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Research Article

The Role of Kisspeptin in Intracytoplasmic Sperm Injection Cycles in a Group of Infertile Iraqi Females

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Abstract

Background: Infertility is a global issue that not only affects individuals but also impacts society as a whole. Kisspeptin has emerged as a key player in the understanding of certain facets of infertility. However, research on the role of kisspeptin in fertility, particularly in assisted reproductive treatment, remains limited.

Objectives: To investigate the potential association between follicular fluid kisspeptin levels and various reproductive parameters, including the number and maturity of oocytes, fertilization rate, pregnancy detection, anti-Müllerian hormone levels, and the age of females.

Methods: Sixty infertile couples with an age range of 20 – 40 years participated in this study. They complained of unexplained infertility, had no hormonal or male factors, all with the same stimulation protocol, with follicular fluid collected on the day the oocytes were retrieved. These follicular fluid samples were centrifuged and then frozen at -20ºC until the day an enzyme-linked immunosorbent assay (ELISA) for kisspeptin was done. The study was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University, Baghdad, between the 1st of January to the 1st of July, 2023.

Results: A significant negative correlation was found between follicular kisspeptin levels and female age. Kisspeptin levels positively correlated with the number of retrieved oocytes, mature oocytes, fertilized oocytes, and AMH levels. Higher kisspeptin levels were observed in pregnant women (707.7 ± 235.28 pg/mL) than in non-pregnant ones (648.9 ± 215.27 pg/mL), but the difference was not significant. A strong positive correlation was found between follicular and serum kisspeptin.

Conclusion: This study suggested that kisspeptin may play a key role in ovarian function, as its levels were associated with oocyte quality and ovarian reserve. The observed relationship between kisspeptin levels and both female age and reproductive outcomes highlights its potential as a biomarker in assisted reproduction.

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Keywords: Kisspeptin; Follicular Fluid; Fertilization; Oocytes; Ovarian Follicle.

Introduction

Infertility is commonly defined as the inability to conceive after one year of regular, unprotected sexual intercourse. It is regarded as a global health concern, with recent estimates suggesting that one in every six couple's experiences infertility at some point in their lives (1, 2, 3). This condition can stem from a variety of male or female factors and often leads affected couples to seek medical assistance, including advanced reproductive technologies such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Despite its wide usage, the success of ICSI depends on several factors, including the quality and maturity of retrieved oocytes, fertilisation rates, and embryo development (3). Over the past two decades, a neuropeptide known as kisspeptin has emerged as a crucial regulator of reproductive function. Kisspeptin belongs to the RF-amide peptide. Family, characterized by an arginine-phenylalanine sequence at the C-terminal. It was first identified in 1996 and initially recognized as a tumour metastasis suppressor gene, hence named "metastin" (4, 5). Its biological action begins when it binds to its receptor, G-protein-coupled receptor 54 (GPR54) (6). In 2003, mutations in GPR54 were linked to hypogonadotropic hypogonadism, highlighting kisspeptin's pivotal role in reproductive physiology (7, 8). Kisspeptin-expressing neurons are found in various hypothalamic regions, including the periventricular nucleus (PeN), anteroventral periventricular nucleus (AVPV), and the arcuate nucleus (ARC) (9). These neurons regulate key reproductive events such as the onset of puberty, gonadotropin secretion, ovulation, and even brain sexual differentiation (10). Furthermore, research has shown that kisspeptin not only acts centrally but may also be locally expressed in the ovaries, influencing oocyte maturation and follicular development (11, 12, 13). Kisspeptin and its receptor have been detected in

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ovarian tissues of both hamsters and humans, with expression levels showing cyclical variations — peaking during the pre-ovulatory phase under the influence of gonadotropins (14,15,16,17,18). The follicular fluid (FF), bathes the oocyte, plays a vital role in supporting oocyte growth and maturation. FF components are derived from both the blood-follicle barrier and secretions from granulosa and theca cells (19). The FF contains different constituents that are important for maturation of the oocytes. These include growth hormone, insulin-like growth factor, estradiol, interleukins, enzymes, and others (20,21,22,23). Recent evidence has indicated the presence of kisspeptin in the FF, raising interest in its potential as a marker of oocyte quality and follicular activity. Studies have attempted to correlate FF kisspeptin levels with antral follicle counts, oocyte maturity, and fertilization success (9,24). In 1992, ICSI has revolutionized the management of infertility, particularly for male-related causes such as oligospermia, asthenospermia, or azoospermia. In females, ICSI has also been applied successfully in cases involving tubal factor infertility, endometriosis, or unexplained infertility (25,26,27). Given the increasing concern over infertility and its psychological burden—especially among males where infertility has been rising in prevalence—investigating biomarkers like kisspeptin may offer new insights into optimizing assisted reproductive technologies (28,29,30,31,32).

This study aimed to investigate the potential association between follicular fluid kisspeptin levels and various reproductive parameters, including the number and maturity of oocytes, fertilization rate, pregnancy detection, anti-Müllerian hormone levels, and the age of females.

Subjects, Materials and Methods

This is a cross-sectional, observational study in which 60 infertile couples had participated at the High Institute for Infertility Diagnosis and Assisted Reproductive Technology / Al-Nahrain University, Baghdad between the 1st of January to and the 1st of July, 2023. The studied infertile couples were infertile for periods between 2-15 years.

All were with unexplained infertility, no male factor had been involved, no endocrine causes, such as, hyperprolactinemia or thyroid abnormality; and no causes, such as, endometriosis, polycystic ovary syndrome, etc. All these causes had been excluded.

All female subjects followed an antagonist protocol, commencing on the second day of their menstrual cycle. On the first day of their cycle, blood samples were collected to measure the concentration of estradiol (E2) in serum.

On day 2, additional blood samples were obtained to quantify the levels of anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH); On the day of oocyte retrieval, blood samples were obtained to determine the level of Kisspeptin (KP) in their serum, with the help of the ELISA kit (Human Kiss1 ELK biotechnology); (Colorado-USA), the analytical sensitivity of the kit = 13.1 pg ⁄ml.

On day 11 of the cycle, the cases received Human Chorionic Gonadotropin (hCG) (Ovtriville) in a dose of 250 micrograms ⁄ 0.5 ml (Merck). The oocyte was retrieved after 34 hours, guided by the ultrasound. The follicular fluid sample that contains blood was discarded. Any follicular fluid that did not contain an ovum, was discarded. At that time, the follicular fluid was collected, centrifuged at 1500 rpm for 10 minutes, and frozen at -20º C, until the day it was being tested for the kisspeptin. After three days, the embryo transplantation was done. These females received progesterone vaginal suppositories (Cyclogest 400 mg) for 14 days. Then the B-hCG test was done for confirmation of pregnancy.

The study protocol was approved by the Committee of the College of Medicine, University of Baghdad (protocol number 236).

Statistical Analysis All data were arranged by using Microsoft Excel and analysis was done by using the IBM SPSS-29 (IBM Statistical Packages for Social Sciences - Version 29, Chicago, IL, USA).

All values were calculated as mean ± SD. The t-test and ANOVA were used for statistical analysis, and a p-value of less than 0.05 was considered statistically significant. Additionally, the correlation coefficient was calculated to assess the strength and direction of relationships between variables.

Results

Thirty of the women who participated in this study, showed diminished ovarian reserve, (AMH), less than 1.1ng/mL and antral count less than 7 follicles. The other 30 women showed good ovarian reserve — this classification was according to the Bologna criteria. As shown in Table 1, the correlation between follicular fluid kisspeptin levels and oocyte parameters is significant. Table 2 further compares the mean levels of kisspeptin in follicular fluid based on oocyte maturity and fertilization status. Table 3 shows the relationship between follicular fluid kisspeptin levels and the age of the females in the study. Table 4 presents the comparison of kisspeptin levels in follicular fluid and serum between pregnant and non-pregnant patients.

Table 5 shows a strong positive correlation between follicular fluid and serum kisspeptin levels.

Table (1): Correlation between follicular fluid kisspeptin levels and oocyte parameters

Variable	r	p
Number of retrieved oocytes	0.462	0.001
Number of mature oocytes (MII)	0.438	0.002
Number of fertilized oocytes	0.410	0.004
Anti-Müllerian Hormone (AMH) (ng/mL)	0.487	0.001
Female age (years)	-0.332	0.019

Table (2): Comparison of follicular kisspeptin fluid levels based on oocyte maturity and fertilization status

Group	Kisspeptin Level (ng/mL) Mean ± SD	p-value
Mature oocytes (MII)	14.5 ± 3.21	0.003
Immature oocytes (non-MII)	11.1 ± 3.70	
Fertilized oocytes	15.2 ± 3.00	0.001
Non-fertilized oocytes	10.8 ± 4.11	

An independent samples t-test was used in comparison

Table (3): Correlations between maternal age and follicular fluid KP level

		Kisspeptin (pg/ml)	
		Serum	Follicular fluid
Age (years)	r	-0.048	-0.302*
	P	0.716	0.019*

*Correlation was assessed using Pearson's correlation coefficient (r).

Table (4): Mean values of follicular and serum KP in pregnant and non-pregnant cases

		No. patients	Kisspeptin (pg/ml) (Mean ± SD)	
			Follicular fluid	Serum
Pregnancy	Pregnancy	8	707.7 ± 235.28	387.2 ± 199.75
	No pregnancy	52	648.9 ± 215.27	370.7 ± 250.36
P value			0.481	0.834

#Significant difference between two independent means using the Students' t-test at a 0.05 level.

Table (5): The correlation between follicular fluid and serum KP

	P	r
Serum vs. Follicular fluid Kisspeptin (pg/dL)	0.0001	0.562**

*Correlation is significant at the 0.05 level.**Correlation is highly significant at the 0.001 level.

Discussion

Ovarian follicles are primarily primordial follicles, as described by Findlay *et al.* in Australia (33). However, these follicles may sporadically develop into more advanced stages, such as tertiary and ovulatory follicles, even before the onset of puberty. These developments are regulated by reproductive hormones released during the menstrual cycle. Recent insights, including a study by Dudek et al in Poland, suggest that kisspeptin also plays a key role in the development of primordial follicle, indicating a regulatory function in ovarian physiology (2,34).

The current study revealed a statistically significant positive correlation between KP levels and the number of ovarian follicles. This finding aligns with a study conducted by Abbara *et al.* in the UK, which demonstrated that elevated KP levels are associated with an increased number of retrieved oocytes (35). Similarly, Taniguchi et al in Japan found a positive correlation between follicular fluid KP levels and the number of oocytes retrieved (13).

In our study, KP levels in follicular fluid showed a positive correlation with the number of metaphase II (MII) oocytes, which is consistent with the findings of Trevisan *et al.* in Brazil, who also reported a significant association between KP levels and oocyte maturation (17).

It is believed that KP influences oocyte maturation indirectly via stimulation of FSH release (36,37). Despite growing evidence supporting this role (14), it remains uncertain whether KP derived from the hypothalamus acts directly on oocytes, or whether local expression of KP in granulosa cells during the pre-ovulatory gonadotropin surge mediates this effect. The specific role of granulosa cell-derived KP in oocyte maturation still requires further investigation. Consequently, additional studies are needed to establish a definitive link between KP and oocyte maturation.

The present study observed a positive but statistically non-significant correlation between follicular KP levels and the number of fertilized oocytes. While this correlation was not strong, other studies provide insight. For example, Qin *et al*. in Thailand reported that both serum and follicular KP are related to oocyte maturation and fertilization, affecting embryo quality and fertilization rates, although they found no direct effect of follicular KP on fertilization (39). In contrast, a study by Goktan et al in Türkiye found no significant relationship between KP expression in cumulus cells and either oocyte maturation or fertilization (38).

These inconsistencies may stem from sample size limitations, study duration, or variations in inclusion criteria. A larger cohort and stricter parameters might be needed to better understand the role of KP in oocyte fertilization. Notably, our study showed a significant negative correlation between age and follicular KP levels, with the highest KP levels found among women aged 20–29. This is consistent with findings of Eral *et al.* in Türkiye, where serum KP levels were highest among women aged 20–24 and declined with increasing age (37,39).

Our data also suggest a positive but statistically non-significant correlation between follicular KP levels and pregnancy rate. This observation is in line with a prior study by Qin *et al.* in Thailand, which highlighted the influence of KP on oocyte maturation and fertilization, but not as a direct predictor of pregnancy (39). Interestingly, a study from Hungary by Kiss et al reported that follicular KP, positively correlated with pregnancy outcomes (35). On the other hand, Goktan *et al.* in Türkiye concluded that KP had no significant effect on either fertilization or pregnancy rate (37). This discrepancy may be due to the lack of studies directly evaluating follicular fluid KP in relation to pregnancy outcomes, and because KP alone may not serve as a reliable predictor of pregnancy success.

Finally, our study found a strong, positive, and significant correlation between serum and follicular KP levels. This supports the findings of Qin *et al*. in Thailand, who also demonstrated that serum KP levels could reflect follicular KP levels and estradiol concentrations in infertile women (40). This relationship could be clinically useful for predicting follicular KP levels noninvasively via serum measurements.

Limitations:
This study had a relatively small sample size, which may limit the generalizability of the findings. It also lacked longitudinal follow-up to assess long-term pregnancy outcomes. Additionally, the cross-sectional design precludes establishing causality between kisspeptin levels and reproductive parameters.

Conclusion:
This study highlights the potential role of follicular kisspeptin as a biomarker of ovarian function, showing its association with oocyte quantity and ovarian reserve. While it was not a strong predictor of fertilization or pregnancy outcomes, its correlation with serum levels suggests noninvasive monitoring potential. The findings underscore kisspeptin's relevance in ovarian physiology, but its clinical utility remains uncertain.

Authors' contributions:
Study conception & design: (Hiba R. Qassim & Najeeb H. Mohammed). Literature search: (Hiba M. Rasheed). Data acquisition: (Hiba R. Qassim). Data analysis & interpretation: (Hiba R. Qassim). Manuscript preparation: (Hiba R. Qassim). Manuscript editing & review: (Najeeb H. Mohammed).

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Data availability:
Upon reasonable request, the corresponding author will make the data sets generated and/or analyzed during the current work available.

Authors' declaration:
We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's Approval-Ethical Clearance: The project was approved by the local ethical committee in (Place where the research was conducted or samples collected and treated) according to the code number (236) on (23/ 04/ 2023).

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دور الكيسبيبتين في دورات الحقن المجهري لدى مجموعة من الإناث العراقيات المصابات بالعقم

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الخلاصة
الخلفية: يُعد العقم مشكلة عالمية لا تؤثر على الأفراد فحسب، بل تؤثر أيضًا على المجتمع ككل. وقد برز الكيسبيبتين كعامل رئيسي في فهم جوانب معينة من العقم. ومع ذلك، لا تزال الأبحاث حول دور الكيسبيبتين في الخصوبة، وخاصةً في علاجات الإنجاب المساعد، محدودة.
الأهداف: دراسة العلاقة المحتملة بين مستويات الكيسبيبتين في السائل الجريبي ومختلف المعايير التناسلية، بما في ذلك عدد البويضات ونضجها، ومعدل الإخصاب، واكتشاف الحمل، ومستويات الهرمون المضاد لمولر، وعمر الإناث.
المنهجية: شارك في هذه الدراسة ستون زوجًا يعانون من العقم، تتراوح أعمارهم بين ٢٠ و٤٠ عامًا. اشتكوا من عقم غير مبرر، ولم تكن لديهم أي عوامل هرمونية أو ذكورية، وجميعهم اتبعوا بروتوكول التحفيز نفسه، حيث جُمعت عينات من السائل الجريبي في يوم سحب البويضات. طُردت هذه العينات بالطرد المركزي، ثم جُمدت عند درجة حرارة -٢٠ درجة مئوية حتى تاريخ إجراء اختبار الممتز المناعي المرتبط بالإنزيم (ELISA) للكشف عن هرمون الكيسبيبتين. أُجريت الدراسة في المعهد العالي لتشخيص العقم وتقنيات الإنجاب المساعدة، جامعة النهرين، بغداد، في الفترة من ١ يناير إلى ١ يوليو ٢٠٢٣.
النتائج: وُجد ارتباط سلبي كبير بين مستويات الكيسبيبتين الجريبي وعمر الأنثى. وارتبطت مستويات الكيسبيبتين ارتباطًا إيجابيًا بعدد البويضات المسترجعة، والبويضات الناضجة، والبويضات المخصبة، ومستويات AMH. ولوحظت مستويات الكيسبيبتين أعلى لدى النساء الحوامل (707.7 ± 235.28 بيكوغرام/مل) مقارنةً بغير الحوامل (648.9 ± 215.27 بيكوغرام/مل)، إلا أن الفرق لم يكن ذا دلالة إحصائية. وُجد ارتباط إيجابي قوي بين الكيسبيبتين الجريبي والمصلي.
الإستنتاج: تشير هذه الدراسة إلى أن الكيسبيبتين قد يلعب دورًا رئيسيًا في وظيفة المبيض، إذ ارتبطت مستوياته بجودة البويضات واحتياطي المبيض. وتُبرز العلاقة الملحوظة بين مستويات الكيسبيبتين وعمر الأنثى ونتائجها الإنجابية إمكاناته كمؤشر حيوي في الإنجاب المساعد.
الكلمات المفتاحية: كيسبيبتين، السائل الجريبي، الإخصاب، البويضات، الجريبات المبيضية.