RESEARCH ARTICLE

The Comparison of the Effect of Recombinant FSH in Antagonist Protocol on Serum and Follicular Fluid Kisspeptin between PCOS and non-PCOS Infertile Women during ICSI

Ghada B. Raaf*, Amal A. Mohammed, Mufeeda A. Jawad

High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq

Received: 22nd October, 2021; Revised: 09th December, 2021; Accepted: 13th February, 2022; Available Online: 25th March, 2022

ABSTRACT

Background: Reproduction is a highly delicate organized process accomplished by the controlled interaction of the higher centers in the brain, the gonads, and different body parts.

Kisspeptin is a neuropeptide that acts as a key regulator controlling the hypothalamic-pituitary-gonadal axis and organizing the reproduction process.

Gonadotropins treatment used in controlled ovarian hyperstimulation increases the follicular count in an attempt to improve the pregnancy rate. However, one of its drawbacks is the increase in ovarian hyperstimulation syndrome risk.

Published data proved that kisspeptin has a fundamental role in regulating follicular growth and maturation at central and peripheral levels highlighting its possible role in diagnosing and treating infertility problems.

Objective: To study the influence of gonadotropin (recombinant FSH medication) used in ovarian hyperstimulation program on the level serum and follicular fluid kisspeptin level in PCOS and non-PCOS patients.

Subject, Materials, and Method: This is a cross-sectional study performed in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Naharin University, from September 2020 to July 2021. The study includes a sample of (80) infertile Iraqi women aged (18–40 years) scheduled for an intracytoplasmic sperm injection program after being stimulated by GnRH antagonist protocol. On the day of oocytes retrieval, blood and follicular fluid samples were drawn from each participant to measure kisspeptin level using ELIZA technique.

Results: In PCOS patients, significant positive correlations were found between recombinant FSH medication with serum and follicular fluid kisspeptin level ($p < 0.05$). Serum kisspeptin was insignificantly higher in the PCOS patient ($p = 0.838$) while follicular fluid kisspeptin was insignificantly lower in the PCOS women ($p = 0.651$). There were significant correlations between serum and follicular kisspeptin in the PCOS and the control groups ($p = 0.000$) and ($p = 0.002$), respectively.

Conclusions: Serum and follicular fluid kisspeptin are positively correlated with the cumulative recombinant FSH medication used by infertile polycystic ovarian syndrome women undergoing ICSI, addressing the possible role of kisspeptin in improving the pregnancy rate in PCOS patients with a lower incidence of ovarian hyperstimulation syndrome.

Keywords: Kisspeptin, Polycystic ovarian syndrome, Recombinant FSH medication.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.1.6

How to cite this article: Raaf GB, Mohammed AA, Jawad MA. The Comparison of the Effect of Recombinant FSH in Antagonist Protocol on Serum and Follicular Fluid Kisspeptin between PCOS and non-PCOS Infertile Women during ICSI. International Journal of Drug Delivery Technology. 2022;12(1):33-38.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Infertility is considered one of the major health problems that have a serious psychological and financial burden on infertile couples.[1]

Since 1978 different assisted reproductive technologies (ART) have been developing consistently.[2] The primary goals of ART are not limited to the birth of a healthy child but also to finish the pregnancy with the lowest complications risk to the mother.[3] Previous studies proved that using ovulation induction protocols in vitro fertilization (IVF)leads to better pregnancy outcomes than the natural cycle.[4] Controlled ovarian hyperstimulation (COH) main objective is to increase the cohort of recruited follicles, so increase the number of retrieved oocytes and subsequently the embryos to be selected and transferred.[4]

GnRH antagonist is one of the protocols used in (COH), which works by competitively blocking GnRH receptors in

*Author for Correspondence: ghada.b.alanssari@gmail.com

the hypothalamus that will rapidly suppress gonadotropins release from the pituitary gland.[5] The main advantage of the GnRH antagonist protocol is its ability to minimize premature LH surge during ovarian hyperstimulation and decrease the retrieved oocyte number, which will lower E2 level at day of HCG administration, ending with a better pregnancy rate with lower risk of developing ovarian hyperstimulation syndrome.[5]

Reproduction is a complex function regulated by the delicate incorporation of the hypothalamic-pituitary-ovarian(HPO) axis and controlled by a complex system of inhibitory and excitatory mediators.[6] In the hypothalamus, the (GnRH) secreting neurons secret GnRH in two modes, the tonic mode important for sex steroid production and ovarian follicular growth, and the surge mode essential for ovulation induction. GnRH stimulates the anterior pituitary gland to secret FSH and LH, which are responsible for controlling sex steroid (estrogen and progesterone) secretion from the ovaries ending in normal ovulation.[7,8]

However, the tonic secretion of GnRH/gonadotropins is regulated by the negative feedback mechanism of estrogen secreted from the growing follicles in the ovary. At the same time, the surge mode of GnRH secretion is influenced by the positive feedback mechanism of the high level of estrogen secreted at the pre-ovulatory stage.[9]

The major conflux is that GnRH neurons do not express estrogen receptor α, needed for estrogen's positive and negative feedback effect.[9]

The novel discovery of kisspeptin in being an upstream regulator of GnRH secretion fill the gap between sex steroid hormones secreted from the ovaries and its feedback mechanisms on the GnRH secretion from the hypothalamus.[10]

Scientific evidence showed that kisspeptin plays a fundamental role in regulating the reproductive process as it controls gonadotropins secretion.[9]

Kisspeptin is a peptide encoded by the *KISS1*gene., All kisspeptin precursors are produced from the cleavage of the prepro-kisspeptin, which is a 145-amino acid peptide, that is further cleaved into four peptides (KP-14, KP-13, KP-10, and KP-54). Kisspeptin binds to its receptor the G-protein-coupled receptor (GPR54/KISS1R),[11] which is expressed in different body parts like the hypothalamus, the pituitary, the ovaries, the pancreas, and adipose tissues; recently, kisspeptin has been discovered to be expressed in the endometrium and placenta.[9]

The Intra-ovarian kisspeptin has a highly delicate expression pattern in human ovaries. The ovarian kisspeptin distribution is believed to have significant temporal specificity, proposing that the intr-ovarian kisspeptin/ KISS1R system carries out many complex functions which differ according to the menstrual phases.[12] Hu *et al*. (2018) declared that any abnormality in the kisspeptin system might lead to the dysregulation of the kisspeptin, which will negatively impact the ovarian function ending with female infertility.[9]

Polycystic ovarian syndrome (PCOS) is a polygenic, multifactorial, systemic, abnormal steroid state disorder.[13] PCOS is the most common cause of females' menstrual irregularities, ovarian dysfunction, and subfertility.[14]

The Rotterdam consensus defines PCOS as the presence of two out of the three criteria: clinical or biochemical hyperandrogenism, menstrual irregularities (oligo or anovulation), and Polycystic ovarian morphology (PCOM).[15]

Published scientific evidence stated that PCOS is a heterogeneous syndrome, characterized by abnormal GnRH secretion that leads to an increase in LH secretion with no significant alteration in the FSH level, that might be linked to the decrease in the sensitivity of the GnRH pulse generator to estrogen feedback and enhance LH secretion. Moreover, the cumulative effect of altered GnRH stimulatory and inhibitory neurotransmitters in the hypothalamic-pituitary center were believed to contribute to the elevated level of GnRH and LH in PCOS women.[16]

Kisspeptin has been proven to stimulate the pulsatile release of GnRH, so it is scientifically reasonable that the circulating level of kisspeptin may be positively related to LH level.[17]

Yang *et al*. (2017) and Hu *et al*. (2018)[18,19] declared that intra-ovarian kisspeptin downregulates the initial recruitment of the primary follicles to secondary follicles by two mechanisms; the first one is by decreasing FSH receptor expression in small ovarian follicles. The second one is by increasing the circulatory level of anti-Müllerian hormone (AMH) secreted from the small antral follicles and the secondary follicles to inhibit the reactivation of primordial follicles. In conclusion, KP may have a fundamental role in improving the quality of oocytes rather than quantity.

Published scientific studies stated that the administration of kisspeptin 54 resulted in successful oocytes maturation in PCOS patients with lower ovarian hyperstimulation syndrome risk. Moreover, scientists proved the importance of serum and follicular fluid in obtaining a better success rate in assisted reproductive technologies.

SUBJECT, MATERIALS, AND METHOD

This study is a cross-sectional study performed in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies of Al-Naharin University, from September 2020 to July 2021. This study includes (80) infertile Iraqi females aged (18–40) years and undergoing an ICSI program; the sample was divided into two groups. The PCOS group (n = 40) and the non-PCOS group (n = 40). All patients underwent a complete infertility assessment which includes (history taking, physical examination, and routine infertility investigations). Infertile females of both groups were stimulated using the flexible antagonist protocol, in a ranging daily dose of (150-450 IU) rFSH (Gonal F). the growth of the ovarian follicles was monitored using transvaginal sonography (TVS). When ovarian follicles reached (13-14 mm) in size, GnRH antagonist was given (Cetrotide®, Merck Serono), and when three or more of the dominant follicles reached (18 mm) in diameter HCG (Ovitrelle® 250 microgram, Merck Serono) was given to induce final oocytes maturation.

After (34–36) hours from HCG injection, oocytes were retrieved using a transvaginal probe under transvaginal ultrasound guidance.

The venous blood sample was taken on the day of oocytes retrieval, then was put in a dry sterile plain tube, allowed to coagulate for 30 minutes at room temperature then centrifuged at 5000 rpm for 10 minutes. The supernatant serum was aspirated and put in small tubes to be stored in the freezer at -20°C for subsequent analysis of measurement of kisspeptin.

Follicular fluid was collected using double-lumen from the very first aspirated follicles of the infertile females undergoing ICSI cycles, then was put in a plain sterile tube to be centrifuged at 5000 rpm for 10 minutes. The supernatants were taken to other sterile tube and stored at –20°C for subsequent assay for kisspeptin level. Follicular fluid samples which were heavily contaminated with blood were all discarded.

Kisspeptin in serum and follicular fluid was measured using a commercially available human kisspeptin 1 (KISS-1) kit which uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology. The ELISA kits used were human kisspeptin 1(KISS-1) ELISA KIT (cat. No. YHB1811Hu, SHANGHAI YEHUA Biological Technology) with a sensitivity of 10.1 ng/L, intra-assay coefficients of variation of less than 10%, and inter-assay coefficients of variation of less than 12%.

RESULTS

The demographic features of the study patients are shown in Tables 1-3. No significant differences have been found between the mean age and mean BMI between the PCOS and non-PCOS groups ($p > 0.05$)

Table 2 showed That the majority of the two studied groups presented with primary infertility (77.5%) of the PCOS group compared to (80%) of the non-PCOS group and that most of the two studied groups had no previous history of IVF trial (PCOS 82.5%) (NON- PCOS 92.5%).

Regarding the causes of infertility, the majority of the participants (44%) complained from both male and female factors for their infertility (combined cause), (27%) suffered

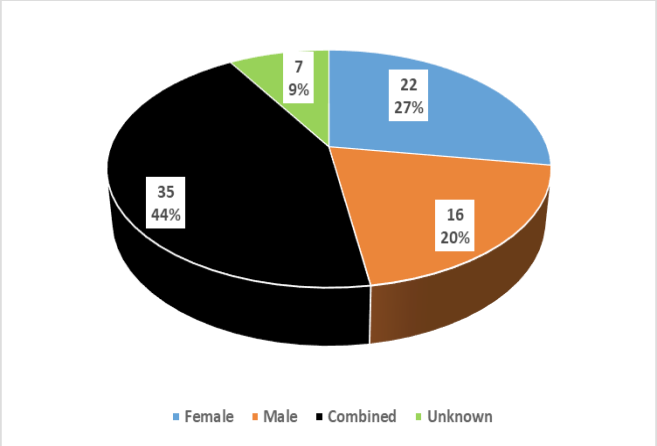

Figure 1: The causes of infertility.

Table 1: Two samples t-test show differences between means of age, BMI, and duration of infertility according to studied group

Variable	*Group*	*N*	*Mean*	*Std. Deviation*	*p-value*
Age	PCOS	40	30.73	5.033	0.264
	Non-PCOS	40	32.08	5.677	
BMI	PCOS	40	26.130	4.1731	0.307
	Non-PCOS	40	27.037	3.6927	
	Non-PCOS	40	7.18	4.930	

Table 2: Chi-square test shows an association between type of infertility and previous IVF according to the studied group.

		PCOS		*Non-PCOS*		
Variable		*N*	*%*	*N*	*%*	*p-value*
Type of infertility	Primary	31	77.5%	32	80.0%	0.785
	Secondary	9	22.5%	8	20.0%	
Previous ICSI trial	Yes	7	17.5%	3	7.5%	0.176
	No	33	82.5%	37	92.5%	

Table 3: Differences between means of hormones and other studied variables according to groups of the study

Variable	*group*	*N*	*Mean*	*Std. Deviation*	*p-value*
FSH	PCOS	40	5.85	1.97353	0.117
	Non-PCOS	40	6.62	2.37754	
LH	PCOS	40	5.54	4.11986	0.259
	Non-PCOS	40	4.69	2.32429	
E2(CD3)	PCOS	40	44.79	20.66282	0.362
	Non-PCOS	40	55.86	48.91908	
E2 at trigger	PCOS	40	1910.70	2511.09234	0.035
	Non-PCOS	40	1036.01	566.37665	

E2 = estradiol, CD3 = menstrual cycle day 3.

from female factors only, (16%) from malefactors solely, and only (7%) of the study sample had unknown cause for their infertility (Figure 1).

The basal hormonal level of the study sample is shown in Table 3; no significant differences had been found between the two study groups regarding (FSH, LH, and E2 at cycle day 3) hormones, in contrary to E2 hormone, which was measured at the day of HCG administration (trigger day), the significant difference had been obtained between PCOS and non-PCOS patients (p= 0.035).

No significant difference was observed in the cumulative dose of recombinant FSH given between the two groups (p > 0.05), as shown in Table 4.

No significant differences were observed in serum, and follicular fluid kisspeptin mean between the two studied groups (p > 0.05) (Table 5).

In Table 6, the correlations of serum and follicular kisspeptin level with serum hormones and the cumulative recombinant FSH (cumulative r FSH) in the PCOS group showed a significant correlation between serum and follicular fluid kisspeptin level (p= 0.000). Also a significant correlation had been observed between serum KP and cumulative r FSH dose (p = 0.001) and follicular fluid KP and cumulative r FSH dose (p = 0.013). no significant correlations had been found among other variables(p > 0.05).

In the non-PCOS group, a significant correlation had been found between serum and follicular fluid kisspeptin (p=0.002), and no significant correlations had been observed between serum and follicular fluid KP with neither the cumulative r FSH dose nor with hormones (p > 0.05) (Table 7).

DISCUSSION

In this study, for the purpose of comparison, the study sample had been divided into two groups, the PCOS and non- PCOS groups. No significant differences had been observed between the two groups regarding demographic features, which include the body mass index (BMI), age, type of infertility, and previous history of IVF since published literature declared that serum KP level might be correlated to the (BMI) and age.16,19 The influence of BMI and age on serum and follicular fluid kisspeptin in this study has been discarded. To the best of our knowledge, no previous report has been published regarding the correlation of follicular kisspeptin with both age and BMI.

Although most of the PCOS patients are well known to suffer a disturbing hypothalamic-pituitary-ovarian axis in the form of an abnormal FSH/LH ratio.[20] In this study, no significant difference had been found between the PCOS and non- PCOS groups.

The results of this study showed that the mean serum kisspeptin level was insignificantly higher in the PCOS group (mean = 223.75) compared to the non-PCOS group (mean=215.90), which is similar to the results of other studies.[21-23] However, other literature declared a significantly higher serum kisspeptin level in PCOS patients proposing that the hyperactive kisspeptin system in PCOS women might enhance the activity of the hypothalamic-pituitary-ovarian

Table 4: Differences between means of total recombinant FSH dose according to groups of the study

Variable	*group*	*N*	*mean*	*Std. Deviation*	*p-value*
Cumulative recombinant FSH dose	PCOS	40	948.75	992.57	0.315
	Non-PCOS	40	1130.63	557.74	

Table 5: Two samples t-test show differences between means of a kisspeptin serum and kisspeptin follicular fluid to studied group

Variable	*Group*	*N*	*Mean*	*SD*	*P-value*
KP serum	PCOS	40	223.75	184.35	0.838
	Non- PCOS	40	215.90	157.46	
KP follicular fluid	PCOS	40	262.11	31.06	0.651
	Non -PCOS	40	265.58	37.08	

KP = kisspeptin

Table 6: Correlations of serum and follicular kisspeptin level with serum hormones of the PCOS group.

PCOS		*KP serum*	*FSH*	*LH*	*E2(CD3)*	*E2 (trigger)*	*cumulative r FSH*
KP serum	r		.070	-.051	-.110	-.117	.497**
	p		.667	.757	.654	.474	.001
KP follicular fluid	r	.619**	.026	-.075	-.208	-.113	.388*
	p	.000	.872	.647	.394	.489	.013

Table 7: Correlations of serum and follicular kisspeptin level with serum hormones of the non-PCOS group.

Non-PCOS		*KP serum*	*FSH*	*LH*	*E2(CD3)*	*E2 (trigger)*	*cumulative r FSH*
KP serum	r	1	-.110	-.204	-.033	.010	-.122
	PV		.499	.207	.880	.952	.452
KP follicular fluid	r	.474**	.022	-.101	.196	.088	.030
	PV	.002	.893	.535	.358	.590	.852

axis leading to the disturbed hormonal pattern seen in PCOS patients.[17] Scientists believed that kisspeptin and leptin are correlated together in regulating the body metabolism and reproduction and that leptin is found to be positively correlated with BMI.[24] So, in this study, the elevated level of serum kisspeptin seen in the PCOS group might be referred to the increased BMI of the participants since most of the PCOS group BMI was > 25kg/m.

However, in this study, follicular fluid KP was observed to be insignificantly lower in the PCOS females than in the non- PCOS women. Celik *et al*. (2018)[25] declared a significantly lower level of follicular fluid kisspeptin in PCOS than that of the control group, highlighting the role of follicular fluid kisspeptin in the abnormal follicular development seen in PCOS patients.

Published literature explained that the elevated serum estradiol level observed in PCOS patients which is related to the increase cohort of retrieved oocytes, will prohibit KISS1R expression in both the ovaries and the vascular endothelial cells leading to minimize the suppression of endogenous Kp-10 into vascular endothelial growth factor (VEGF) production which is considered the most potent angiogenic factor regulating microvascular permeability important for follicular growth.[26]

Moreover, scientists believed that the marked suppression in ovarian Kiss1 mRNA levels during the peri-ovulatory period in PCOS patients might be attributed to the impaired synthesis of prostaglandin synthesis responsible for ovarian Kiss1 mRNA levels production during the peri-ovulatory period in PCOS.[27]

This study obtained a significant positive correlation between serum and follicular kisspeptin in PCOS and non-PCOS groups. This result is in accordance with Rehman *et al*. (2020) and Taniguchi *et al*. (2017),[28,29] and in contrary to Hu *et al*. (2018), who did not find any correlation between serum and follicular kisspeptin.[9]

In this study, a remarkable significant positive association was obtained between recombinant FSH (Gonal F) medication used for ovarian hyperstimulation in the antagonist protocol with both serum and follicular kisspeptin in the PCOS group, a result which was not obtained in the control group. Similar to what had been announced by Taniguchi, Y. *et al*. (2017), Bódis *et al*. (2020), and Rehman *et al*. (2020), their study results showed that the mean of serum kisspeptin increased in response to ovarian stimulation protocol.[28-30]

It is well known that PCOS is considered a potential risk factor for the development of ovarian hyperstimulation syndrome (OHSS), especially after controlled ovarian stimulation used in ART. OHSS has a negative impact on oocyte and embryo quality and pregnancy success rate.[31]

Abbara *et al*. (2017) demonstrated that the administration of Kisspeptin-54 resulted in the safe triggering of oocytes maturation in PCOS women without increasing the risk of OHSS.[32] Furthermore, published literature stated that intr-ovarian kisspeptin downregulates follicular recruitment by decreasing FSH receptor expression in small ovarian follicles and increasing anti-Müllerian hormone (AMH) level, which inhibits the reactivation of primordial follicles.[9,18] In conclusion, KP may have a fundamental role in improving the quality of oocytes rather than quantity.

Kisspeptin has been proven to play a vital role in regulating reproduction through its fundamental role in triggering LH surge and through its importance in providing a vital milieu for normal oocyte viability and growing competency ending in a successful fertilization and post-fertilization growth. In this study, the significant association of serum and follicular fluid kisspeptin with gonadotrophin used in controlled ovarian hyperstimulation used for PCOS patients highlights that kisspeptin might have a role in treating infertility problems in accordance with proper adjustment of gonadotrophins dose will result in better pregnancy outcome with lower complication risk.

CONCLUSIONS

Serum and follicular fluid kisspeptin are significantly and positively correlated with rFSH used in antagonist protocol during ICSI in PCOS infertile women, which might improve oocyte quality and decrease the risk of ovarian cancer hyperstimulation risk.

REFERENCES

1. Patel A, Sharma PS, Kumar P, Binu VS. Sociocultural determinants of infertility stress in patients undergoing fertility treatments. Journal of human reproductive sciences. 2018 Apr;11(2):172.
2. Silva SG, Bertoldi AD, Silveira MF, Domingues MR, Evenson KR, Santos IS. Assisted reproductive technology: prevalence and associated factors in Southern Brazil. Revista de saude publica. 2019 Jan 31; 53:13.
3. Esteves SC, Roque M, Bedoschi GM, Conforti A, Humaidan P, Alviggi C. Defining low prognosis patients undergoing assisted reproductive technology: POSEIDON criteria—the why. Frontiers in endocrinology. 2018 Aug 17; 9:461.
4. Cota AM, Oliveira JB, Petersen CG, Mauri AL, Massaro FC, Silva LF, Nicoletti A, Cavagna M, Baruffi RL, Franco JG. GnRH agonist versus GnRH antagonist in assisted reproduction cycles: oocyte morphology. Reproductive Biology and Endocrinology. 2012 Dec;10(1):1-0.
5. Wang R, Lin S, Wang Y, Qian W, Zhou L. Comparisons of GnRH antagonist protocol versus GnRH agonist long protocol in patients with normal ovarian reserve: a systematic review and meta-analysis. PloS one. 2017 Apr 24;12(4): e0175985
6. Campisi SC, Humayun KN, Wasan Y, Soofi SB, Islam M, Hussain A, Shakeel A, Vandermorris A, Söder O, Bhutta ZA. The relationship between pubertal timing and under-nutrition in rural Pakistan. Journal of Adolescence. 2021 Apr 1; 88:58-66.
7. Okamura H, Tsukamura H, Ohkura S, Uenoyama Y, Wakabayashi Y, Maeda KI. Kisspeptin and GnRH pulse generation. Kisspeptin Signaling in Reproductive Biology. 2013:297-323.
8. Wawrzkiewicz-Jałowiecka A, Kowalczyk K, Trybek P, Jarosz T, Radosz P, Setlak M, Madej P. In Search of New Therapeutics—Molecular Aspects of the PCOS Pathophysiology: Genetics, Hormones, Metabolism and Beyond. International Journal of Molecular Sciences. 2020 Jan;21(19):7054.
9. 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. 2018 May;25(3): 326-43.
10. Trevisan CM, Montagna E, de Oliveira R, Christofolini DM, Barbosa CP, Crandall KA, Bianco B. Kisspeptin/GPR54 system: what do we know about its role in human reproduction? Cellular Physiology and Biochemistry. 2018;49(4):1259-76.
11. Hussain MA, Song WJ, Wolfe A. There is kisspeptin–and then there is kisspeptin. Trends in Endocrinology & Metabolism. 2015 Oct 1;26(10):564-72.
12. Cao Y, Li Z, Jiang W, Ling Y, Kuang H. Reproductive functions of Kisspeptin/KISS1R Systems in the Periphery. Reproductive Biology and Endocrinology. 2019 Dec;17(1):1-9.
13. Khan MJ, Ullah A, Basit S. Genetic basis of polycystic ovary syndrome (PCOS): current perspectives. The application of clinical genetics. 2019; 12:249.
14. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocrine reviews. 2015 Oct 1;36(5):487-525.
15. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF. Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. The Journal of Clinical Endocrinology & Metabolism. 2006 Nov 1;91(11):4237-45.
16. Ibrahim RO, Omer SH, Fattah CN. The correlation between hormonal disturbance in PCOS women and serum level of kisspeptin. International journal of endocrinology. 2020 Jan 7;2020.
17. Tang R, Ding X, Zhu J. Kisspeptin and polycystic ovary syndrome. Frontiers in endocrinology. 2019 May 10; 10:298.
18. Yang J, Feng T, Li S, Zhang X, Qian Y. Human follicular fluid shows diverse metabolic profiles at different follicle developmental stages. Reproductive Biology and Endocrinology. 2020 Dec;18(1):1-1.
19. Asalah AK, El-Sayed MA, Abdel-Aleem DI, EL Nagar WM, Gobran A. Serum Kisspeptin Level in normal pregnancy and preeclampsia with and without obesity. Zagazig University Medical Journal. 2021 Mar 1;27(2):343-51.
20. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. Reproductive Biology and Endocrinology. 2016 Dec;14(1):1-7.
21. Emeka Ozay O, Ozay AC, Acar B, Cagliyan E, Seçil M, Küme T. Role of kisspeptin in polycystic ovary syndrome (PCOS). Gynecological Endocrinology. 2016 Sep 1;32(9):718-22.
22. Allawi FS, Daghestani MH, Daghestani MH, Eldali A, Warsy AS. rs4889 polymorphism in KISS1 gene, its effect on polycystic ovary syndrome development and anthropometric and hormonal parameters in Saudi women. Journal of biomedical science. 2018 Dec;25(1):1-6.
23. Daghestani MH, Daghestani MH, Daghestani M, Ambreen K, Albalawi FS, AlNeghery LM, Warsy AS. Influence of KISS1 gene polymorphisms on the risk of polycystic ovary syndrome and its associated variables, in Saudi women. BMC endocrine disorders. 2020 Dec;20(1):1-0.
24. Clarke H, Dhillo WS, Jayasena CN. Comprehensive review on kisspeptin and its role in reproductive disorders. Endocrinology and Metabolism. 2015 Jun 1;30(2):124-41.
25. Celik N, Aydin S, Ugur K, Yardim M, Acet M, Yavuzkir S, Celik O. Patatin-like phospholipase domain-containing 3-gene (adiponutrin), preptin, kisspeptin and amylin regulates oocyte developmental capacity in PCOS. Cellular and Molecular Biology. 2018 Dec 31;64(15):7-12.
26. Zhai J, Liu J, Zhao S, Zhao H, Chen ZJ, Du Y, Li W. Kisspeptin-10 inhibits OHSS by suppressing VEGF secretion. Reproduction. 2017 Oct 1;154(4):355-62.
27. Gaytan F, Gaytan M, Castellano JM, Romero M, Roa J, Aparicio B, Garrido N, Sanchez-Criado JE, Millar RP, Pellicer A, Fraser HM. 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 Mar;296(3): E520-31.
28. Rehman R, Zafar A, Ali AA, Baig M, Alam F. Impact of serum and follicular fluid kisspeptin and estradiol on oocyte maturity and endometrial thickness among unexplained infertile females during ICSI. PloS one. 2020 Oct 28;15(10):e0239142.
29. Taniguchi Y, Kuwahara A, Tachibana A, Yano Y, Yano K, Yamamoto Y, Yamasaki M, Iwasa T, Hinokio K, Matsuzaki T, Irahara M. Intra-follicular kisspeptin levels are related to oocyte maturation and gonadal hormones in patients who are undergoing assisted reproductive technology. Reproductive medicine and biology. 2017 Oct;16(4):380-5.
30. Bódis J, Sulyok E, Kőszegi T, Prémusz V, Várnagy Á, Koppán M. 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. Journal of International Medical Research. 2020 Oct;48(4):0300060519879330.
31. Sun B, Ma Y, Li L, Hu L, Wang F, Zhang Y, Dai S, Sun Y. Factors associated with ovarian hyperstimulation syndrome (OHSS) severity in women with polycystic ovary syndrome undergoing IVF/ICSI. Frontiers in endocrinology. 2020;11.
32. Abbara A, Clarke S, Islam R, Prague JK, Comninos AN, Narayanaswamy S, Papadopoulou D, Roberts R, Izzi-Engbeaya C, Ratnasabapathy R, Nesbitt A. 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 Sep 1;32(9):1915-24.