Azhar Int J Pharm Med Sci 2025; Vol 5 (1):118-127
Research Article

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Association between Kisspeptin and Foxa2 in regulation of trophoblast function and placentation: possible role in pregnancy viability

Aya M. Selmy [1, *], Laila A. Rashed [2], and Doha E.H. Ellakwa [1]

[1] Department of Biochemistry and Molecular Biology, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.
[2] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt.

***** Correspondence: Ayona3368@gmail.com ; Tel.: 01023112615.

Article history: Received: 06-02-2024 **Revised: 07-05-2024** **Accepted: 08-09-2024**

Abstract: Several unique processes, including embryo implantation, decidualization, placentation, and parturition, are necessary for a pregnancy to proceed successfully in humans and animals. Since the endometrium expresses particular genes that control endometrial receptivity, it is essential throughout the implantation window. One such gene is Forkhead Box A2 (FOXA2), which codes for a transcription factor clearly expressed in the human uterus' glandular epithelium (GE). Kisspeptin, a neuropeptide renowned for its direct control over the gonadotrophic axis, including placentation and pregnancy, is another significant role. The KISS1 gene regulates kisspeptin. This research aims to investigate how KISS1 and FOXA2 regulate trophoblast cell function during placentation and contribute to the viability of the pregnancy. In order to look into this, we examined maternal plasma samples from ninety first-trimester pregnant women who were split equally into three groups: Group A was made up of thirty women who had recently had spontaneous abortions; Group B was made up of thirty women who had problems related to placental bleeding; and Group C was the control group, consisting of thirty women who had normal pregnancies. The presented research demonstrated a statistically significant relationship between trophoblast cell function and kisspeptin and foxa2 relative expression, affecting pregnancy viability. These findings demonstrate the significant functions that foxa2 and kisspeptin play during the first trimester of pregnancy, particularly in regulating trophoblast cell function and promoting placental formation.

Keywords: kisspeptin, FOXa2, trophoblast, placenta.

1. INTRODUCTION

The placenta plays a vital role in supporting the growth of the embryo and is crucial for a healthy pregnancy outcome. This is because it serves as the location where the mother and the fetus exchange substances, as well as performing important immunological and endocrine tasks [1]. Abnormal placentation is recognized as a fundamental factor contributing to a range of pregnancy problems, including miscarriage [2]. During pregnancy in humans, the development of the placenta is highly dependent on the rapid proliferation, invasion, and migration of trophoblast cells [3]. Invading the uterus, Extravillous trophoblast cells change the spiral arteries [4]. Compounding this challenge is the fact that many pregnancy disorders, rooted in inadequate placentation, begin to manifest during the first trimester when ethical limitations constrain in vitro studies on placental tissue [5].

As a consequence, our understanding of placental development largely relies on studies conducted in mice. While these studies have identified key regulatory genes and biological processes in placental development, discrepancies between murine and human placentation exist, emphasizing the need for improved human model systems [6]. Hence, it is crucial to prioritize the examination of structural modifications throughout pregnancy, the functions of different types of trophoblasts, and the essential regulatory pathways that govern the formation of the placenta, as well as the development and specialization of trophoblast cells [7].

Cite this article: Selmy, A.M., Rashed, L.A., and Ellakwa, D. H., Association between Kisspeptin and Foxa2 in regulation of trophoblast function and placentation: possible role in pregnancy viability. Azhar International Journal of Pharmaceutical and Medical Sciences, 2025; 5(1):118-127. doi: 10.21608/aijpms.2024.268303.1255

DOI : 10.21608/aijpms.2024.268303.1255

https://aijpms.journals.ekb.eg/

Kisspeptins are peptide molecules that are derived from the KISS-1 gene. They were initially discovered in 1996 in Hershey, Pennsylvania (USA) as a gene that can inhibit the spread of cancer cells in humans. Kisspeptins interact with the G-protein coupled receptor 54 (GPR54), also known as the KISS-1 receptor (KISS-1R), to activate their biological activity [8]. Kisspeptin is well known for being crucial to the central control of human reproduction and pubertal onset. Additional research supported the theory that kisspeptin could control the HPG axis and that idiopathic hypogonadotropic hypogonadism develops due to a mutation in KISS-1R [9].

In the past decade, studies have demonstrated that kisspeptin plays a role in controlling both the surge and pulsatile releases of GnRH. Additionally, it plays vital functions in the regulation of gonadotropin secretion, brain sexual differentiation, triggering of ovulation, and the metabolic control of fertility [10]. KISS1 and KISS1R have significant expression levels in the placenta, similar to HCG. Furthermore, kisspeptins can be isolated from human placental extracts. The peptide product of the kisspeptin gene (KISS-1), formerly referred to as "metastin," was first identified in 1996 in malignant melanoma cells lines as a gene that suppresses the spread of tumors. Kisspeptin is expressed in many organs, such as the liver, gonads, pancreas, limbic system, and hypothalamus. However, it is most prevalent in the placenta and is considered crucial for pregnancy [11].

FOXA2, also known as Forkhead box A2 protein, emerges as a pivotal regulator in uterine biology, particularly in glandular epithelia. According to studies, its expression is essential for the uterine gland's development and successful implantation. Foxa2 plays a vital role in early uterine morphogenesis, as evidenced by the significant defects in gland development when it is deleted in newborns [12].

This work aims to examine how KISS1 and FOXA2 regulate the function of trophoblast cells during placentation and whether or not this has any bearing on the viability of pregnancy. To clarify the connection between kisspeptin, FOXA2, and trophoblast cell function, maternal plasma samples from first-trimester pregnant women—including those who experienced spontaneous abortions, placental bleeding, and normal pregnancies—were examined. In addition, Understanding the molecular processes behind recurrent spontaneous abortion and elucidating the functions of kisspeptin and FOXA2 in controlling trophoblast function and placenta development.

2. METHODS

2.1. Study population

The original article included ninety pregnant cases in this experimental case-control pilot study. The Faculty of Pharmacy Ethics Committee (Girls) approved the study at Al-Azhar University, Cairo, Egypt. The research was carried out between January 2019 and April 2022. The research subjects provided their informed consent. The Department of Obstetrics and Gynecology, Faculty of Medicine, Al-Azhar University, Cairo, provided all the samples. Three subject groups comprising ninety women in their first trimester of pregnancy were enrolled: group A (n=30, women with recently confirmed miscarriage), group B (n=30, women suffered from placental bleeding (threatened abortion) and group C/ control group (n=30, uncomplicated pregnancy women). The research subjects provided their informed consent, indicating their willingness to participate in the study. The gestational age of the study women ranged from 7 to 12 weeks of pregnancy, and it was measured clinically depending on menstrual cycle 1st day.

Enrolled subjects had an age range from 23 to 44 years, with singleton, intrauterine pregnancy. Exclusion criteria were as follows: (a) history of medical illness (diabetes mellitus, hypertension, renal failure, or hepatic, cardiac, or autoimmune diseases), (b) ectopic pregnancy, (c) gestational age >12 weeks at the time of blood sampling, (d) abortion due to infection or trauma. Every patient received a complete evaluation, including a detailed personal, current, obstetric, and menstrual history. Vital signs, including blood pressure, temperature, pulse, and body mass index (BMI) in kilograms per square meter (Kg/m2), were assessed as part of a general examination. The assessment of the chest, heart, abdomen, and upper and lower limbs was also included in the general examination. During a local pelvic examination, the perineum was examined for indications of trauma, lesions, anomalies, infections, or ulcers. A speculum examination was also carried out to identify the bleeding source (uterus, cervix, or vagina), evaluate the amount of bleeding, and check the cervix for abnormalities like polyps or ulcers. Typical prenatal testing included a complete blood count, cytomegalovirus (CMV) antibody test, Rh and ABO blood typing, rubella antibody test, hepatitis B surface antigen test, fasting and postprandial blood sugar level tests, and a comprehensive urine analysis.

2.2. Plasma collection

Blood samples from the study participants were collected and processed according to a standard protocol. Each participant had 5 milliliters of venous blood collected in a sterile setting. Commercially accessible EDTA tubes, frequently used for

anticoagulant purposes, were used to collect the blood. Following collection, the blood samples were centrifuged for five minutes at 1800xg. The density-based separation of the blood components depends on this centrifugation stage. Consequently, three separate layers developed inside the tubes due to centrifugation: the top layer held the plasma, the middle layer held the buffy coat, a mixture of white blood cells and platelets, and the bottom layer held the red blood cells.

The top layer, the supernatant, was meticulously separated from the lower layers to acquire plasma samples for analysis. The plasma samples were further filtered through a membrane with 0.2 mm pores to guarantee their cleanliness. An unclouded and cell-free plasma sample remains after this filtration stage, which helps eliminate any leftover cells or debris. Ultimately, a temperature of -80°C was used to keep the plasma samples. Preserving biological samples for long-term storage at this freezing temperature is standard practice without sacrificing their integrity. The plasma samples were frozen by being stored at -80°C, which stopped any deterioration or alterations to the biomolecules contained inside. These meticulous procedures for collecting blood, centrifuging, filtering, and storing it were used to guarantee the integrity and quality of the plasma samples for the study's later analyses.

2.3. Gene expression analysis

2.3.1. RNA isolation and cDNA synthesis:

Total RNA was extracted from the acquired blood plasma using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. A Thermo Scientific NanoDrop ND2000 spectrophotometer, located in the United States, was used to assess the sample's concentration and purity. We transformed the extracted RNA into complementary DNA (cDNA) in accordance with the manufacturer's instructions using the SensiFASTTM cDNA Synthesis Kit (Bioline, Germany). These were the settings under which the correct procedure was carried out: annealing at 25 °C for 10 min, then transcription at 42 °C for 15 min, inactivation at 85 °C for 5 min, and finally holding at 4 °C using a Perkin Elmer heat cycler at Applied Biosystems.

2.3.2. Quantitative real-time polymerase chain reaction (qRT-PCR):

For the study of gene expression, quantitative real-time polymerase chain reaction (qRT-PCR) was used. The following primers were used, along with Applied Biosystems' Power Up SYBR green PCR Master Mix: Kisspeptin: Forward primer: 5'-GTAGATCCAACTCACTGGTTTCGTGGCAG-3', Reverse primer: 5'-GCTAAGCTTTCACTGCCCCGCACCTG-3'. FOXa2: Forward primer: 5'-CACGGCTCCCAGCATACTTT-3', Reverse primer: 5'-CACGGCTCCCAGCATACTTT-3' CyclophyllineA: Forward primer: 5'-CCCACCGTGTTCTTCGACAT-3', Reverse primer: 5'-CCAGTGCTCAGAGCACGAAA-3'.

The ABI 7500 detection system was used to conduct the qRT-PCR reactions under the following conditions: 45 cycles of denaturation at 95°C for 15 seconds, annealing, and an extension step at 60°C for 1 minute, after an initial activation step of 10 minutes at 95°C. The integrity of the RNA samples was confirmed by detecting cyclophilin A gene (PPIA) expression under all conditions. Utilizing the comparative Ct method for relative quantification, 2^(-ΔΔCT), the gene expression was determined after adjusting the expression levels to PPIA [13].

2.3.3. The ultrasound evaluations:

The procedures were carried out by proficient sonographers using a Voluson E8 or E10 machine (GE Medical Systems, Zipf, Austria). A transvaginal ultrasound probe with a frequency range of 6-12 MHz was used for 3D ultrasound examinations. The complete placenta and the embryo's blood vessels were visualized using power Doppler ultrasonography (PD US). In order to reduce measurement errors and motion-related artifacts, every participant was told to hold their breath for about 30 seconds while the images were being taken. Every ultrasound test was performed in compliance with international guidelines for the safe application of Doppler ultrasonography in the first trimester of pregnancy [14].

2.4. Statistical analysis

We used SPSS Inc.'s Statistical Package for the Social Sciences, version 23.0, as their statistical software for the data analysis. With its array of statistical tools and methodologies, this program is frequently utilized in the social sciences for data analysis. Figures or charts were used to display the qualitative elements, which were portrayed as percentages. On the other hand, mean values and corresponding standard deviations were used to portray quantitative data. T.

To determine the degree of trust in the results, an acceptable error margin of 5% was used. This means that if the probability of any differences or connections in the data occurring by chance alone was less than or equal to 5%, the findings were

deemed statistically significant. A p-value was computed to ascertain statistical significance, A 95% confidence interval was selected. In this inquiry, a p-value of 0.05 or lower was considered statistically significant, indicating that the observed results were unlikely to be due to random chance. We ensured thorough data analysis and interpretation by following these statistical techniques and standards, which made it possible to draw significant results from the study.

3. RESULTS

This study conducted using the data from 90 pregnant women in their first trimester; we divided them into three different groups: Group A represented cases facing sudden abortion, Group B represented cases facing placental bleeding, and finally, group C represented standard pregnant cases as a control group. The average maternal age in group A was 34.6 years, ranging from 24 to 44.The average maternal age in group B was 34.4 years, ranging from 23 to 43. The average maternal age in group C was 31.5 years, with a range of 26 to 40. In group A, the average gestational age was nine weeks, with a range of 7 to 12 weeks. In group B, the average gestational age was 8.8 weeks, with a range of 8 to 10 weeks. The average gestational age in group C, with respect to gestational diabetes, was 8.4 weeks (ranging from 7 to 10 weeks); in both group A and group B, there were 18 positive cases (60%) and 12 negative cases(40%) in each group of them .in group C, there were only six positive cases (20%). In comparison, the other 24 cases were negative. These results are depicted in table 1 and figures 1.

Table 1. Comparison between groups according to demographic data.

Demographic data	Group A *(n=30)*	Group B *(n=30)*	Group C *(n=30)*	F-test	p-value
Maternal age					
Mean ± SD	34.60±6.69	34.40±6.54	31.50±4.74	0.961	0.414
Range	24-44	23-43	26-40		
Gestational age (weeks)					
Mean ± SD	9.20±1.69	8.80±1.03	8.40±1.07	1.108	0.368
Range	7-12	8-10	7-10		
Gestational diabetes					
Negative	12 (40.0%)	12 (40.0%)	24 (80.0%)	3.900	0.174
Positive	18 (60.0%)	18 (60.0%)	6 (20.0%)		

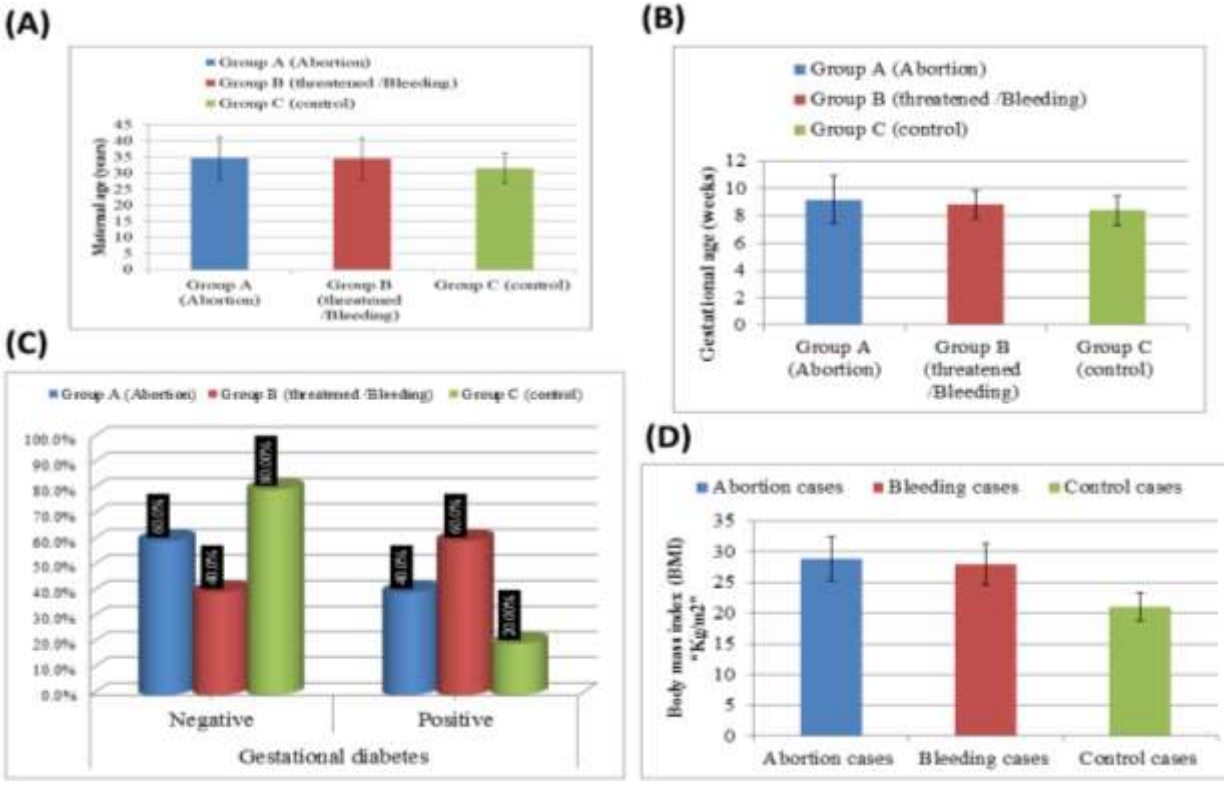

Figure 1. (A) Comparison of the examined groups according to Maternal age; (B) Comparison of the study groups according to the weeks of gestation. (C) A comparative evaluation of the groups under investigation in relation to gestational diabetes; (D) An assessment comparing the groups being studied based on their body mass index (kg/m2).

Table 2. Comparison between all the studied groups: (A) according to Body mass index (BMI); (B) according to systolic blood pressure (mmHg)

(A) according to Body mass index (BMI)								
(BMI) "Kg/m2"	Abortion (n=30)	Bleeding (n=30)	Control (n=30)	Test value	P-value	Multiple Comparison		
						P1	P2	P3
Mean ± SD	28.90±3.54	28.00±3.40	21.10±2.25	56.22	<0.001**	0.267	<0.001**	<0.001**
Range	22-35	23-34	18-25					
(B) according to systolic blood pressure (mmHg) .								
(SBP) mmHg	**Group A (n=30)**	**Group B (n=30)**	**Group C (n=30)**	**Test value**	**P-value**	**Multiple Comparison**		
						P1	**P2**	**P3**
Mean ± SD	143.00±15.79	126.00±11.77	90.00±14.38	110.8	<0.001**	<0.001**	<0.001**	<0.001**
Range	120-170	105-145	70-110					

In the abortion group (A), the mean body mass index (BMI) was 30 with a standard deviation of 3.45 (minimum 22 and highest 35). The mean BMI in the bleeding group (B) was 28 with SD 3.4 (minimum 23 maximum 34), whereas the control group's mean BMI was 21 with SD 2.25 (minimum 18 maximum 25). Table 2 and Figure 1 present these findings. Group A demonstrated a mean systolic blood pressure (SBP) of 143 mmHg, ranging from 120 to 170 mmHg. In contrast, Group B exhibited a mean SBP of 126 mmHg, ranging from 105 to 145 mmHg. The average SBP in group C ranged from 70 to 110 mmHg. These results are shown in Table 2, which offers a thorough summary of the SBP values for each group.

Concerning the relative expression of kisspeptin in group A, the relative expression (Rq) mean value ranged from 0.11 to 0.33. The relative expression (Rq) mean value in group B was 0.53, ranging from 0.27 to 0.79. The relative expression (Rq) mean value for group C was 1.02, ranging from 1 to 1.04.

Group A exhibited a mean relative expression (Rq) of 0.20 (range from 0.12 to 0.34) for FOXa2 relative expression. The mean Rq value for Group B was 0.55, ranging from 0.28 to 0.86. The average Rq value in group C was 1.04, ranging from 1 to 1.08. Table 3 presents a comprehensive analysis of each group's relative expression levels of FOXa2 and kisspeptin, illustrating the outcomes. The extensive data presented in Tables 2 and 3 enables a thorough examination of the variations in systolic blood pressure and the relative expression levels of FOXa2 and kisspeptin among the various groups.

Table 3. The relative expression (Rq) of Kisspeptin and FOXa2 in the three groups.

PCR	**Group A**	**Group B**	**Group C**
Kisspeptin (Rq)	Mean : 0.20 SD : 0.07 Range : 0.11-0.33	Mean :0.53 SD : 0.18 Range : 0.27-0.79	Mean :1.02 SD :0.01 Range :1-1.04
FOXa2 (Rq)	Mean : 0.20 SD : 0.07 Range : 0.12-0.34	Mean :0.55 SD : 0.20 Range :0.28-0.86	Mean :1.04 SD : 0.03 Range :1-1.08

Table 4. Association between placental/ trophoblastic abnormality by ultrasound and Kisspeptin among the studied groups.

Group A and B (60 cases)	**Placental/ trophoblastic abnormality by US**		**t-test**	**p-value**
	Negative *(n=18)*	**Positive *(n=42)***		
Kisspeptin (mean±SD)	0.46±0.21	0.21±0.12	5.281	<0.001**
FOXA2 (mean±SD)	0.43±0.17	0.23±0.10	-3.361	<0.001**

Forty two instances in total with placental problems specifically linked to aberrant trophoblast cell function were found in groups A and B. These problems, which included molar pregnancy, subchorionic hematoma, and irregular spiral artery blood flow, were identified by transvaginal ultrasonography.Subsequent investigation revealed that in these instances, the mean relative quantity (Rq) of kisspeptin was 0.21 and the Rq of FOXa2 was 0.23. A thorough summary of the outcomes is given

in Table 5, which presents these findings. The investigation also found 18 cases with problems resulting from causes other than aberrant trophoblast cell function. The mean Rq of FOXa2 was 0.43 and the mean Rq of kisspeptin was 0.46 for these instances. Together with Figures 1 and 2, which visually illustrate the data for easier comprehension and comparison, Table 5 also shows these results. A thorough examination of the variations in kisspeptin and FOXa2 expression between patients with placental difficulties linked to aberrant trophoblast cell activity and instances with complications resulting from other variables is made possible by the extensive data presented in Table 4 and Figure 2.

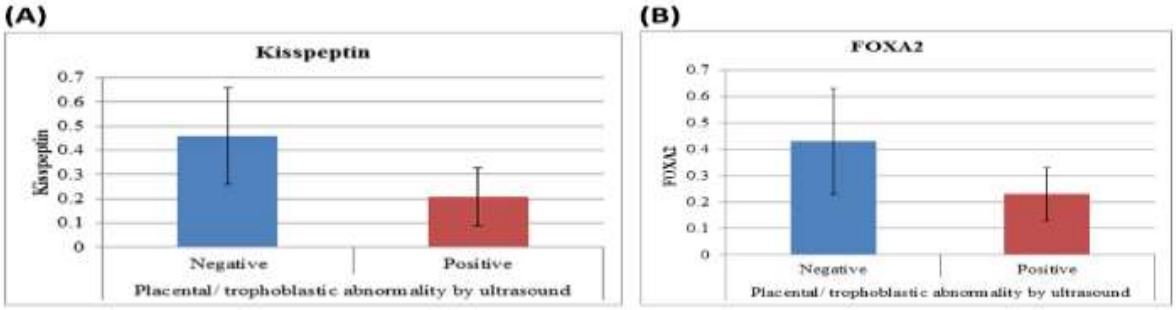

Figure 2. (A) Association between placental/ trophoblastic abnormality by ultrasound and kisspeptin among the studied group. (B) Association between placental/ trophoblastic abnormality by ultrasound and FOXa2 among the studied group.

4. DISCUSSION

As regard the body mass index (BMI) , the current study align with those of Metwally et al., who found a link between an elevated body mass index (BMI) and an increased risk of Miscarriage in the first trimester of pregnancy [15]. More specifically, they discovered that miscarriage risk was considerably higher in women with a BMI over 25 kg/m2.

On the other hand, these results are at odds with those of Hornberger et al., who stated that no correlation between maternal obesity and the incidence of unexpected early pregnancy loss [16]. This disparity implies that different studies have reached different results about the connection between early pregnancy loss and maternal obesity. Notably, the variations reported in these results could be attributed to different techniques, sample sizes, and populations investigated. To fully comprehend the intricate connection between maternal obesity and the risk of Miscarriage during the first trimester of pregnancy, more investigation and research are required.

Our results proved that there is no notable difference between cases as regard maternal age and this was Compatible with Dadkhah et al. findings in studying the females facing threatened Miscarriage in advanced maternal age [17]. However, these findings contradicted Mbugua et al., who asserted that advanced mother age impacts the pregnancy process and increases the likelihood of miscarriage [18]. As regard gestational diabetes mellitus (GDM), the current study is compatible with Pace NP et al who found no remarkable correlation between Miscarriage and GDM [19]. This outcome, however, did not support the findings of Hedayati H et al., who argued that GDM induces severe difficulties for both the mother and the fetus [20]. Concerning mean arterial blood pressure (MAP), we did not find any statistically consequential differences between the groups in this study ($p>0.05$). The low number of women who participated or the timing of the pregnancy stage may cause the differences between the current results and those from forenamed studies. According to this analysis, the positive ultrasound group's mean relative kisspeptin expression was statistically significantly lower at 0.21±0.12 than in the negative ultrasound group at 0.46±0.21, with a p-value of less than 0.001.

This finding is consistent with SMETS E.M. et al., who noted that proper expression of kisspeptin and its receptors is essential for standard placental function [21]. Additionally, PARK D.W. et al., who discovered kisspeptin and GPR-54 expression in human trophoblasts, support this study [22]. When comparing the abortion group to the standard or placental bleeding group, the kisspeptin level reduction was found to be the least significant (P value = 0.0001), indicating a possible significant link between the kisspeptin level and the continuance of a regular pregnancy. Its decline could be a risk factor for poor placentation. Furthermore, these findings align with the research conducted by Hu et al., 2021, which suggests that kisspeptin holds potential as a reliable biomarker for early pregnancy miscarriage [23]. Additionally, Hiden U et al. have reported that during the first trimester, syncytiotrophoblasts have significant quantities of Kisspeptin [24]. However, this study was not in agreement with Gorkem U et al., Who found that serum kisspeptin levels were higher in spontaneous abortion group than in normal pregnancy group and had no significant predictive value for miscarriage [25] .

Hiden et al., reported that the placenta is believed to be the source of increased kisspeptin expression in typical pregnancy cases because it returns to non-pregnant levels immediately after birth [26]. In this study, the expression of FOXA2 was observed to have the most substantial drop in the abortion group compared to both the standard and placental bleeding groups. The findings were consistent with those of León S et al., who discovered that FOXA2 expression was surprisingly increased in the glandular epithelium (GE) throughout the first trimester [27]. The present investigation corroborated the findings of Hancock et al., who determined that there is an increase in FOXA2 expression in the epithelial cells during the initial stages of pregnancy [28]. Whirledge et al. mentioned that the precise molecular processes that control the stage-specific transcriptional profile in the uterus during pregnancy are not fully comprehended because of the overlapping expression patterns [29]. According to Whirledge et al., the exact molecular mechanisms that regulate the particular transcriptional profile in the uterus during pregnancy are not entirely understood due to the overlapping patterns of gene expression .this was in agreement with Ellery PM et al ., who stated that The syncytiotrophoblast enlarge and surround the uterine glands throughout the implantation process, eroding into their lumens to facilitate connections between the developing placenta's intervillous space and the uterine glands [30].

According to research by Moser et al., the invasion of spiral arteries is comparable to the uterine glands occupied by "endo glandular trophoblasts," a type of trophoblast cell that are thought to nourish the growing fetus with nutrients from the glands before the uteroplacental circulation becomes functional at the end of the first trimester [31]. In this study, subchorionic hematomas accounted for half of the abnormality's ultrasonography saw. This result is in line with the study by Ben-Haroush et al., which found that in patients between 10 to 20 weeks of gestational age, subchorionic hematoma and hemorrhage are the most frequent causes of vaginal bleeding [32]. Bhoil et al., noted that the blood flow in the spiral arteries was lower in threatened abortion compared to the normal pregnancy cases [33].

The study's weaknesses stem from its relatively small sample size, which restricted the generalizability of the findings. Furthermore, the study may not represent the general population because it concentrated on a particular group of pregnant women. Moreover, the timing of the pregnancy stage and the small number of participants in some analyses might have affected the results. Furthermore, the lack of discernible variations in maternal age and gestational diabetes mellitus (GDM) among the various cases calls into question earlier conclusions and emphasizes the necessity of additional research. Moreover, variables like sample size and the timing of pregnancy stages may impact the lack of statistically significant differences in mean arterial blood pressure (MAP) between groups. These limitations emphasize the need for more research to clarify the complex relationships among maternal factors, placental function, and pregnancy outcomes.

5. CONCLUSIONS

Kisspeptin and FOXA2 are vital in regulating trophoblast cell function and placenta formation during pregnancy trimester. This regulation plays a vital part in proper placentation, which depends on the link between endometrial glands and trophoblast cell types. FOXA2 serves as a transcription factor for the endometrial glands' role regulation; on the other hand, Kisspeptin is concerned with controlling trophoblast cells. The proper relation between Kisspeptin and FOXA2 is a crucial factor in placenta formation and pregnancy viability. Their expression plays a critical indicator for poor placentation and recurrent spontaneous abortion. These findings emphasize the significance of studying their interaction to determine the causes and develop new interventions for different complications**.**

Funding: This original article did not receive funding from any public or private agency.

Acknowledgments: NA

Conflicts of Interest: All authors state that they do not have any competing interests

Ethical Statement: The original article was performed according to the ethical rules and policies set out by the Ethics Committee No. 21 of the Faculty of Pharmacy (Girls), Al-Azhar University (No 197), on 25 March 2019.

Author Contribution: Aya M. Selmya was responsible for writing the manuscript, collecting data, performing the study, and data analysis. **Laila A. Rashedb and Doha E.H. Ellakwa's** role was supervising the study flow, from creating the idea to finalizing the article data and writing by making significant edits to the manuscript.

List of Abbreviations: FOXA2: Forkhead Box A2, GE: Glandular epithelium, CMV: cytomegalovirus, KISS1: kisspeptin, BMI: body mass index, MAP: mean arterial blood pressure, SBP: systolic blood pressure, GDM: gestational diabetes mellitus, Rq: relative quantity, CDNA: complementary

Deoxyribonucleic acid, qRT-PCR: quantitative real-time polymerase chain reaction.

REFERENCES

1. Papuchova H, Latos PA. Transcription factor networks in trophoblast development. Cell Mol Life Sci. 2022 Jun 3;79(6):337.

2. Knöfler M, Haider S, Saleh L, Pollheimer J, Gamage TKJB, James J. Human placenta and trophoblast development: key molecular mechanisms and model systems. Cell Mol Life Sci. 2019 Sep;76(18):3479-3496.

3. Liang L, Chen Y, Wu C, Cao Z, Xia L, Meng J, He L, Yang C, Wang Z. MicroRNAs: key regulators of the trophoblast function in pregnancy disorders. J Assist Reprod Genet. 2023 Jan;40(1):3-17.

4. Abbas Y, Turco MY, Burton GJ, Moffett A. Investigation of human trophoblast invasion in vitro. Hum Reprod Update. 2020 Jun 18;26(4):501-513.

5. Lawless L, Qin Y, Xie L, Zhang K. Trophoblast Differentiation: Mechanisms and Implications for Pregnancy Complications. Nutrients. 2023 Aug 12;15(16):3564.

6. Tobita T, Kiyozumi D, Muto M, Noda T, Ikawa M. Lvrn expression is not critical for mouse placentation. J Reprod Dev. 2019 Jun 14;65(3):239-244.

7. Frost JM, Amante SM, Okae H, Jones EM, Ashley B, Lewis RM, Cleal JK, Caley MP, Arima T, Maffucci T, Branco MR. Regulation of human trophoblast gene expression by endogenous retroviruses. Nat Struct Mol Biol. 2023 Apr;30(4):527-538.

8. Tsoutsouki J, Patel B, Comninos AN, Dhillo WS, Abbara A. Kisspeptin in the Prediction of Pregnancy Complications. Front Endocrinol (Lausanne). 2022 Jul 19;13:942664.

9. Szydełko-Gorzkowicz M, Poniedziałek-Czajkowska E, Mierzyński R, Sotowski M, Leszczyńska-Gorzelak B. The Role of Kisspeptin in the Pathogenesis of Pregnancy Complications: A Narrative Review. Int J Mol Sci. 2022 Jun 14;23(12):6611.

10. Stopa LRS, de Souza CF, Martins AB, Lopes GM, Costa NO, Gerardin DCC, de Carvalho GG, Zaia DAM, Zaia CTBV, Uchoa ET, Leite CM. Neonatal overfeeding reduces estradiol plasma levels and disrupts noradrenergic-kisspeptin-GnRH pathway and fertility in adult female rats. Mol Cell Endocrinol. 2021 Mar 15;524:111147.

11. Abbara A, Al-Memar M, Phylactou M, Daniels E, Patel B, Eng PC, Nadir R, Izzi-Engbeaya C, Clarke SA, Mills EG, Hunjan T, Pacuszka E, Yang L, Bech P, Tan T, Comninos AN, Kelsey TW, Kyriacou C, Fourie H, Bourne T, Dhillo WS. Changes in Circulating Kisspeptin Levels During Each Trimester in Women With Antenatal Complications. J Clin Endocrinol Metab. 2022 Jan 1;107(1):e71-e83.

12. Kelleher AM, Behura SK, Burns GW, Young SL, DeMayo FJ, Spencer TE. Integrative analysis of the forkhead box A2 (FOXA2) cistrome for the human endometrium. FASEB J. 2019 Jul;33(7):8543-8554.

13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec;25(4):402-8.

14. Hitzerd E, Reijnders IF, Mulders AGMGJ, Koning AHJ, Reiss IKM, Danser AHJ, Steegers-Theunissen RPM, Simons SHP, Koster MPH. Larger First-Trimester Placental Volumetric Parameters Are Associated With Lower Pressure and More Flow-Mediated Vasodilation of the Fetoplacental Vasculature After Delivery. Front Physiol. 2020 Jan 24;11:6.

15. Metwally M, Ong KJ, Ledger WL, Li TC. Does high body mass index increase the risk of miscarriage after spontaneous and assisted conception? A meta-analysis of the evidence. Fertil Steril. 2008 Sep;90(3):714-26.

16. Hornberger, K.L. et al. "Maternal Obesity Is Not Associated with Increased Miscarriage Rates Following Euploid Blastocyst Transfer." Fertility and Sterility 108.3 (2017): e251–e252.

17. Dadkhah F, Kashanian M, Eliasi G. A comparison between the pregnancy outcome in women both with or without threatened abortion. Early Hum Dev. 2010 Mar;86(3):193-6.

18. Mbugua Gitau G, Liversedge H, Goffey D, Hawton A, Liversedge N, Taylor M. The influence of maternal age on the outcomes of pregnancies complicated by bleeding at less than 12 weeks. Acta Obstet Gynecol Scand. 2009;88(1):116-8.

19. Pace NP, Vassallo J, Calleja-Agius J. Gestational diabetes, environmental temperature and climate factors - From epidemiological evidence to physiological mechanisms. Early Hum Dev. 2021 Apr;155:105219.

20. Hedayati H., Khazaee T., Mogharrab M., Sharifzadeh GH.R..Prevalance of gestational diabetes mellitus and overt diabetes in pregnant wome in Birjand. modern care journal. 2012;8(4 (32)):238-244.

21. Smets EM, Deurloo KL, Go AT, van Vugt JM, Blankenstein MA, Oudejans CB. Decreased plasma levels of metastin in early pregnancy are associated with small for gestational age neonates. Prenat Diagn. 2008 Apr;28(4):299-303. doi: 10.1002/pd.1969. PMID: 18317999.

22. Park DW, Lee SK, Hong SR, Han AR, Kwak-Kim J, Yang KM. Expression of Kisspeptin and its receptor GPR54 in the first trimester trophoblast of women with recurrent pregnancy loss. Am J Reprod Immunol. 2012 Feb;67(2):132-9.

23. Hu KL, Zhang Y, Yang Z, Zhao H, Xu H, Yu Y, Li R. Predictive value of serum kisspeptin concentration at 14 and 21 days after frozen-thawed embryo transfer. Reprod Biomed Online. 2019 Jul;39(1):161-167.

24. Hiden U, Wadsack C, Prutsch N, Gauster M, Weiss U, Frank HG, Schmitz U, Fast-Hirsch C, Hengstschläger M, Pötgens A, Rüben A, Knöfler M, Haslinger P, Huppertz B, Bilban M, Kaufmann P, Desoye G. The first trimester human trophoblast cell line ACH-3P: a novel tool to study autocrine/paracrine regulatory loops of human trophoblast subpopulations--TNF-alpha stimulates MMP15 expression. BMC Dev Biol. 2007 Dec 19;7:137.

25. Arslan, Emine, Umit Gorkem, and Cihan Togrul. "Is There An Association Between Kisspeptin Levels And Gestational Diabetes Mellitus?" Gynecology Obstetrics & Reproductive Medicine 26.3 (2020): 179–183.

26. Hiden U, Bilban M, Knöfler M, Desoye G. Kisspeptins and the placenta: regulation of trophoblast invasion. Rev Endocr Metab Disord. 2007 Mar;8(1):31-9.

27. León S, Fernandois D, Sull A, Sull J, Calder M, Hayashi K, Bhattacharya M, Power S, Vilos GA, Vilos AG, Tena-Sempere M, Babwah AV. Beyond the brain-Peripheral kisspeptin signaling is essential for promoting endometrial gland development and function. Sci Rep. 2016 Jul 1;6:29073.

28. Hancock JM, Li Y, Martin TE, Andersen CL, Ye X. Upregulation of FOXA2 in uterine luminal epithelium and vaginal basal epithelium of epiERα-/-(Esr1fl/flWnt7aCre/+) mice†. Biol Reprod. 2023 Mar 13;108(3):359-362.

29. Whirledge SD, Kisanga EP, Oakley RH, Cidlowski JA. Neonatal Genistein Exposure and Glucocorticoid Signaling in the Adult Mouse Uterus. Environ Health Perspect. 2018 Apr 5;126(4):047002.

30. Ellery PM, Cindrova-Davies T, Jauniaux E, Ferguson-Smith AC, Burton GJ. Evidence for transcriptional activity in the syncytiotrophoblast of the human placenta. Placenta. 2009 Apr;30(4):329-34.

31. Moser G, Weiss G, Gauster M, Sundl M, Huppertz B. Evidence from the very beginning: endoglandular trophoblasts penetrate and replace uterine glands in situ and in vitro. Hum Reprod. 2015 Dec;30(12):2747-57.

32. Ben-Haroush A, Yogev Y, Mashiach R, Meizner I. Pregnancy outcome of threatened abortion with subchorionic hematoma: possible benefit of bed-rest? Isr Med Assoc J. 2003 Jun;5(6):422-4.

33. Bhoil R, Kaushal S, Sharma R, Kaur J, Sharma T, Thakur R, Sharma R. Color Doppler ultrasound of spiral artery blood flow in mid first trimester (4-8 weeks) in cases of threatened abortion and in normal pregnancies. J Ultrason. 2019 Dec;19(79):255-260. doi: 10.15557/JoU.2019.0038. Epub 2019 Dec 31. PMID: 32021706; PMCID: PMC6988462.