Reproduction, Fertility and Development*, VOL20,N(2), pp. 269–274.Binder, D., Shine, S., Turkaspa, I., Leuren, J., Dor J.,(1998) Cortisol concentrations in follicular fluid of low responder patients. *J.Hum Reprod* VOL.1 ,PP.27–9.

Breen, K.M., Billings, H.J., Wagenmaker, E.R., Wessinger, E.W.and Karsch FJ., (2005) endocrine basis for disruptive effects of cortisol on preovulatory events *.J. Endocrinology* , VOL. 146,pp.2107–2115

Carlsson, I.B., Scott, J.E., Visser, J.A., Ritvos, O., Themmen, A.P., Hovatta, O.,(2006) Anti Mullerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro. *J.Hum. Reprod*. ,VOL.21 , PP2.23–7. doi.org/10.1016/j. fertnstert.2018.07.442.

Cook, CL., Siow, Y., Brenner, AG., Fallat, ME.,(2002) ,Relationship between serum Mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. *J.Fertil Steril* ,VOL. 77,pp. 141–146

Couse, J.F., Yates, M.M., Deroo, B.J., Korach, K.S.,(2005) Estrogen receptor beta is critical to granulosa cell differentiation and the ovulatory response to gonadotropins. *Endocrinology* VOL146, PP.3247–3262.

Dickerson S, Kemeny ME.,(2004) Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol Bull. VOL 3,PP.355–9.

Ellen Anckaert, Johan Schiettecatte, Marleen Mees, Johan Smitz ,(2002) Clinical Validation of a Fully Automated 17β-Estradiol and Progesterone Assay (VIDAS®) for Use in Monitoring Assisted Reproduction Treatment on follicular development and the preovulatory surge of LH. *J.Reprod. Sci*., VOL.63,pp. 167–175

Marieb EN, Hoehn K(2007). Human anatomy & Physiology. 7th ed. San

Nielsen, S.E., Ahmed, I., Cahill, L.,(2013a) Sex and menstrual cycle phase at encoding influence emotional memory for gist and detail. *Neurobiol. J.Learn Mem*.,VOL .106, PP. 56-65.

Nielsen, S.E., Ahmed, I., Cahill, L.,(2014) Postlearning stress differentially affects memory for emotional gist and detail in naturally cycling women and women on hormonal contraceptives. Neurosci, *J.Behav.*. Vol. 128,PP .482-493.

Olooto, Wasiu Eniola, Amballi, Adebayo Adetola, and Banjo, Taiwo Abayomi .,(2012) A review of Female Infertility; important etiological factors and management, *J. of Microbiology and Biotechnology* ,VOL 2,N (3), pp:379-385.

Pigny, P., Jonard, S., Robert, Y., Dewailly, D.,(2006) Serum anti-Mullerian hormone as asurrogate for antral follicle count for definition of the polycystic ovary syndrome. *J. Clin. Endocrinol Metab* ,VOL.91 , PP.941–5.

Rehman ,R., Khan, R., Baig, M., Hussain, M., Fatima, SS.,(2014) Estradiol progesterone ratio on ovulation induction day: a determinant of successful pregnancy outcome after intra cytoplasmic sperm injection. Iran *J. Reprod Med* , VOL. 12 ,PP. 633-40.

Simopoulou, M., Giannelou, P., Bakas, P., Gkoles, L., Kalampokas, T., Pantos, K. and Koutsilieris, M. (2016) Making ICSI safer and more effective: a review of the human oocyte and ICSI practice *in vivo*,VOL 30, N (4), pp. 387-400

Yong, P., Martin, C., Thong, J.,(2000) A comparison of psychological functioning in women at different stages of in vitro fertilization treatment using the mean affect adjective check list. *J. Assist Reprod Genet*. ,VOL.17,N.(10) ,PP.553-556.