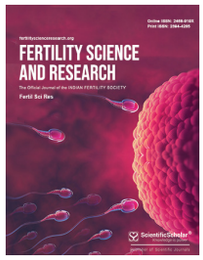
ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

https://fertilityscienceresearch.org

Fertility Science and Research



Original Article

Association of Follicular Fluid Kisspeptin-54 Levels and Ovarian Response to Stimulation

Erin R. Hupy[1], Michael Wolfe[2], Gillian Epp Luellen Luevano[1], Courtney Marsh[1]

[1]Departments of Obstetrics and Gynaecology, [2]Cell Biology and Physiology, University of Kansas Medical Centre, Kansas City, KS, USA

***Corresponding author:**
Erin R. Hupy,
Department of Obstetrics and Gynaecology, University of Kansas Medical Centre, Kansas City, KS, USA

hupy.erin@mayo.edu

Received: 21 January 2025
Accepted: 30 April 2025
Published: 12 June 2025

DOI
10.25259/FSR_3_2025

Quick Response Code:

ABSTRACT

Objectives: There is demand for new biomarkers of fertility, particularly among those utilizing assisted reproductive technology. This study aims to evaluate the association of follicular fluid kisspeptin-54 (Kp-54) levels and response to controlled ovarian hyperstimulation in women undergoing egg retrieval for in vitro fertilization.

Material and Methods: Study participants included patients who underwent egg retrieval at the University of Kansas in 2019. They were separated into categories based on response to controlled ovarian hyperstimulation: moderate (6-15 oocytes) and high (≥ 20 oocytes). Follicular fluid was evaluated for Kp-54 concentration using enzyme-linked immunoassay (ELISA).

Results: Baseline characteristics were similar between the moderate (n = 23) and high (n = 22) responder groups. Mean Kp-54 concentrations were not significantly different between the moderate (0.2077 ± 0.124 ng/mL) and high (0.1905 ± 0.0886 ng/mL) responder groups (p = 0.5971). There were no significant correlations between Kp-54 concentration and age, body mass indec (BMI), gravidity, parity, or live birth rate. The moderate and high responder groups also showed no difference in the number of live birth rate.

Conclusion: This is the first study to our knowledge that compares follicular fluid Kp-54 concentrations between moderate and high responders to ovarian stimulation. Kp-54 may hold potential within the field of assisted reproductive technology, and further investigation is needed to determine its utility as a fertility biomarker.

Keywords: Biomarker, Controlled ovarian stimulation, Fertility, In vitro fertilisation, Kisspeptin

INTRODUCTION

Kisspeptin-54 (Kp-54) is a neuropeptide produced by the hypothalamus and is thought to be an important regulator of several biological processes. Kp-54 is encoded by the *KISS-1* gene, which was initially identified in melanoma cells as a metastasis suppressor gene.[1] It was originally named "metastin" due to its inhibitory effect on chemotaxis, invasion, and metastasis.[2] It has since been identified in many other tissues throughout the body, including the pancreas, intestine, ovaries, and placenta.[2–4] Kp-54 is secreted by kisspeptin neurones, which in humans exist primarily in the infundibular nucleus of the hypothalamus.[5] The target of Kp-54 is a G-protein coupled receptor called GPR-54, which is similarly expressed in diverse tissues. The seemingly widespread role of Kp-54 led researchers to suspect diverse functions outside of melanoma regulation; this was later confirmed when a study by de Roux *et al.* demonstrated that a mutation in GPR-

54 led to hypogonadotropic hypogonadism.[6] A similar study by Seminara *et al.* showed that GPR-54 mutations result in autosomal recessive idiopathic hypogonadotropic hypogonadism in humans and mice.[7] Kp-54 later emerged as a potent activator of gonadotropin-releasing hormone (GnRH) neurones, further implicating the role of Kp-54 in reproductive function.[8–10]

Kp-54 has since been identified as a key member of the hypothalamic-pituitary-gonadal (HPG) axis, though its entire regulatory role remains undetermined. Kp-54 likely influences the secretion of GnRH by integrating central and peripheral signals; GnRH secretion can then stimulate the release of luteinising hormone and follicle-stimulating hormone.[11] These gonadotropins stimulate the release of sex steroid hormones, which ultimately exert feedback on the hypothalamus and complete the regulatory loop. Though GnRH neurones in the hypothalamus are known to respond to sex steroid hormone levels, the mechanism by which this occurs is unclear because these neurones lack oestrogen and progesterone receptors.[12,13] Kp-54 may represent the "missing link" that connects the players of the HPG axis, as Kp-54 neurones express sex steroid receptors and project over GnRH neurones, which express Kp-54 receptors.[14–18]

Recent studies have identified even broader functions of Kp-54, including the regulation of puberty,[6,7,10] ovulation,[19–21] placentation,[22–25] and pregnancy.[26–29] In the realm of assisted reproductive technology (ART), Kp-54 has already been used as an effective oocyte maturation trigger with very low rates of ovarian hyperstimulation syndrome.[30,31] It is currently regarded as the most potent stimulator of GnRH secretion.[32,33] It has also been shown to increase granulosa cell receptivity to gonadotropin stimulation during in vitro fertilisation (IVF).[34]

In light of Kp-54's many physiologic roles, there has become interest in exploring its potential as a fertility biomarker or even a therapeutic target in addressing reproductive disorders. Because of this, a deeper understanding of Kp-54's role in the ovary and HPG axis is desired. Several studies have measured serum Kp-54 concentrations in association with clinical features such as pregnancy, polycystic ovarian syndrome, and precocious puberty.[22,35–38] Very little is known about Kp-54 concentrations in follicular fluid, though this information could be useful in providing insight into the role of Kp-54 in the local environment of the ovary and maturing oocyte. Furthermore, a deeper understanding of the relationship between follicular fluid Kp-54 (ff Kp-54) levels and response to controlled ovarian hyperstimulation in IVF patients could be informative in the prediction of fertility outcomes.

We hypothesised that there is an association between ff Kp-54 concentration and response to controlled ovarian stimulation. To test this hypothesis, we compared the ff Kp-54 concentrations of IVF patients with moderate versus high responses to controlled ovarian hyperstimulation. We also evaluated whether ff Kp-54 levels vary with age, BMI, gravidity, parity, or pregnancy outcomes.

MATERIAL AND METHODS

Sample Collection

This study was approved by the Institutional Review Board of the University of Kansas Medical Centre. All participants provided written consent following a thorough informed consent discussion. A power calculation was performed to assess the number of participants needed to detect a 30% difference between the moderate and high responders based on data presented by Bodis *et al.*[39] We recruited study participants from patients aged 18–55 undergoing IVF at the University of Kansas Centre for Advanced Reproductive Medicine in 2019. A total of 45 patients were enrolled. During routine egg retrieval by a reproductive endocrinologist, the "first pass" follicular fluid (fluid surrounding the first aspirated oocyte) was collected and reserved before mixing with flushing media. The sample was placed in a specimen cup and stored on ice. The remaining oocytes were collected in the standard fashion, completing the oocyte retrieval per protocol. The follicular fluid was later categorised into a study group based on the patient's response to stimulation – those who produced 6–15 oocytes were considered moderate responders, while those who produced ≥ 20 oocytes were considered high responders. These categories were set according to definitions established in literature (Fanton *et al.*)[40]; patients with < 6 or 16–19 oocytes retrieved were excluded accordingly. The follicular fluid sample was then immediately transported to the laboratory of Dr. Michael Wolfe, where it was transferred to a 15 ml conical tube via a p1000 micropipette. The sample was then centrifuged for 5 minutes at 0.8 RCF at 4°C. The supernatant was removed and redistributed into microcentrifuge tubes. The samples were stored in a −80°C freezer until the time of sample analysis.

Sample Analysis and Statistics

When participant recruitment was complete and all samples had been collected, follicular fluid samples were retrieved from the −80°C freezer and thawed over ice. Each sample was evaluated for Kp-54 concentration using an enzyme-linked immunoassay by Phoenix Pharmaceuticals. Assays were completed in duplicate, and Kp-54 concentrations were measured by absorbance at 450 nm on a SpectraMax iD5 plate reader. The data was displayed on SoftMax Pro and calculated into Kp-54 concentration (ng/ml) using the average of the duplicate samples and according to a standard curve. Independent sample t-tests were used to assess differences in age, BMI, gravidity, parity, and ff Kp-54 concentration between the moderate and high responder groups. Correlations

between ff Kp-54 concentration and age, BMI, gravidity, parity, and pregnancy outcomes were calculated using Pearson's correlation coefficients. The birth outcome of interest was defined as the rate of live births per embryo transfer.

RESULTS

Of the 45 study participants, 23 were categorised into the moderate responder group and 22 were categorised into the high responder group. The mean ff Kp-54 concentration in the moderate responder group was 0.2077 ± 0.124 ng/ml, compared to 0.1905 ± 0.0886 ng/ml in the high responder group. These concentrations were not statistically significantly different (p = 0.5971).

Baseline characteristics of age, BMI, gravidity, and parity were evaluated. The mean age of participants in the moderate responder group was 34.9130 ± 3.4066 and 33.1364 ± 4.1332 in the high responder group. The mean BMI was 26.6227 ± 5.5835 for moderate responders and 28.4300 ± 5.5035 for high responders. Similarly, there was little difference between moderate and high responder groups with respect to gravidity and parity. The mean gravidity for moderate responders was 0.9333 ± 1.0998 and for high responders was 0.6923 ± 1.1821, whereas the mean for parity was 0.2000 ± 0.4140 for moderate responders and 0.3846 ± 0.6504 for high responders. No statistically significant differences in these baseline characteristics were noted between the comparison groups [Table 1]. Lastly, associations between ff Kp-54 concentration and age, BMI, gravidity, parity, and live birth per embryo transfer were assessed [Table 2]. No statistically significant associations were identified.

Table 1: Baseline characteristics between moderate and high responders.

	Moderate responders	High responders	p
Age	34.9130 ± 3.4406	33.1364 ± 4.1332	0.1253
BMI	26.6227 ± 5.5835	28.4300 ± 5.5034	0.2978
Gravidity	0.9333 ± 1.0998	0.6923 ± 1.1821	0.5812
Parity	0.2000 ± 0.4140	0.3846 ± 0.6504	0.372

Table 2: Association between Kp-54 and age, BMI, gravidity, parity, and live birth per embryo transfer (ET).

	r	p
Age	–0.05872	0.6983
BMI	–0.02957	0.8507
Gravidity	–0.13335	0.4904
Parity	–0.10447	0.5896
Live birth rate per ET	–0.21297	0.1553

DISCUSSION

In this study population, we found no statistically significant differences in the ff Kp-54 concentration between moderate and high responders to ovarian stimulation. Similarly, no statistically significant associations were noted between ff Kp-54 concentration and age, BMI, gravidity, parity, or birth outcomes. Kp-54 has a variety of functions throughout the body, including roles in puberty,[6,7,10] ovulation,[19–21] placentation,[22–25] and pregnancy.[26–29] Kp-54 is already in use as an oocyte maturation trigger and may have other applications in ART.[30,31] This study sought to better understand how knowledge of Kp-54 concentrations at the level of the ovarian follicle might predict treatment outcomes.

This study is limited by ff Kp-54 concentrations that were lower and more similar than expected, resulting in an underpowered study design. This was unanticipated, as our initial power calculation was appropriate and indicated a need for 8 participants in each group; over 45 total patients were recruited. Of note, the Kp-54 assays used in these studies were from different manufacturers. Strengths of the study include the similarity of the study populations at baseline. A study with more participants who have a more diverse age, BMI, gravidity, and parity would likely be more informative.

Though ff Kp-54 concentrations were found to be similar in patients with moderate and high response to controlled ovarian stimulation, there remains biological plausibility for Kp-54 as a fertility marker in other applications. Given its known physiologic roles in fertility and reproduction, many questions remain: How much could Kp-54 concentrations vary using different assays? Could there be different utilities for serum versus follicular fluid Kp-54 levels? Is one better than the other for predicting early pregnancy outcomes or IVF outcomes? If Kp-54 were to become commercially available (i.e., could be ordered easily in the hospital setting), could we correlate these levels with β-hCG or another hormonal marker for better predictive value?

CONCLUSION

In this study, we aimed to identify whether a difference in ff Kp-54 concentrations exists between moderate and high responders to controlled ovarian hyperstimulation. We found that no such difference exists in this study population. We also identified no association between ff Kp-54 concentration and age, BMI, gravidity, parity, or pregnancy outcomes. Additional investigation is needed to better understand the role of Kp-54 in the ovarian follicle and how it may be targeted to improve fertility outcomes.

Author contribution

Lane Christenson: Laboratory resources; Elaine Phillips: Laboratory support; Sharon Fitzgerald: Manuscript editing.

Acknowledgements: Lane Christenson, PhD; Elaine Phillips, Sharon Fitzgerald, MPH.
Ethical approval: The research/study was approved by the Institutional Review Board at the University of Kansas Medical Centre, approval number 24-010101, dated 16th October 2021.
Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent.
Financial support and sponsorship: Nil.
Conflicts of interest: There are no conflicts of interest.
Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

1. Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, *et al*. KiSS-1, a Novel Human Malignant Melanoma Metastasis-Suppressor Gene. J Natl Cancer Inst 1996;88:1731–7.
2. Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, *et al.* Metastasis Suppressor Gene KiSS-1 Encodes Peptide Ligand of a G-Protein-Coupled Receptor. Nature 2001;411:613–7.
3. Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden J-M, Le Poul E, *et al*. The Metastasis Suppressor Gene KiSS-1 Encodes Kisspeptins, the Natural Ligands of the Orphan G Protein-coupled Receptor GPR54. J Biol Chem 2001;276:34631–6.
4. Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A, *et al*. AXOR12, a Novel Human G Protein-coupled Receptor, Activated by the Peptide KiSS-1. J Biol Chem 2001;276:28969–75.
5. Rometo AM, Krajewski SJ, Lou Voytko M, Rance NE. Hypertrophy and Increased Kisspeptin Gene Expression in the Hypothalamic Infundibular Nucleus of Postmenopausal Women and Ovariectomized Monkeys. J Clin Endocrinol Metab 2007;92:2744–50.
6. de Roux N, Genin E, Carel J-C, Matsuda F, Chaussain J-L, Milgrom E. Hypogonadotropic Hypogonadism Due to Loss of Function of the KiSS1-Derived Peptide Receptor GPR54. Proc Natl Acad Sci 2003;100:10972–6.
7. Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS, Shagoury JK, *et al*. The GPR54 Gene as a Regulator of Puberty. N Engl J Med 2003;349:1614–27.
8. Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, *et al*. A Role for Kisspeptins in the Regulation of Gonadotropin Secretion in the Mouse. Endocrinology 2004;145:4073–7.
9. Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T. Peripheral Administration of Metastin Induces Marked Gonadotropin Release and Ovulation in the Rat. Biochem Biophys Res Commun 2004;320:383–8.
10. Navarro VM, Fernández-Fernández R, Castellano JM, Roa J, Mayen A, Barreiro ML, *et al.* Advanced Vaginal Opening and Precocious Activation of the Reproductive Axis by KiSS-1 Peptide, the Endogenous Ligand of GPR54. J Physiol 2004;561:379–86.
11. Latif R, Rafique N. Serum Kisspeptin Levels Across Different Phases of the Menstrual Cycle and their Correlation with Serum Oestradio. Neth J Med 2015;73:175–8.
12. Hrabovszky E. Neuroanatomy of the Human Hypothalamic Kisspeptin System. Neuroendocrinology 2014;99:33–48.
13. Leonardi CEP, Dias FCF, Adams GP, Araujo ER, Singh J. Kisspeptin Induces Ovulation in Heifers Under Low Plasma Progesterone Concentrations. Theriogenology 2020;141:26–34.
14. Franceschini I, Lomet D, Cateau M, Delsol G, Tillet Y, Caraty A. Kisspeptin Immunoreactive Cells of the Ovine Preoptic Area and Arcuate Nucleus Co-express Estrogen Receptor Alpha. Neurosci Lett 2006;401:225–30.
15. Greenwald-Yarnell ML, Marsh C, Allison MB, Patterson CM, Kasper C, *et al*. ERα in Tac2 Neurons Regulates Puberty Onset in Female Mice. Endocrinology 2016;157:1555–65.
16. Han S-K, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, *et al*. Activation of Gonadotropin-Releasing Hormone Neurons by Kisspeptin as a Neuroendocrine Switch for the Onset of Puberty. J Neurosci 2005;25:11349–56.
17. Herbison AE, d'Anglemont de Tassigny X, Doran J, Colledge WH. Distribution and Postnatal Development of Gpr54 Gene Expression in Mouse Brain and Gonadotropin-Releasing Hormone Neurons. Endocrinology 2010;151:312–21.
18. Huang X, Harlan RE. Absence of Androgen Receptors in LHRH Immunoreactive Neurons. Brain Res 1993;624:309–11.
19. Goto Y, Endo N, Nagai K, Ohkura S, Wakabayashi Y, Tanaka A, *et al*. Ovarian and Hormonal Responses to Follicular Phase Administration of Investigational Metastin/Kisspeptin Analog, TAK-683, in Goats. Reprod Domest Anim 2014;49:338–42.
20. Zhai J, Ding L, Zhao S, Li W, Sun Y, Su S, *et al*. Kisspeptin: A New Marker for Human Pre-ovulation. Gynecol Endocrinol 2017;33:560–3.
21. Smith JT, Popa SM, Clifton DK, Hoffman GE, Steiner RA. Kiss1 Neurons in the Forebrain as Central Processors for Generating the Preovulatory Luteinizing Hormone Surge. J Neurosci 2006;26:6687–94.
22. Horikoshi Y, Matsumoto H, Takatsu Y, Ohtaki T, Kitada C, Usuki S, *et al*. Dramatic Elevation of Plasma Metastin Concentrations in Human Pregnancy: Metastin as a Novel Placenta-Derived Hormone in Humans. J Clin Endocrinol Metab 2003;88:914–9.
23. Hu K-L, Chang H-M, Zhao H-C, Yu Y, Li R, Qiao J. Potential Roles for the Kisspeptin/Kisspeptin Receptor System in Implantation and Placentation. Hum Reprod Update 2018;25:326–43.
24. Matjila M, Millar R, van der Spuy Z, Katz A. Elevated Placental Expression at the Maternal–Fetal Interface But Diminished Maternal Circulatory Kisspeptin in Preeclamptic Pregnancies. Pregnancy Hypertens 2016;6:79–87.
25. Radovick S, Babwah AV. Regulation of Pregnancy: Evidence for Major Roles by the Uterine and Placental Kisspeptin/KISS1R Signaling Systems. Semin Reprod Med 2019;37:182–90.
26. Armstrong RA, Reynolds RM, Leask R, Shearing CH, Calder AA, Riley SC. Decreased Serum Levels of Kisspeptin in Early Pregnancy are Associated with Intra-uterine Growth Restriction and Pre-eclampsia. Prenat Diagn 2009;29:982–5.

27. Jayasena CN, Abbara A, Izzi-Engbeaya C, Comninos AN, Harvey RA, Gonzalez Maffe J, *et al.* Reduced Levels of Plasma Kisspeptin During the Antenatal Booking Visit Are Associated With Increased Risk of Miscarriage. J Clin Endocrinol Metab 2014;99:E2652–60.
28. Torricelli M, Novembri R, Conti N, De Falco G, De Bonis M, Petraglia F. Correlation with Placental Kisspeptin in Postterm Pregnancy and Apoptosis. Reprod Sci 2012;19:1133–7.
29. Wu S, Zhang H, Tian J, Liu L, Dong Y, Mao T. Expression of Kisspeptin/GPR54 and PIBF/PR in the First Trimester Trophoblast and Decidua of Women with Recurrent Spontaneous Abortion. Pathol Res Pract 2014;210:47–54.
30. Abbara A, Clarke SA, Dhillo WS. Novel Concepts for Inducing Final Oocyte Maturation in In Vitro Fertilization Treatment. Endoc Rev 2018;39:593–628.
31. Jayasena CN, Abbara A, Comninos AN, Nijher GMK, Christopoulos G, Narayanaswamy S, *et al.* Kisspeptin-54 Triggers Egg Maturation in Women Undergoing In Vitro Fertilization. J Clin Invest 2014;124:3667–77.
32. Quaas AM, Legro RS. Pharmacology of Medications Used for Ovarian Stimulation. Best Pract Res Clin Endocrinol Metab 2019;33:21–33.
33. Roa J, Castellano JM, Navarro VM, Handelsman DJ, Pinilla L, Tena-Sempere M. Kisspeptins and the Control of Gonadotropin Secretion in Male and Female Rodents. Peptides 2009;30:57–66.
34. Owens LA, Abbara A, Lerner A, O'floinn S, Christopoulos G, Khanjani S, *et al.* The Direct and Indirect Effects of Kisspeptin-54 on Granulosa Lutein Cell Function. Hum Reprod 2017;33:292–302.
35. Liu J, Qu T, Li Z, Yu L, Zhang S, Yuan D, *et al.* Serum Kisspeptin Levels in Polycystic Ovary Syndrome: A Meta-Analysis. J Obst Gynaecol Res 2021;47:2157–65.
36. Mut A, Erel CT, İnan D, Öner YÖ. Serum Kisspeptin Levels Correlated with Anti-mullerian Hormone Levels in Women with and Without Polycystic Ovarian Syndrome. Gynecol Endocrinol 2020;37:1–5.
37. Tsoutsouki J, Patel B, Comninos AN, Dhillo WS, Abbara A. Kisspeptin in the Prediction of Pregnancy Complications. Front Endocrinol 2022;13.
38. Xue J, Song W, Si M, Sun C, Li K, Wang W, *et al.* Serum Kisspeptin and AMH Levels Are Good References for Precocious Puberty Progression. Dufau ML, editor. Int J Endocrinol 2020;2020:1–6.
39. 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. J Int Med Res. 2020;48:300060519879330.
40. Fanton M, Cho JH, Baker VL, Loewke K. A Higher Number of Oocytes Retrieved is Associated with an Increase in Fertilized Oocytes, Blastocysts, and Cumulative Live Birth Rates. Fertil Steril 2023;119:762–9.

How to cite this article: Hupy ER, Wolfe M, Luevano GEL, Marsh C. Association of Follicular Fluid Kisspeptin-54 Levels and Ovarian Response to Stimulation. Fertil Sci Res. 2025;12:15. doi: 10.25259/FSR_3_2025