Impact of serum and follicular fluid kisspeptin and estradiol on oocyte maturity and endometrial thickness among unexplained infertile females during ICSI

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Impact of serum and follicular fluid kisspeptin and estradiol on oocyte maturity and endometrial thickness among unexplained infertile females during ICSI

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Abstract

Objective

To relate serum and follicular fluid (FF) kisspeptin and estradiol levels in different stages of stimulation during Intracytoplasmic Sperm Injection (ICSI) with oocyte maturity and endometrial thickness among unexplained infertile females.

Methods

This cross-sectional study was carried out at the Australian Concept Infertility Medical Centre from March 2017 till March 2018. Fifty unexplained infertile females, booked for ICSI, were included in the study. Serum kisspeptin and estradiol were estimated by Enzyme-Linked Immunosorbent Assay in all four stages; 1: follicular stimulation, 2: ovulation induction, 3: oocyte pickup, and 4: embryo transfer. FF was aspirated during oocyte retrieval (stage 3) for the analysis of KP and estradiol. Pregnancy outcomes were categorized as non-pregnant, preclinical abortion, and clinical pregnancy.

Results

The age of the study subjects was 32.04 ± 2.29 (Mean±SD) years, with mean BMI of 28.51 ± 4.15 (Mean±SD) kg/m². Mean serum kisspeptin and estradiol levels increased in all subjects as the stimulation proceeded stages 1–3; however, the mean dropped after retrieval of the oocytes (stage 4). Out of 27 female subjects who completed the cycle, 17 remained non-pregnant, 4 had preclinical abortion, and 6 acquired clinical pregnancy. The FF kisspeptin concentration was significantly higher than serum concentrations and positively correlated with serum and FF estradiol concentrations. FF-kisspeptin correlated with serum kisspeptin in Stage 3 (r = 0.930, p<0.001), maturity of oocyte (r = 0.511, p = 0.006) and endometrial thickness (r = 0.522, p = 0.005). Kisspeptin in stage 3 was also found to correlate with endometrial thickness (r = 0.527, p = 0.005) and with estradiol (r = 0.624, p = 0.001) independently.
Conclusion

Increase in serum and FF-kisspeptin and estradiol levels from stages 1 to 3, resulted in an optimum endometrial thickness, probability of fertilization of oocytes and chances of clinical pregnancy in Assisted Reproductive Techniques /ICSI cycles of unexplained infertile females.

Introduction

Regulation of fertility by hypothalamo-pituitary-ovarian (HPO) axis involves the pulsatile release of Gonadotropin-Releasing Hormone (GnRH) from the hypothalamus, stimulating the release of Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) from the anterior pituitary that in turn acts on the ovaries to control gametogenesis [1]. Kisspeptin (KP) is a neuropeptide that acts via a G-protein coupled receptor and has been found to be the upstream regulator of GnRH release. Hence it plays important roles in female reproduction such as the onset of puberty, ovulation, implantation, placentation and metabolic regulation of fertility [1–3]. The role of KP in puberty onset is supported by the observations that loss of function due to mutations in KP result in the absence of pubertal development, whereas girls with central precocious puberty are found to have higher KP levels [4].

Furthermore, expression of genes encoding KP in ovarian tissue and granulosa-lutein cells as well as on the glandular and luminal epithelial cells of the endometrium, signifies the role of KP in the regulation of reproductive functions [5, 6]. During the ovarian cycle, KP levels increase from the follicular stage to the luteal stage [4]. Estrogen released by the ovaries acts through its receptors present on KP secreting neurons and, in turn, contributes as the negative feedback on KP secretion [6].

Infertility is the inability to conceive after one year of regular unprotected intercourse [1, 7], an attribute of the dysfunction of the HPO, genetic factors, hormonal imbalances and environmental influences [8]. However, in 10–30% of couples seeking infertility treatment, the cause cannot be elucidated, referred to as ‘Unexplained Infertility’ (UI). In UI cases, the tubal patency, anatomy of the uterine cavity, serum progesterone levels in the mid-luteal stage and the semen analysis (male partner) are normal [9]. Treatment options for unexplained infertile females are superovulation and Artificial reproductive techniques (ART), which In Vitro Fertilization (IVF) and ICSI [10].

 Constituents of FF indicate variations in the ovarian follicle cells in response to the gonadotrophin’s administration, by producing various biochemically active substances. These may affect oocytes’ sustainability and growing capability and are also linked to fertilization outcomes and early post-fertilization development [11]. Literature reveals that follicular fluid (FF) produced by the granulosa cells secrete hormones such as growth hormone, insulin-like growth factor-I, estradiol (E2), and progesterone [11]. Fluctuations in serum and FF-KP levels have been observed during the preovulatory and luteal stages of the reproductive cycle [11–14]. Furthermore, at ovulation induction (OI) during ART, higher serum KP levels are known to be associated with a better pregnancy outcome [6]. Low serum KP levels in UI females [9], and significant correlation of FF-KP with E2 concentrations and follicular maturity in infertile females undergoing ICSI [13], show a link between serum and FF KP concentrations and estradiol with the success of ICSI.

Therefore, we aim to determine the relationship between serum and FF-KP and estradiol concentrations with oocyte maturity and endometrial thickness (determinants of successful conception) among unexplained infertile females during ICSI.
Material and methods

This cross-sectional study was carried out at the Australian Concept Infertility Medical Center (ACIMC) from March 2017 to March 2018. Ethical approval was obtained from the Institute Review Board of the ACIMC and Aga Khan University, Pakistan (ERC#3331-BBS-ERC-14), and all participants signed written consent.

Women with unexplained infertility (n = 50); duration of infertility more than two years with regular menstrual cycles (21–35 days), normal endocrine profile (normal levels of FSH (<11 IU/L) in early follicular phase (day 2–5), normal Prolactin levels (<20 mg/L) in serum, normal thyroid levels), pelvic ultrasonography with patent fallopian tubes, intact ovaries and uterus, no prior ovarian surgery with normal semen profile of their husband [9, 15] age 24 to 35 years booked for ICSI treatment were included in the study.

Females with uterine fibroids, endometriosis, polycystic ovaries, uterine and cervical lesions, metabolic disorders, and those following the short agonist and antagonist protocol for ICSI, were excluded. Male causes of infertility based on low sperm parameters (sperm count/motility/morphology) according to Kruger’s strict criteria were also omitted [16].

Treatment protocol

For the downregulation of the hormones, daily injections of Deca-Peptyl (Gonadotropin-releasing hormone agonist) were administered to the patients from Day 21 of the previous cycle. The treatment protocol was carried out in stages (1–4) with sequence of dropouts from the study is given in Fig 1.

Sample collection

Serum samples. The venous blood samples were collected for estimation of serum KP and estradiol at the beginning of stimulation (stage 1), at the time of hCG administration on OI day (stage 2), on OPU (stage 3) and ET days (stage 4). After collecting blood, the serum fraction and the plasma fraction were separated rapidly by centrifuge and frozen at −80°C until assayed. Serum samples were used to detect the concentration of the hormone, using commercially available ELISA kits, following the manufacturer’s protocol. The ELISA kits used were: serum KP (Cat. No: 95611, Glory BioScience, USA) with analytical sensitivity of 10.16 ng/L and intra and inter assay coefficients of variation of less than 10% and 12%, respectively, serum FSH (Kit Cat. No DKO010; DiaMetra) with the inter assay coefficient of variation, <8% and intra assay coefficient of variation <9.7% and serum LH (Kit Cat. No DKO010; Dia Metra) with inter assay coefficient of variation of <7.91%; intra assay coefficient of variation of <9.21%. Serum estradiol was determined using a commercially available kit for Human estradiol (E2) Enzyme Immunoassay Kit (Catalog # 07BC-1111 by MP BioCheck, Inc.).

Sample of follicular fluid

On the day of oocyte retrieval, two-dimensional mean diameter the follicles, were measured by TVS followed by ultrasound guided aspiration in order to pool FF samples from around one to five follicles of both ovaries, each 18-mm diameter or larger. Samples that appeared blood stained or with oocytes were discarded. For each patient, about 20–40 ml of FF were transferred to sterile conical tubes (BD Falcon, Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 1500 x G for 10 minutes at room temperature. Supernatant was aliquoted in 2ml cryovials and was stored at -80°C refrigerator until further analyzed for hormones [18].
Fig 1. Flow chart of the treatment protocol during the study period. Stage 1: Follicular stimulation (FS) (n = 50). Down-regulation was followed by controlled ovarian stimulation (COS) by gonadotrophin injections (Only Inj Puregon) for fourteen days. The dose was titrated with respect to basal FSH and follicular monitoring by transvaginal ultrasound. Out of the 50 females, 44 females reached the ovarian induction stage (stage 2). Cycle of 4 females was cancelled due to inadequate response to stimulation, whereas two did not opt to continue the treatment. Stage 2:
Pregnancy outcome. Luteal support of included females (after egg collection) was maintained by progesterone vaginal pessaries (Cyclogest 400 mg) twice a day. We assessed ß-hCG at the end of the treatment approximately 14 days after ET as the outcome marker. TVS was performed 14 days after receiving positive results of ß-hCG to confirm the presence of gestational sac and cardiac activity. Based on ß-hCG and TVS results, women were grouped as: Non-pregnant (NP) if women had ß-hCG 5–25 mIU/ml, Preclinical Abortion (PA) if women had ß-hCG >25 mIU/ml and no fetal cardiac activity on TVS. Women with ß-hCG >25 mIU/ml and evidence of cardiac activity on TVS were grouped as Clinical pregnancy (CP) [20].

Statistical analysis

All of the clinical and hormonal data were entered and statistically analyzed on SPSS IBM Statistical Package for the Social Sciences (IBM, SPSS version 21;IBM Corp Inc., Armonk, NY, USA). Descriptive analysis of continuous variables was expressed as mean ± standard deviation/error and median (interquartile range). For statistical comparison of variables among stages or groups of patients (NP, CA, CP), analysis of variance (ANOVA) was performed, where p value <0.05 and <0.01 was considered statistically significant and highly significant, respectively. Spearman correlation coefficient was utilized to detect the effect of hormones on the outcome, p value <0.05 and <0.01 was considered statistically significant and highly significant, respectively.

Results

The mean age of the study subjects was 32.04 ± 2.29 SD years, with mean BMI of 28.51 ± 4.15 SD kg/m². Clinical characteristics of all participants (n = 50) show mean FSH 7.14 ± 1.55 IU/ml and LH 5.98 ± 1.44 IU/ml. The antral follicle count before COS was 7.91 ± 1.9. The mean dose of puregons (rFSH/day) was 175 ± 24.34 in 44 subjects, OPU of them performed on 13th, 14th, or 15th day of stimulation. All the collected eggs were treated and then transferred to the incubator for about 1–2 hours prior to insemination by ICSI procedures. During this phase, follicular fluid (FF) was acquired from all follicles (measuring 18 mm or more on ultrasound) from each ovary of the patient [18]. Stage 4: Embryo transfer (ET) (n = 27). After confirmation of fertilization and cleavage (18 to 24 hours after egg collection), embryos were graded from 1–5 [19]. The embryo transfer of 27 females (blastocysts) was done on Day 5 using Sims-Wallace Embryo Replacement Catheter, under ultrasound guidance.

Comparison of outcome groups

The mean number of embryos transferred to 27 subjects were 1.63 ± 0.56. Out of these 27 female subjects, 17 remained NP, 4 had PA, and 6 acquired CP. In these subjects, the number and maturity of oocytes were 7.89 ± 1.85 and 6.89 ± 2.01, respectively, and was highest in the CP group. Table 1 describes comparison of study variables in outcome groups; NP, PA, CP). The number of mature follicles and endometrial thickness were significantly more in CP subjects when compared with NP subjects (p<0.01). The FF- KP and estradiol levels were significantly higher in CP subjects compared to NP subjects (p<0.05) (Table 1).
Fig 2 depicts the mean serum KP concentrations were observed to increase within the subjects as the stimulation preceded; however, the mean dropped after retrieval of the oocytes (stage 3). KP showed an increasing trend from stage 1 to stage 3 and was significantly higher in stages 2 & 3 compared to the first stage \((p < 0.01)\). A highly significant correlation of KP1 was observed with KP2 \((r = 0.845, p < 0.001)\), KP3 \((r = 0.833, p < 0.001)\) and KP4 \((r = 0.718, p < 0.001)\) among 27 subjects who reached the end of the stimulation successfully.

Estradiol also showed an increasing trend from stage 1 to stage 3, and was significantly higher in the latter two when compared with the first stage \((p < 0.01)\). E1 demonstrated a moderately significant correlation with E2 \((r = 0.445, p = 0.020)\), E3 \((r = 0.501, p = 0.008)\) and E4 \((r = 0.484, p = 0.011)\) \((Fig 2)\).

Comparison of serum KP and estradiol in various stages of stimulation

Serum E2, E3 and E4 were significantly higher in CP compared to PA subjects \((p<0.01)\). Similarly, serum KP2, KP3 and KP4 levels were significantly higher in CP subjects when compared to NP subjects \((p<0.05)\) \((Fig 3)\).

Serum KP and estradiol levels in NP, PA, and CP subjects showed significantly higher concentrations in OI, OPU and ET (stages 2, 3 and 4) as compared to stage 1 \((p<0.01)\), and the highest levels were found in the 3rd stage. Follicular KP and estradiol levels were highest in the CP females.

Correlation of serum and FF-KP and estradiol with determinants of pregnancy outcomes

A moderate association was found between FF-KP and FF estradiol \((r = 0.584, p = 0.001)\) \((Fig 4A)\). The FF- KP was observed to correlate with the oocyte maturity \((r = 0.511, p = 0.006)\) \((Fig 4B)\) and KP3 \((r = 0.930, p = 0.000)\) \((Fig 4C)\). Furthermore, KP3 was positively associated with E3 \((r = 0.624, p = 0.001)\) \((Fig 4D)\) and endometrial thickness \((r = 0.527, p = 0.005)\) \((Fig 4E)\).

Discussion

Kisspeptin is known to control the pulsatile surge of GnRH release, ovulation, implantation, placentation, and metabolic regulation of fertility \([2]\). Wang and Moenter, \(2020\) suggested
that arcuate and anteroventral periventricular KP neurons perform definite functions in facilitating the response to systemic variations in estradiol levels, standardizing cyclicity and LH rise [21]. Our study showed that ART was not successful in females with lower serum and FF-KP levels. The results are in accordance with KP's role in eliciting the LH surge required for oocyte maturation and ovulation. Jamil et al. (2017) reported a significantly higher KP, LH, basal and peak estradiol after ICSI treatment, in a positive pregnancy subjects compared to the negative pregnancy subjects [6]. Besides, endometrial thickness, oocyte numbers (matured and fertilized), embryo quality, and potential for developing to the blastocyst stage were
notably better in the positive pregnancy group [6]. Moreover, they reported a positive correlation between raised KP and positive pregnancy outcome, and a borderline correlation of KP with the endometrial thickness [6]. A recent study reported no significant difference in serum and FF KP levels in patients undergoing in vitro fertilization [2]. However, they observed significant increase in serum KP levels in patients treated with ovarian hyperstimulation.
Interestingly, in contrast to our results, there was no significant difference in KP levels between pregnancy negative and positive subjects either in serum or FF after ovarian hyperstimulation [22]. Furthermore, no correlation was found between FF and serum KP in all patients and pregnancy positive patients. They didn’t find any significant correlation between estradiol and FF and serum KP levels [22]. They also reported that by employing multivariate stepwise linear regression, the number of oocytes was considerably affected by the hyperstimulation-induced increase in serum KP. Few studies have reported improvement in oocyte maturation after the administration of synthetic KP that caused a controlled LH rise in women at high risk of ovarian hyperstimulation syndrome (OHSS) [23, 24].

Our study observed that serum KP levels increased from FS to OPU stages of ART, followed by a decrease in ET stage. The findings are consistent with the results of another study [13]. A Saudi study showed a significant increase in serum KP and E2 levels from the early follicular to the preovulatory and the luteal stage among normal menstruating female medical students.
In contrast to our results, they documented an insignificant correlation between KP and E2 in all three stages. At present, we don’t have any rationale for this difference. Recently, Yu et al. (2019) reported role of KP as a placental marker by finding correlation of KP with pregnancy outcomes, and noticed that women with biochemical pregnancy loss had lower KP levels [25]. Muntau et al. (2017) also reported higher KP levels among the CP group as compared to NP and PA groups [9]. However, in their study, blood samples were taken from study participants only before initiation of stimulation for ICSI.

We found that FF KP concentration was higher than serum KP, similar to Taniguchi et al., confirming the presence of KP receptors in the granulosa cells and secretion by the ovary [13]. They also found that increase in KP correlated with E2 production in all stages of the ovarian cycle, suggesting that higher KP and E2 levels are supportive of attaining pregnancy. Emerging evidence on FF- KP levels and its relationship with the female reproductive system reflects the importance of intraovarian KP in follicular development, oocyte maturation, and ovulation. Decreased concentration of follicular KP can adversely affect ovarian function, female reproduction, and fertility [26]. It has been observed that granulosa cells (GC) are one of the major sites for KP synthesis in rats, as Kiss1mRNA expression was found to be significantly higher in the GCs compared with the theca cells and other ovarian cells [26]. In our study, KP levels in FF were higher than maximum serum KP levels in women, which is similar to a study that also observed higher FF KP concentration than serum levels [13].

It has been observed that the high serum (peak) E2 around the time of ovulation impacts the number, and maturity of oocytes and the thickness of endometrial lining [23, 24]. Its measurement on OI day for predicting success in the treatment of ICSI has also been recognized [19]. Our results showed maximum serum E2 levels at the time of OPU (stage 3) in CP subjects. Higher E2 levels observed on the day of OI were maintained in luteal phase of conception cycles in another study [27].

The serum KP concentration, the number of fertilized oocytes, and the endometrial thickness were found to be more significant factors contributing in unexplained infertile women in becoming pregnant as compared to those who did not conceive. Our results are similar to a study that included infertile females with different infertility causes [28]. The result of a clinical trial using KP-54 as an inductor of oocyte maturation (in IVF treatment) showed that there is a dose-dependent increase in the number of mature oocytes, even though the rate of oocyte maturation remained similar across the doses [29]. Another clinical trial study indicated that synthetic KP-54 is a promising choice for oocyte maturation in IVF treatment, especially in patients at high risk of OHSS [23]. Till now, several trials used synthetic KP-54 for oocyte maturity, and they suggest its suitability for oocyte maturation induction, specifically in OHSS. Nevertheless, there is a need to compare KP with current oocyte maturation methods [30].

In our study, the number of oocytes and the endometrial thickness was the highest in those attaining CP, consistent with the highest serum and FF- KP levels in the similar women. This suggests the positive effect of high levels of FF-KP on the number of oocytes, their fertilization, endometrial thickness, favorable implantation of an embryo, and the success of ART in achieving pregnancy. Literature supports the efficacy of Kisspeptin-54 in triggering the maturation of oocytes specially in high risk cases of OHSS [23]. We found a significant positive association between FF- KP level and estradiol concentration, furthermore, FF-KP was also observed to correlate with the maturity of oocytes. These results agree with a previous study that reported the positive correlation between FF-KP with serum and FF estradiol levels and the number of mature eggs [13]. These outcomes suggest a link between FF KP concentration and egg maturity.

Our results indicate that KP plays a role in oocyte maturity, and its decreased levels in serum and FF not only affect the normal fertility process but also have the potential to influence IVF/ICSI outcomes. Further studies are required to understand the role of KP that could...
Direct its employment as a biomarker in treating infertile subjects and explore its utility in COS [4].

Limitations
The present study has a few limitations, such as small sample size and the cross-sectional nature of the study. Furthermore, the genetic and immunological causes of infertility could not be investigated in our study. We need to design longitudinal studies with larger sample size. The generalizability of the results is not possible since it was a unicentric study. We tried to understand the relationship between serum and FF - KP and estradiol levels with the determinants of pregnancy outcome.

Conclusions
In ICSI cycles, serum KP and increased from the initiation of stimulation to egg collection (from stages 1 to 3), with a higher concentration depicted in FF. The stimulated granulosa increased the production of estradiol, which was reflected in both serum and FF concentrations, during these stages. Estradiol production reached its maximum in the OI phase. A strong positive correlation of KP3 and E3 with the determinants of successful conception; number of fertilized oocytes and endometrial thickness emphasize on the role of KP and estradiol in successful conceptions. Thus increase in serum and FF of KP and estradiol levels assisted in acquiring an optimum endometrial thickness, and fertilization of oocytes with more chances of clinical pregnancy in ART /ICSI cycles of UI females.

We recommend further clinical trials with more UI females to determine the impact of serum and FF - KP and estradiol on UI females’ outcome measures during ICSI. This will help us understand the therapeutic options for KP in treating infertility, especially the unexplained cases.

Supporting information
S1 Table. Comparison of kisspeptin and estradiol at the time of follicular stimulation with other phases of stimulation.
(DOCX)

S2 Table Comparison of kisspeptin and estradiol in outcome groups in various phases of stimulation.
(DOCX)

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References
1. Zeydabad Nejad S, Ramezani Tehrani F, Zadeh-Vakili A. The Role of Kisspeptin in Female Reproduction. International journal of endocrinology and metabolism. 2017; 15(3):e44337. https://doi.org/10.5812/ijem.44337 PMID: 29201072
2. Hu KL, Chang HM, Zhao HC, Yu Y, Li R, Qiao J. Potential roles for the kisspeptin/kisspeptin receptor system in implantation and placentation. Human reproduction update. 2019; 25(3):326–43. https://doi.org/10.1093/humupd/dmy046 PMID: 30649364
3. Hu KL, Zhao H, Chang HM, Yu Y, Qiao J. Kisspeptin/Kisspeptin Receptor System in the Ovary. Frontiers in endocrinology. 2017; 8:365. https://doi.org/10.3389/fendo.2017.00365 PMID: 29194609
4. Trevisan CM, Montagna E, de Oliveira R, Christofolini DM, Barbosa CP, Crandall KA, et al. Kisspeptin /GPR54 System: What Do We Know About Its Role in Human Reproduction? Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology. 2018; 49(4):1259–76. https://doi.org/10.1159/000493406 PMID: 30205368
5. Gaytan F, Gaytan M, Castellano JM, Romero M, Roa J, Aparicio B, et al. KiSS-1 in the mammalian ovary: distribution of kisspeptin in human and marmoset and alterations in KiSS-1 mRNA levels in a rat model of ovulatory dysfunction. American journal of physiology Endocrinology and metabolism. 2009; 296(3):E520–31. https://doi.org/10.1152/ajpendo.90895.2008 PMID: 19141682
6. Jamil Z, Fatima SS, Arif S, Alam F, Rehman R. Kisspeptin and embryo implantation after ICSI. Reproductive biomedicine online. 2017; 34(2):147–53. https://doi.org/10.1016/j.rbmo.2016.11.004 PMID: 27908602
7. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod 2007; 22(6):1506–12. https://doi.org/10.1093/humrep/dem046 PMID: 17376819
8. Hanson B, Johnstone E, Dorais J, Silver B, Peterson CM, Hotaling J. Female infertility, infertility-associated diagnoses, and comorbidities: a review. Journal of assisted reproduction and genetics. 2017; 34(2):167–77. https://doi.org/10.1007/s10815-016-0836-8 PMID: 27817040
9. Mumtaz A, Khalid A, Jamil Z, Fatima SS, Arif S, Rehman R. Kisspeptin: A Potential Factor for Unexplained Infertility and Impaired Embryo Implantation. International journal of fertility & sterility. 2017; 11(2):99–104. https://doi.org/10.1002/rmb2.12056 PMID: 28670427
10. Gunn DD, Bates GW. Evidence-based approach to unexplained infertility: a systematic review. Fertility and sterility. 2016; 105(6):1566–74.e1. https://doi.org/10.1016/j.fertnstert.2016.02.001 PMID: 26902860
11. Mendoza C, Ruiz-Requena E, Ortega E, Cremades N, Martinez F, Bernabeu R, et al. Follicular fluid markers of oocyte developmental potential. Hum Reprod. 2002; 17(4):1017–22. https://doi.org/10.1093/humrep/dem107 PMID: 11925399
12. Katagiri F, Kotani M, Hirai T, Kagawa J. The relationship between circulating kisspeptin and sexual hormones levels in healthy females. Biochem Biophys Res Commun. 2015; 458(3):663–6. https://doi.org/10.1016/j.bbrc.2015.02.021 PMID: 25684182
13. Taniguchi Y, Kuwahara A, Tachibana A, Yano Y, Yano K, Yamamoto Y, et al. Intra-follicular kisspeptin levels are related to oocyte maturation and gonadal hormones in patients who are undergoing assisted reproductive technology. Reprod Med Biol. 2017; 16(4):380–5. https://doi.org/10.1002/rmb2.12056 PMID: 29259492
14. Latif R, Rafique NJ. Serum kisspeptin levels across different phases of the menstrual cycle and their correlation with serum oestradiol. Neth J Med. 2015; 73 (4):175–8. PMID: 25968289
15. Kamath MS, Deepthi MK. Unexplained infertility: An approach to diagnosis and management. Current Medical Issues. 2016; 14(4):94.
16. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Human reproduction update, 2010; 16(3):231–45. https://doi.org/10.1093/humupd/dmp048 PMID: 19934213
17. Rehman R, Fatima SS, Hussain M, Khan R, Khan TA. Effect of endometrial thickness on pregnancy outcome after intracytoplasmic sperm injection. J Pak Med Assoc. 2015; 65(5):448–51. PMID: 26028374
18. Petean CC, Ferriani RA, dos Reis RM, de Moura MD, Jordao AA Jr, Navarro PA. Lipid peroxidation and vitamin E in serum and follicular fluid of infertile women with peritoneal endometriosis submitted to controlled ovarian hyperstimulation: a pilot study. Fertility and sterility. 2008; 90(6):2080–5. https://doi.org/10.1016/j.fertnstert.2007.10.072 PMID: 18249400
19. Rehman R, Irfan T, Jawed S, Hussain M, Ali R. Embryo quality in intracytoplasmic sperm injection: A quasi experimental design in Pakistan. Pak J Med Assoc 2018; 68(10): 1451–55 PMID: 30317340

20. Rehman R, Jawaid S, Gul H, Khan R. Impact of peak estradiol levels on reproductive outcome of intracytoplasmic sperm injection. J Pak Med Assoc. 2014; 30(5):986–91.

21. Wang L, Moenter SM. Differential roles of hypothalamic AVPV and arcuate kisspeptin neurons in estradiol feedback regulation of female reproduction. Neuroendocrinology. 2020; 110(3–4):172–84. https://doi.org/10.1159/000503006 PMID: 31466075

22. Bódis J, Sulyok E, Kőszegi T, Prémusz V, Várnagy Á, Koppán MJ. Serum and follicular fluid levels of serotonin, kisspeptin, and brain-derived neurotrophic factor in patients undergoing in vitro fertilization: an observational study: Neurohormones in patients receiving IVF. J Int Med Res. 2019:030006519879330.

23. Abbara A, Jayasena CN, Christopoulos G, Narayanaswamy S, Izzi-Engbeaya C, Nijher GM, et al. Efficacy of kisspeptin-54 to trigger oocyte maturation in women at high risk of ovarian hyperstimulation syndrome (OHSS) during in vitro fertilization (IVF) therapy. The Journal of Clinical Endocrinology & Metabolism. 2015; 100(9):3322–31. https://doi.org/10.1210/jc.2015-2332 PMID: 26192876

24. Abbara A, Clarke S, Islam R, Prague JK, Comninos AN, Narayanaswamy S, et al. A second dose of kisspeptin-54 improves oocyte maturation in women at high risk of ovarian hyperstimulation syndrome: a Phase 2 randomized controlled trial. Human Reproduction. 2017; 32(9):1915–24. https://doi.org/10.1093/humrep/dex253 PMID: 28654728

25. Yu H, Liu J, Guo H, Chen C, Han Y, Cui Y. Prognostic value of repeated serum kisspeptin measurements in early first trimester pregnancy: a preliminary study. Reprod Biomed Online. 2019; 38(3):465–71. https://doi.org/10.1016/j.rbro.2018.11.014 PMID: 30718048

26. Hu K-L, Zhao H, Chang H-M, Yu Y, Qiao J. Kisspeptin/kisspeptin receptor system in the ovary. Frontiers in endocrinology. 2018; 8:365. https://doi.org/10.3389/fendo.2017.00365 PMID: 29354093

27. Rehman R, Tariq S, Tariq S, Hashmi F, Baig M. Mid-luteal estradiol levels of poor/good responders and intracytoplasmic sperm injection. Pak J Med Sci. 2017; 33(1):182–6. https://doi.org/10.12669/pjms.331.11692 PMID: 28367196

28. Rehman R, Jamil Z, Khalid A, Fatima SS. Cross talk between serum Kisspeptin-Leptin during assisted reproduction techniques. PaK J Med Sci. 2018; 34(2):342–6 https://doi.org/10.12669/pjms.342.14078 PMID: 29805405

29. Jayasena CN, Abbara A, Comninos AN, Nijher GM, Christopoulos G, Narayanaswamy S, et al. Kisspeptin-54 triggers egg maturation in women undergoing in vitro fertilization. The Journal of clinical investigation. 2014; 124(8):3667–77. https://doi.org/10.1172/JCI75730 PMID: 25036713

30. Abbara A, Clarke SA, Dhillo WS. Novel Concepts for Inducing Final Oocyte Maturation in In Vitro Fertilization Treatment. Endocrine reviews. 2018; 39(5):593–628. https://doi.org/10.1210/er.2017-00236 PMID: 29982525