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Association of Serum Kisspeptin Levels and Pregnancy Outcomes in Assisted Reproductive Technology

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Abstract

Kisspeptin, a neuropeptide produced by syncytiotrophoblast cells, has been hypothesized to regulate successful placental formation. Several studies have shown a positive correlation between maternal serum kisspeptin levels and successful pregnancy outcomes in second and third-trimester pregnancy, but trends in early pregnancy have not been well-established. In this prospective case-control study, we examine whether kisspeptin levels correlate with serum beta-human chorionic gonadotropin (bhCG), progesterone, or pregnancy outcomes at the time of pregnancy testing in cis-gendered females (n= undergoing assisted reproductive technology. Pregnancy testing occurred at an average of 33 days after the last menstrual period for non-IVF protocols and an average of 12 days after blastocyst transfer for IVF protocols. Outcome groups included: viable first-trimester pregnancy (n=18), early miscarriage (n=12), ectopic pregnancy (n=5), biochemical pregnancy (n=16), and not pregnant (n=12). Descriptive statistics including Chi-squared or Fisher's Exact, ANOVA and Pearson's correlation coefficient were utilized. Kisspeptin values did not show a significant difference between outcome groups. A weak positive correlation was demonstrated between kisspeptin and bhCG in biochemical and viable pregnancy but not for other outcomes. In contrast, a significant correlation between kisspeptin and measured progesterone value or exogenous progesterone, regardless of administration route, was not observed. In this study population, a single serum measurement of kisspeptin very early in pregnancy was not significantly different between pregnancy outcomes. The utility of kisspeptin as a pregnancy biomarker in the first trimester remains unclear. Additional studies are needed to further investigate the relationship between viable intrauterine pregnancy and serum kisspeptin concentration in the early first trimester and the current findings suggest that it may prove useful in combination with bhCG.

Keywords: Assisted Reproductive Technology, Abortion, Kisspeptins, Pregnancy

Introduction

Assisted reproductive technology is associated with a significant emotional burden as well as an investment of time and money. According to the 2019, CDC Assisted Reproductive Technology National Summary Report, 2% of infants born in the United States were conceived with assisted reproductive technology (1). Fetal viability after embryo transfer is accessed using serial measurements of serum beta-human chorionic gonadotropin (bhCG) until the first ultrasound at four to five weeks post-embryo transfer. About 20% of cases resulting in miscarriage are associated with rising bhCG (2). Additionally, in an ectopic pregnancy (pregnancy of undetermined location), bhCG levels are highly variable and must be combined with transvaginal ultrasound for diagnostic utility (3). Thus, early recognition of viable pregnancies is valuable to counsel patients about clinical options related to an adverse outcome and could inform the timing of subsequent cycles.

Kisspeptin's role in hypothalamic gonadotropin-releasing hormone release has been well documented (4). Kisspeptin is also expressed in high levels by the placenta (5). The syncytiotrophoblast produces kisspeptin, and its receptor (KISS1R) is expressed by both cytotrophoblasts and syncytiotrophoblasts (6, 7). When produced by syncytiotrophoblast placental cells, kisspeptin regulates villous trophoblast invasion into the uterus (6-8). In vitro, studies have shown kisspeptin to decrease trophoblast migration but not proliferation. Kisspeptin reduces the expression of matrix metalloproteinase II, which is essential for extracellular matrix degradation and invasive progression into the maternal decidua (7, 9). Expression of KISS-1 and localization of KISS-1R correlated with cells that had fused and become non-invasive (7). The hypothesis is that placental kisspeptin released into maternal systemic circulation could provide biochemical evidence of trophoblastic cells that have reached adequate invasion. Circulating levels of kisspeptin have been shown to increase 7000-fold during pregnancy. Jayasena et al. and Sullivan-Pyke et al. could distinguish miscarriage from intrauterine pregnancy by serum kisspeptin measurements after the 6th week of pregnancy. However, trends in early pregnancy have not been well documented.

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In the present study, we sought to determine kisspeptin trends at the time of pregnancy testing in women undergoing assisted reproductive technology around multiple pregnancy outcomes and delineate correlations between bhCG, kisspeptin, and progesterone.

Materials and Methods

Patient Selection and Study Design

This case-control study included women seeking assisted reproductive technology at the University of Kansas Center for Advanced Reproductive Medicine between August 2021 to December 2021. Samples were retrospectively analyzed based on the first-trimester outcome. We collected participants' basic descriptive statistics, including age, race, gravidity, parity, and body mass index (BMI). This study was approved by the University of Kansas School of Medicine IRB committee and meets all ethical guidelines.

Treatment Protocols and Medications

The provider determined the ovarian stimulation protocol, which could include estrogen, progesterone, clomid, letrozole, or gonadotropins per the provider's discretion. The mode of delivery of conception materials could include egg retrieval and fertilization via in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), intrauterine insemination, or intercourse. Exogenous progesterone supplementation was added via progesterone injection or vaginal progesterone.

Outcomes Determination

Maternal serum samples were obtained at the time of pregnancy testing. For IVF protocol, this occurred a median of 12 days after blastocyst transfer (n=37). For spontaneous and non-IVF protocols, this happened at a median of 33 days after the last menstrual period (n=27). Positive pregnancy was bhCG > 10 mlU/mL (n=51), and negative pregnancy was bhCG < 10 mlU/mL (n=12). Women who achieved positive pregnancy continued to be monitored by subsequent bhCG measurement and ultrasound at between 6-7 weeks of gestational age. Women who experienced biochemical pregnancy loss (n=16), first-trimester early miscarriage (n=12), ectopic pregnancy (n=5), and first-trimester intrauterine gestation (n=18) were included. Biochemical pregnancy loss was defined as a positive pregnancy with spontaneous demise indicated by falling subsequent bhCG measurements without ultrasound visualization of a yolk sac. First-trimester early miscarriage was defined as a positive pregnancy determined by bhCG and visualization of fatal yolk sac on ultrasound with subsequent demise confirmed by falling bhCG and ultrasound. Ectopic pregnancy was identified by a pregnancy visualized outside of the endometrial cavity by transvaginal ultrasound and/or salpingectomy with chorionic villi confirmed by pathology and/or a bhCG rise of less than 66% every 48 hours, signifying a lack of fetal viability, with methotrexate administered causing a fall in bhCG without a pregnancy visualized in the endometrial cavity. Viable first-trimester pregnancy was defined as an adequate fatal heart rate visualized on ultrasound at 13 weeks gestation.

Collection and Measurement of bhCG, Progesterone, and Kisspeptin Concentrations

Samples were collected in Lithium Heparin Vacutainer tubes containing 100uL/mL blood aprotinin (Phoenix Pharmaceuticals, Inc. Catalog RK-APRO). Samples were centrifuged at 1,600 g for 15 minutes at 4°C, and serum was flash-frozen with liquid nitrogen and stored at -80°C until assayed. The concentration of total kisspeptin (including kisspeptin- 10, 54) was measured by enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals, Inc. Catalog No EK-048056) according to manufacturer instructions. The intra-assay and inter-assay variations were 10% and 15%, respectively, with a detection limit of 0.08 ng/mL. The optical density of wells was measured at 450 nm by SpectraMax iD5 plate reader. Progesterone and bhCG levels were measured by sequential two-step immunoenzyme assay (Beckman Counter DXI 800).

Statistical Analysis

Data were analyzed using SAS version 9.4. A Chi-squared or Fisher's Exact to determine differences in categorical data. Continuous data were analyzed using analysis of variance (ANOVA). Pearson's correlation coefficient was used to analyze the correlation for continuous variables such as kisspeptin and bhCG which were further stratified by the outcome. The study was adequately powered with a sample size of 64.

Results

The outcome group had a total of 64 participant samples. The average age of the participants was 34.4 (SD 7.2). The majority of participants received an IVF protocol. The average BMI was 29.7 (SD 7.5). There was an even split between nulligravid vs. one or more pregnancies. Most were nulliparous (45 out of 64 or 71%). No statistically significant differences were seen between demographic characteristics and outcome groups (Table 1).

Kisspeptin values did not show a significant difference between outcome groups (P=0.6) (Figure 1). Furthermore, kisspeptin was not significantly increased in pregnant women (Figure 1). No significant correlation was observed between kisspeptin and bhCG overall (R=0.2, P=0.2) (Figure 2A). Kisspeptin and bhCG levels did show a weak, but statistically significant positive correlation for viable pregnancy (R=0.5, P=0.02) and biochemical pregnancy (R=0.-6, P=0.03) but not for ectopic (R=-0.7, P=0.2), early miscarriage (R=-0.1, P=0.7), or not pregnant (R=-0.2, P=0.6) (Figure 2 B-F).

Kisspeptin did not show a statistically significant correlation with progesterone regardless of outcome or route of administration (Table 2 and Figure 3 A-F).

Conclusions

Kisspeptin has been shown to increase 900-fold during the first trimester of pregnancy, with levels of kisspeptin positively correlating to levels of placental-derived sex hormones (10). This suggests that the origin of the exponential production of kisspeptin is placentally derived. A recent meta-analysis showed kisspeptin to be of higher diagnostic utility than bhCG in diagnosing miscarriage after the sixth week of pregnancy (11).

The diagnostic utility of kisspeptin has not been shown to extend before six weeks of gestation. In a recent case-control study by Yu et al., kisspeptin and bhCG were measured sequentially at the time of pregnancy testing and then four days afterwards in patients undergoing IVF with ISCI. They found

kisspeptin was not superior to bhCG for diagnosing early miscarriage and biochemical pregnancy, and the trends for kisspeptin were not as clear as bhCG between measurements for these outcomes (12). A similar case-control study by Hu et al. compared serum kisspeptin levels between singleton pregnancies, early miscarriages, twin pregnancies, and biochemical pregnancies at 14 days and 21 days post embryo transfer. At 14 days, this study found that kisspeptin was not significantly increased but had increased by day 21. At day 21, serum kisspeptin levels had a poor predictive value of miscarriage compared with serum bhCG levels (13). The present study observed that kisspeptin values were not statistically different between outcome groups at a median of 12 days post blastocyst transfer/ 33 days from LMP. Therefore, we confirm that kisspeptin levels appear to be of poor prognostic value at this early time point. This is likely due to low levels of circulating kisspeptin that has not increased to sufficient levels to differentiate a viable pregnancy from other outcomes assessed.

In the case of ectopic pregnancy, previous studies are conflicting. Two prior studies showed the diagnostic value of kisspeptin in first-trimester ectopic pregnancy. The first was a case-control prospective study that included patients symptomatic for ectopic pregnancy at the 4^{th}-20^{th} week of gestation. This study showed kisspeptin to be dramatically decreased in ectopic pregnancy vs. healthy controls undergoing a voluntary abortion (14). In agreement, another case-control prospective study showed kisspeptin to discriminate between miscarriage, ectopic, viable pregnancy, and not pregnant at five to six weeks gestation (15). In contrast, a third case-control prospective study that assessed asymptomatic and symptomatic pregnant women between five and six weeks with the pregnancy of unknown location versus intrauterine pregnancy found no significant differences in circulating levels of kisspeptin (16). The present study, to our knowledge, is the only one to access the value of kisspeptin for ectopic pregnancy before the fourth week of pregnancy. We found no significant difference in kisspeptin levels in patients with an ectopic pregnancy compared to other outcomes. We suspect that this is likely due to the early first trimester time point.

This study also found no correlation between levels of kisspeptin and bhCG at 12 days post blastocyst transfer/33 days from LMP for the sample size and in early miscarriage. This is likely due to the difference between bhCG and kisspeptin physiologically during the early stages of pregnancy. While bhCG functions to stimulate progesterone production by the corpus luteum in humans, kisspeptin has been implicated in implantation and decidualization in rodents. A uterine-based kisspeptin/KISSR signaling system is necessary for the embryo adhesion and penetration (but not the embryo apposition) stages of implantation due to the upregulation of glandular leukemia inhibitory factor (LIF) that is essential for mouse blastocyst implantation (17). Additionally, kisspeptin-triggered LIF contributes to uterine decidualization. Kiss1 and its receptor increase with the progression of stromal cell decidualization, and the process is blocked by down-regulating Kiss1 using siRNA against Kiss1 (18). In humans, the kisspeptin receptor is expressed in decidual stromal cells, where it controls cell motility (19). Kisspeptin's roles in implantation and decidualization also provide additional possible confounding kisspeptin sources of production at an early stage of pregnancy. A weak positive correlation between kisspeptin and bhCG was delineated for viable and biochemical pregnancy. This agrees with Yu et al., which showed that decreased kisspeptin and bhCG are found in a biochemical pregnancy, and increased kisspeptin and bhCG are found in a viable pregnancy (12). This suggests that kisspeptin is indeed changing in parallel with the pregnancy outcome. However, the differences are not large enough to be of prognostic value.

Table 1. Demographic data by pregnancy outcome.

Sample Characteristic	Total (n=63)	Biochemical Pregnancy (n=16)	Early Miscarriage (n=12)	Ectopic Pregnancy (n=5)	Not Pregnant (n=12)	Viable Pregnancy (n=18)	p-Value
Age, mean (SD)	34.4 (7.2)	33.9 (6.1)	34.6 (5.7)	36.6 (6.2)	35.1 (4.6)	33.7 (3.3)	0.78
Race, n (%)							
White	54 (85.7)	15 (93.8)	10 (83.3)	4 (80.0)	10 (83.3)	15 (83.3)	0.10
African Am	3 (4.7)	1 (6.3)	0	0	0	0	
Hispanic	2 (3.2)	0	2 (16.7)	0	0	0	
Asian	2 (3.2)	0	0	1 (20.0)	2 (16.7)	1 (5.56)	
Other	2 (3.2)	0	0	0	0	2 (11.1)	
Protocol, n (%)	36 (57.1)	8 (50.0)	7 (19.4)	2 (40.0)	11 (91.7)	8 (44.4)	0.18
IVF	22 (34.4)	7 (43.8)	3 (13.6)	3 (13.6)	1 (8.3)	8 (44.4)	
Non-IVF	5 (7.8)	1 (6.3)	2 (16.7)	0	0	2 (11.1)	
Spontaneous							
BMI mean (SD)	29.7 (7.5)	28.5 (5.8)	29.6 (7.7)	37.0 (9.6)	28.0 (8.7)	29.9 (6.9)	0.24
Gravidity, n (%)							
Zero	31 (49.2)	7 (22.6)	5 (16.3)	4 (6.35)	5 (8.0)	10 (15.9)	0.61
1 or more	32 (50.8)	9 (12.3)	7 (11.1)	1 (1.6)	7 (11.1)	8 (12.7)	
Parity, n (%)	45 (71.4)	13 (20.6)	8 (12.7)	4 (6.4)	7 (11.1)	13 (20.6)	0.73
Zero	18 (28.6)	3 (4.8)	4 (6.4)	1 (1.6)	5 (8.0)	5 (8.0)	
1 or more							

A potential limitation of the current study is the possible confounding effects of the exogenous medications given, especially progesterone. A previous study showed kisspeptin mRNA to be significantly increased when ovariectomized mice were treated with progesterone (18). However, there was no correlation between kisspeptin to progesterone value regardless of outcome or type of progesterone supplementation. This suggests that kisspeptin is regulated by multiple factors and is not significantly changed by progesterone levels or exogenous administration. In this study population, we did not find that kisspeptin provides a significant clinical predictive value for pregnancy outcomes in early first-trimester pregnancy.

The utility of kisspeptin as a pregnancy biomarker in the first trimester remains unclear. Additional studies are needed to further investigate the relationship between viable intrauterine pregnancy and serum kisspeptin concentration during the early first trimester and may prove useful in combination with bhCG.

Table 2. Correlation between kisspeptin and progesterone by the method of progesterone administration.

Type of Progesterone	ρ, (p value)
No Exogenous Progesterone (n=13)	-0.13 (0.66)
Exogenous Progesterone (n=49)	-0.14 (0.32)
Progesterone Injection (n=36)	-0.19 (0.25)
Vaginal Progesterone (n=12)	-0.18 (0.60)

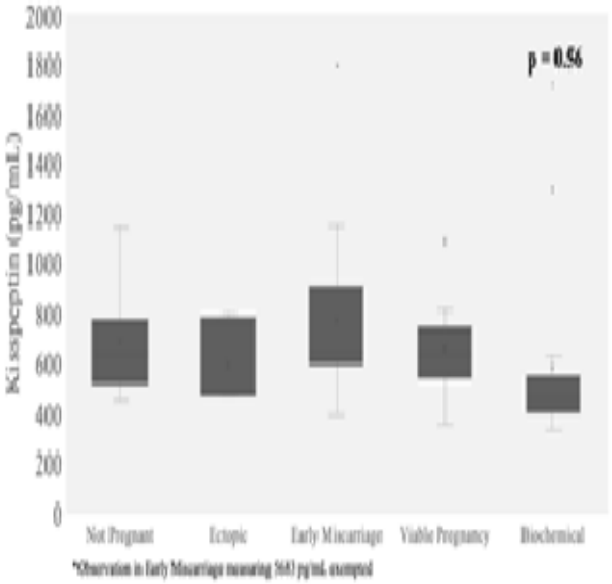

Figure 1. Kisspeptin by pregnancy outcome.

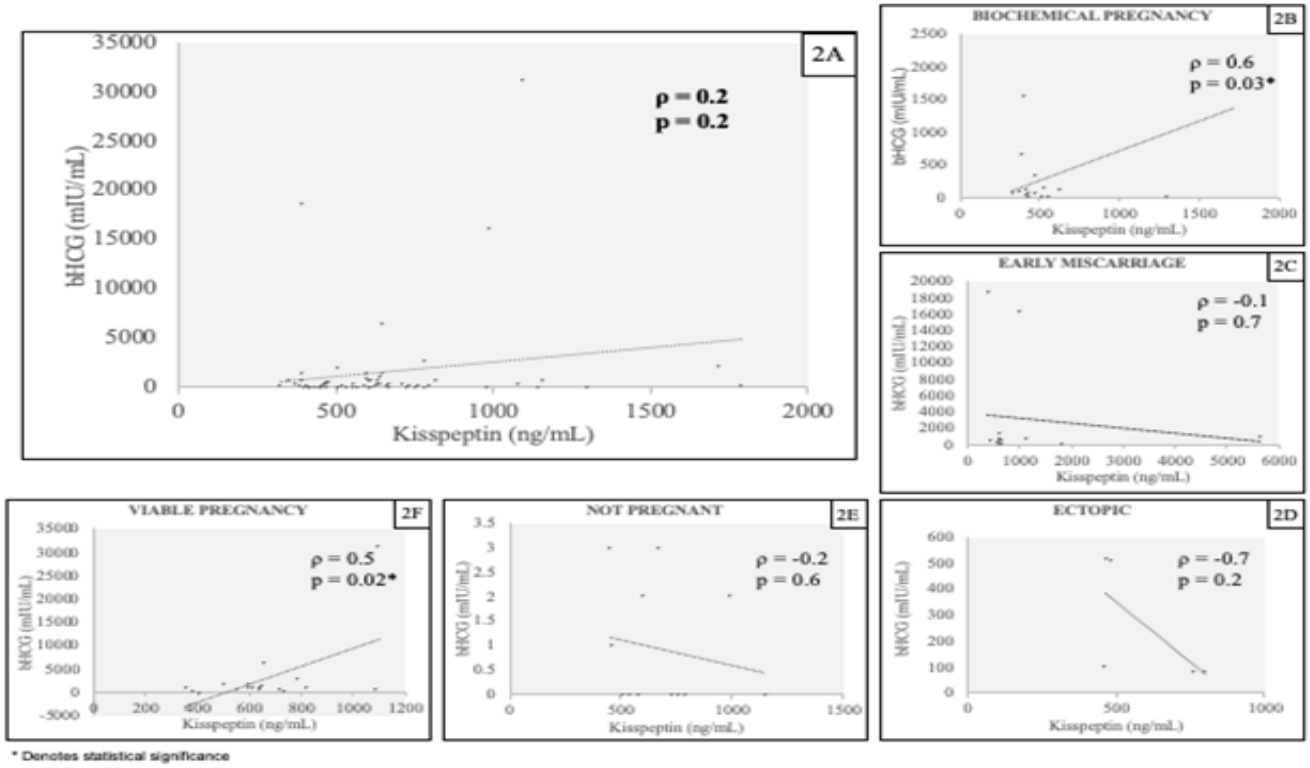

Figure 2. A: Kisspeptin correlation to BhCG for sample size n=63. B-F: Kisspeptin to BhCG correlation by the outcome

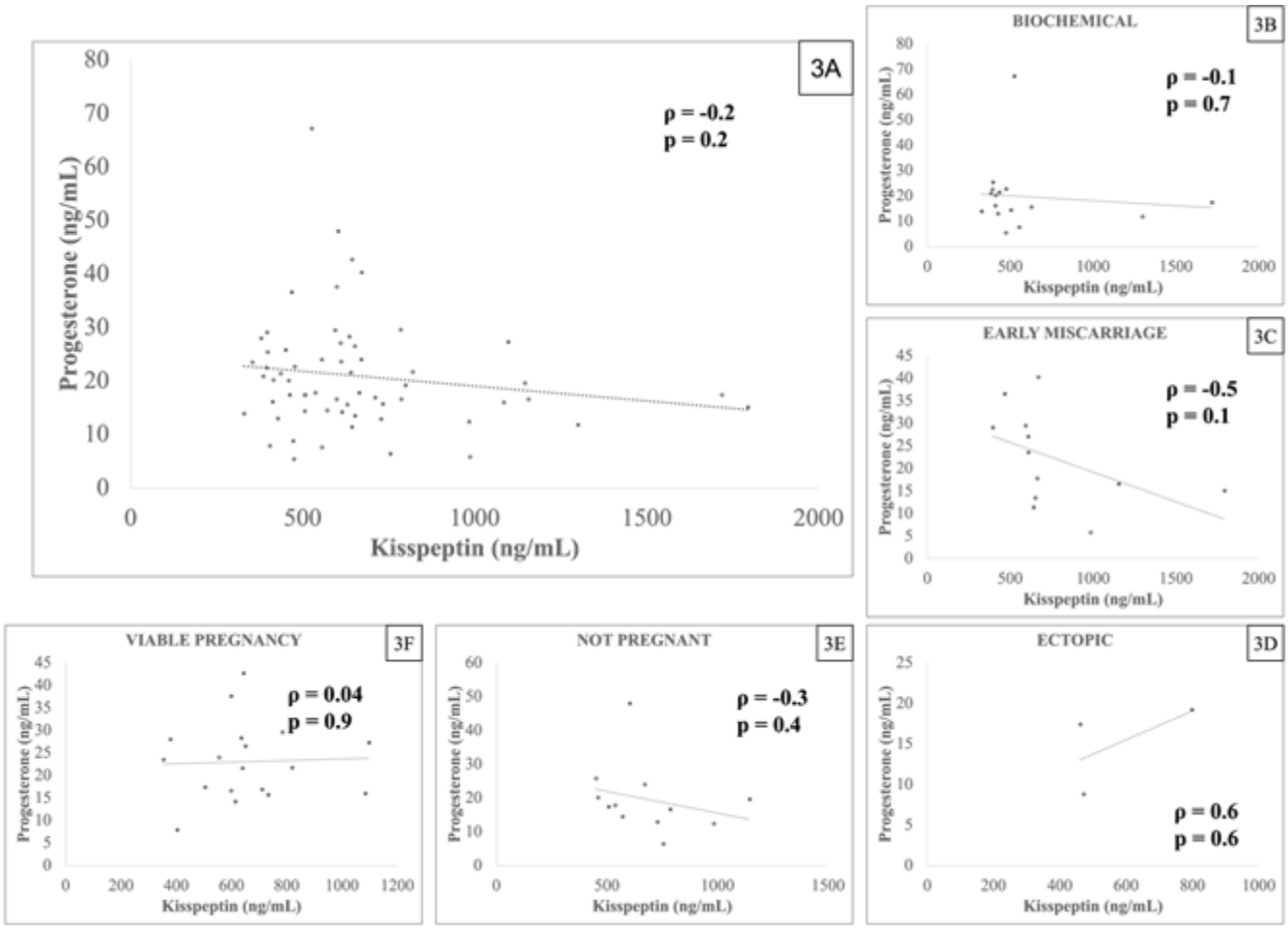

Figure 3. A: Kisspeptin correlation to progesterone for sample n=63. B-F: Kisspeptin to progesterone correlation by outcome

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Ethical issue

Authors are aware of and comply with, best practices in publication ethics specifically concerning authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests, and compliance with policies on research ethics. Authors adhere to publication requirements that the submitted work is original and has not been published elsewhere in any language.

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Competing interests

The authors declare that no conflict of interest would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution to data collection, data analysis, and manuscript writing.

References

1. Pervention CfDCa. 2019 Assisted Repoductive Technology Fertility Clinic and National Summary Report 2019.
2. Horne AW, McBride R, Denison FC. Normally rising hCG does not predict live birth in women presenting with pain and bleeding in early pregnancy. European Journal of Obstetrics and Gynecology and Reproductive Biology. 2011;156(1):120-1.
3. Doubilet PM, Benson CB, Bourne T, et al. Diagnostic criteria for nonviable pregnancy early in the first trimester. New England Journal of Medicine. 2013;369(15):1443-51.

4. Millar RP, Babwah AV. KISS1R: Hallmarks of an Effective Regulator of the Neuroendocrine Axis. Neuroendocrinology. 2015;101(3):193-210.
5. Kotani M, Detheux M, Vandenbogaerde A, et al. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. Journal of Biological Chemistry 2001;276(37):34631-6.
6. Roseweir AK, Katz AA, Millar RP. Kisspeptin-10 inhibits cell migration in vitro via a receptor-GSK3 beta-FAK feedback loop in HTR8SVneo cells. Placenta. 2012;33(5):408-15.
7. Bilban M, Ghaffari-Tabrizi N, Hintermann E, et al. Kisspeptin-10, a KiSS-1/metastin-derived decapeptide, is a physiological invasion inhibitor of primary human trophoblasts. Journal of Cell Science. 2004;117(8):1319-28.
8. Hiden U, Bilban M, Knöfler M, et al. Kisspeptins and the placenta: regulation of trophoblast invasion. Reviews in Endocrine and Metabolic Disorders. 2007;8(1):31-9.
9. Francis VA, Abera AB, Matjila M, et al. Kisspeptin regulation of genes involved in cell invasion and angiogenesis in first trimester human trophoblast cells. PLoS One. 2014;9(6):e99680.
10. Horikoshi Y, Matsumoto H, Takatsu Y, et al. Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placenta-derived hormone in humans. Journal of Clinical Endocrinology and Metabolism. 2003;88(2):914-9.
11. Hu KL, Chen Z, Deng W, et al. Diagnostic Value of Kisspeptin Levels on Early Pregnancy Outcome: a Systematic Review and Meta-analysis. Reproductive Sciences. 2022;29(12):3365-72.
12. Yu H, Liu J, Guo H, et al. Prognostic value of repeated serum kisspeptin measurements in early first trimester pregnancy: a preliminary study. Reproductive Biomed Online. 2019;38(3):465-71.
13. Hu KL, Zhang Y, Yang Z, et al. Predictive value of serum kisspeptin concentration at 14 and 21 days after frozen-thawed embryo transfer. Reproductive Biology and Endocrinology. 2019;39(1):161-7.
14. Romero-Ruiz A, Avendaño MS, Dominguez F, et al. Deregulation of miR-324/KISS1/kisspeptin in early ectopic pregnancy: mechanistic findings with clinical and diagnostic implications. American Journal of Obstetrics and Gynecology. 2019;220(5):480.e1-.e17.
15. Yuksel S, Ketenci Gencer F. Serum kisspeptin, to discriminate between ectopic pregnancy, miscarriage and first trimester pregnancy. J Obstet Gynaecol. 2022;42(6):2095-9.
16. Abbara A, Al-Memar M, Phylactou M, et al. Performance of plasma kisspeptin as a biomarker for miscarriage improves with gestational age during the first trimester. Fertility and Sterility. 2021;116(3):809-19.
17. Calder M, Chan YM, Raj R, et al. Implantation failure in female Kiss1-/- mice is independent of their hypogonadic state and can be partially rescued by leukemia inhibitory factor. Endocrinology. 2014;155(8):3065-78.
18. Zhang P, Tang M, Zhong T, et al. Expression and function of kisspeptin during mouse decidualization. PLoS One. 2014;9(5):e97647.
19. Wu HM, Huang HY, Soong YK, et al. Kisspeptin regulation of human decidual stromal cells motility via FAK-Src intracellular tyrosine kinases. Human Reproduction. 2019;34(7):1291-301.