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Purification of Prolidase enzyme and study of some biochemical variables in Abortifacient women

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Abstract :

Repeat induced abortion is a serious public health issue that has been linked to adverse maternal health outcomes. However, knowledge about repeat induced abortion and its associated factors among reproductive age women. The present study evaluated level of Prolidase, Prolactin, Insulin, HOMA-IR, Vit E, Calcium, and IL6 in aborted women then purification of Prolidase in aborted women. The current study included collecting 90 blood samples divided into 60 samples from female patients with Aborted women of reproductive age aged (18 to 40 years) and thirty samples from healthy women collected from private clinics in Anbar Governorate for a period from January 2023 to October 2023. The results of the study indicate that prolidase, prolactin, Insulin, HOMA-IR, IL6 levels are increased in abortion women as compared to healthy women at p-value<0.05. While vitamin E and Calcium levels were significantly lower as compared to the healthy women at p-value<0.05. The present study concluded increase Prolidase, Prolactin, Insulin, HOMA-IR, Vit E, Calcium, and IL6 level in aborted women. By purifying the prolinase enzyme from abortifaceint women's serum, it was found that there was only one enzyme isoform after purification.

Keywords: Aborted women, Prolidase, Prolactin, Insulin, HOMA-IR, Vit E, Calcium, IL6, and Prolidase enzyme purification .

تنقية أنزيم البرولايديز ودراسة بعض المتغيرات الكيموحيوية لدى النساء المجهضات

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مستخلص:

يُعد الإجهاض المتكرر مشكلة صحية عامة خطيرة ترتبط بنتائج سلبية على صحة الأم. ومع ذلك، فإن المعرفة بالإجهاض المتكرر والعوامل المرتبطة به بين النساء في سن الإنجاب لا تزال غير كافية. الهدف من الدراسة: قامت هذه الدراسة بتقييم مستوى البرولايديز والبرولاكتين والأنسولين و HOMA-IR وفيتامين E والكالسيوم و IL6 لدى النساء المجهضات ثم تم تنقية البرولايديز لديهن. تضمنت الدراسة الحالية جمع 90 عينة دم مقسمة إلى 60 عينة من مريضات في سن الإنجاب تتراوح أعمارهن بين 18 و40 عامًا وثلاثين عينة من نساء سليمات تم جمعهن من عيادات خاصة في محافظة الأنبار للفترة من يناير 2023 إلى أكتوبر 2023. تشير نتائج الدراسة إلى أن مستويات البرولايديز والبرولاكتين والأنسولين و HOMA-IR و IL6 مرتفعة لدى النساء المجهضات مقارنةً بالنساء السليمات عند قيمة $p<0.05$. بينما كانت مستويات فيتامين هـ والكالسيوم أقل بشكل ملحوظ مقارنةً بالنساء الأصحاء عند قيمة $p<0.05$. خلصت هذه الدراسة إلى زيادة في مستويات البرولايديز، والبرولاكتين، والأنسولين، و HOMA-IR، وفيتامين هـ، والكالسيوم، و IL6 لدى النساء المجهضات. بتنقية إنزيم البروليناز من مصل النساء المجهضات، وُجد أن هناك شكلًا واحدًا فقط من الإنزيم بعد التنقية.

الكلمات المفتاحية: النساء المُجهضات، البرولايديز، البرولاكتين، الأنسولين، HOMA-IR، فيتامين E، الكالسيوم، IL6، وتنقية إنزيم البرولايديز

Introduction

Prolidase is a manganese-dependent exopeptidase that hydrolyzes imidodipeptides using C-terminal proline or hydroxyproline [1]. The recycling of proline from imidodipeptides by prolidase please share role in collagen remodeling and extracellular matrix remodeling Our aim was to evaluate the activity of prolidase in serum and fleisler floyd in abortifacients Our findings revealed a direct correlation between serum and follicular fluid prolidase levels approximately 70% of affected women remain undiagnosed or have a delayed diagnosis before the condition is recognized [2]. Prolidase is a cellular metabolic enzyme that belongs to the matrix metalloproteinase family and is responsible for the degradation of the extracellular matrix in the ovary Prolidase is secreted from erythrocytes, leukocytes, dermal fibroblasts, and keratinocytes Prolidase regulates collagen metabolism and extracolor matrix remodeling [2, 3]. In women with abortifacients elevated levels of matrix metalloproteinase may be associated with abnormalities in extracellular matrix remodeling multiple cyst formation and chronic anovulation [4]. Infertility and abortion are significant reproductive health issues that impact individuals, families, and societies worldwide. Infertility, defined as the inability to conceive after one year of unprotected intercourse, affects approximately 12% - 17% of couples globally [5].

Infertility rates vary across populations and have an effect by factors such as age, lifestyle, environmental factors, and underlying medical conditions Agerelated infertility, particularly in women over 37 years, is a growing concern globally, as advancing maternal age is associated with decreased ovarian reserve and higher rates of chromosomal abnormalities , Additionally, lifestyle factors such as smoking, obesity, and sedentary behaviors can contribute to infertility risk [6, 7]. Abortion, which could be spontaneous (miscarriage) and induced, is another area of concern in reproductive health. automatic miscarriages occur in approximately 15% - 20% of clinically recognized pregnancies, often ascribed to chromosomal abnormalities and maternal health conditions Induced abortion, which is a sensitive and complex issue, is a common reproductive choice

globally, influenced by legal, social, cultural, and healthcare factors [8, 9].

Materials and methods

The current study included collecting 90 blood samples divided into 60 samples from female patients with Aborted women of reproductive age aged(18 to 40 years) and thirty samples from healthy women collected from private clinics in Anbar Governorate for a period from January 2023 to October 2023. After the disease was diagnosed by a specialist physician and 4-5 ml of venous blood was withdrawn and placed in tubes and the samples were left at room temperature and then separated in a centrifuge to obtain serum and then stored at -20 degrees Celsius until the required measurements were performed.

Assessment of biochemical variables

Enzymatic activity of Prolidase, Prolactin, Insulin, HOMA-IR, Vit E, Calcium, and IL6 were analyzed by using Enzyme-linked immunosorbent assay (ELISA) kits (Sunlong-ChinaUSA). The measurement of Prolidase, Prolactin, Insulin, HOMA-IR, Vit E, Calcium, and IL6 levels was conducted in accordance by using ELISA technique. The plate was pre-coated with an antibody specific to human Prolidase, Prolactin, Insulin, HOMA-IR, Vit E, Calcium, and IL6. The sample is supplemented with Prolidase, Prolactin, Insulin, HOMA-IR, Vit E, Calcium, and IL6 content. The concentration of human Prolidase, Prolactin, Insulin, HOMA-IR, Vit E, Calcium, and IL6 exhibited a positive correlation with the emergence of color in the substrate solution. The cessation of the process is achieved by the introduction of an acidic stop solution, followed by the quantification of absorbance at a wavelength of 450 nm.

Partial purification of prolidase in the serum of patients with abortion

Partial purification of prolidase in the serum of aborted women was carried out from the patients' sera according to the following steps:

Dialysis

The above protein precipitation product was dialysed to remove salts and short peptides. The product was placed in the dialysis sachet and tied tightly at both ends, then the sachet was placed in the buffer solution for 12 hours at 9 degrees Celsius, taking into

account the alternation of the buffer periodically, after which the product was concentrated using sucrose, where the dialysis sachet was dipped periodically on crystals for 50 minutes at 9 degrees Celsius to eliminate salts and short peptides .After that, the enzymatic activity and protein concentration are measured.

Ion exchange chromatography:

The ion exchanger was prepared by soaking 5g of DEAE-Cellulose in 500mL Beaker with buffer solution and left at 4C◦ for 24 hours. 4.5ml of the protein separated by the precipitation and dialysis step was added and left on the column for 5 minutes to saturate, then the separation was carried out using a beeper containing rising concentrations of NaCl in order to obtain a higher purity enzyme by eliminating differently charged proteins. The resulting fractions (5mL) were collected for each tube and then the enzymatic activity and protein concentration were estimated for each tube. The separated fractions were stored at (-20C◦) to complete the purification process.

Gel filtration chromatography:

A suspension was prepared for filtration by soaking 10g of Sephadex G-150 in a 500mL beaker with buffer solution for 1 day at 4oC, alternating the buffer periodically. 5mL of the sample separated in the previous step was added and left on the column for 5min to soak well. The separation process was started by adding the eluent to the column and then collecting the mature fractions 5mL per tube in order to obtain a higher purity enzyme by separating proteins according to their molecular weights. Enzymatic activity and protein quantification were measured for each tube and samples were stored at -20C◦ for kinetic studies

Statistical analysis

The results were statistically analyzed using Minitab Ver.17 by ANOVA and t-test and the means were compared by Duncun's multiple range test at a probability level of $P \leq 0.05$.

Results and discussion

The results of the study indicate that prolidase levels are increased in abortion women compared as to healthy women that were (5.773 ±2.128, 4.629 ±0.893) respectively, at p-value<0.05 .Furthermore, prolactin, Insulin, HOMA-IR, IL6 levels were elevated in abortion women (17.67 ± 5.88, 40.69 ±16.91, 12.54 ± 6.18) as compared to

healthy women (11.46 ± 5.43, 17.44 ± 5.17, 3.99±1.42, 0.280 ± 0.164), respectively, at p-value<0.05. While vitamin E and Calcium levels were significantly lower (10.73 ± 2.64, 7.99 ±0.59) compared to the healthy women (20.82 ± 2.71, 9.25 ± 0.54),), respectively, at p-value<0.05.

Table (1) Statically analysis results of prolidase enzyme in the serum of Abortion patients compared to control group

Group	Patients (60)	Control (30)	P-value
Prolidase	5.773 ±2.128	4.629 ±0.893	<0.05
Prolactin	17.67 ± 5.88	11.46 ± 5.43	<0.05
Insulin	40.69 ±16.91	17.44 ± 5.17	<0.05
HOMA-IR	12.54 ± 6.18	3.99±1.42	<0.05
Vit E	10.73 ± 2.64	20.82 ± 2.71	<0.05
Calcium	7.99 ±0.59	9.25 ± 0.54	<0.05
IL6	0.648 ± 0.108	0.280 ± 0.164	<0.05

The levels of prolidase enzyme play a role in aborted women, In a study done by [10] it was determined that serum prolidase activity was increased and placental prolidase activity was decreased depending on the possible placental use in patients with early pregnancy loss. The study [11] concluded that the decreased placental activity of prolidase might be an etiopathological factor in women with early pregnancy loss . This result disagree with [12], that showed increase level of prolidase enzyme activities in aborted women.

This study shows some women with a history of recurrent spontaneous miscarriage have raised serum concentrations of prolactin in the follicular phase. It can be assumed that these findings are causally related to the development of miscarriage, especially in women with recurrent spontaneous abortion in whom no other cause for their repeated pregnancy loss was apparent. Increased frequencies of hyperprolactinaemia [13] have been reported in women with miscarriage. Hyperprolactinaemia and hypoprolactinaemia have been causally related with luteal phase defects and with miscarriage[14], a finding which is supported by our observation of a significant excess of hyperprolactinaemia in women with recurrent spontaneous abortion.

The results of this study indicated that the risk of spontaneous abortion was positively related with HOMA-IR level. This result agree with [15] that suggests that IR is a common factor behind the increased risk of spontaneous abortion in women who are overweight/obese. Insulin resistance is often associated with a hypercoagulable state (impaired fibrinolysis) and increased inflammatory cytokine levels Insulin resistance has been demonstrated to increased expression of PAI-1. PAI-1 activity is known to elevate levels of serum insulin and it induces a hypofibrinolytic state. This creates a thrombotic milieu at the maternofetal interface with high risk of miscarriage . Insulin resistance is known to play a critical role in the ovarian androgen excess and therefore might promote miscarriage by increasing circulating testosterone concentrations [16, 17] .

Pregnant women have quicker metabolism, increased production of free radicals, and increased lipid peroxidation. Thus, low levels of vitamin E can lead to the production of excessive free radicals, leading to placental aging, endothelial vascular damage, which increases the incidence of high-risk infections in pregnancy . It can also damage the lining of the fetal cell membranes, increasing the risk of premature rupture of the embryo [18]. The results of calcium deficiency in women with pregnancy loss compared to healthy blood indicate that the mineral is linked to maintaining pregnancy throughout its duration due to its association with hypothyroidism and parathyroidism in performing its function, as its deficiency causes both the mother and the fetus to have high blood pressure, lack of development and maintenance of the skeleton, preeclampsia, fetal growth problems, high blood pressure, and decreased blood flow in the uterus, all of which lead to pregnancy loss and its danger [19]. The study emphasized the importance of research and pathways related to the role of IL-6 and the clinical aspects of treatment, as it is elevated in most cases of inflammation and can be used as a potential target for treatment, as it mediates basic functions such as regeneration of the phenomenon and enhancing the body's defense mechanism, the prolonged inflammatory response resulting from Abortion, and its clear association with high insulin, obesity,

and type 2diabetes [20].

Partial purification of prolidase the serum of patients

Enzyme from serum of patients with leukodystrophy was carried out in several steps as shown in Table (2), where the first step included protein precipitation using solid ammonium sulfate salt with a saturation rate of 71%, the enzyme yield was 1.085times, enzyme activity was 7.16IU/mL, the percentage of purification was . 77.03%, while the specific activity of the enzyme was 0.117unit/mg. In the next step, Dialysis was performed to remove short proteins, amino acids and salts using buffer solution for 12 hours at 4oC. Enzyme yield at this stage was 2.18times and enzyme activity were 5.9IU/mL, while the percentage purification and specific activity were 63.51% and 0.256unit/mg, respectively. Third step of the purification process included using DEAE-Cellulose Ionic exchange chromatography to separate the enzyme isoforms using graduated concentrations of NaCl salt, Enzyme yield during this stage was 3.76 times with an enzymatic activity of 5.29IU/mL, the percentage purity was 60% and the specific activity was 0.440unit/mg as shown in Figure(1). As for last step in the partial purification of the enzyme, Sephadex G-150 gel filtration chromatography was used, the degree of purification during this step was 6.153 times, the enzymatic activity reached 4.32IU/mL, while the percentage and specific activity of the enzyme were 30.9% and 0.72unit/mg respectively, as shown in Figure (2).

Table (2) Steps of prolidase purification from serum patients

Purification steps	Volume Ml	Activity Iu/ml	Total activity	Protein Concentr Ati on mg/ml	Specif Activit U/mg	Yield	Fold of purif -icatio	Tot Al Prot Ein
Crude	10	8.36	83.6	71	0.117	100	1	710
precitation	9	7.16	64.44	56	0.127	77.03	1.085	504
Dialysis	9	5.9	53.1	23	0.256	63.51	2.18	207
Ion exchange	5	5.29	26.45	12	0.440	31.63	3.76	60
Gel filtration	5	4.32	25.92	6	0.72	30.9	6.153	30

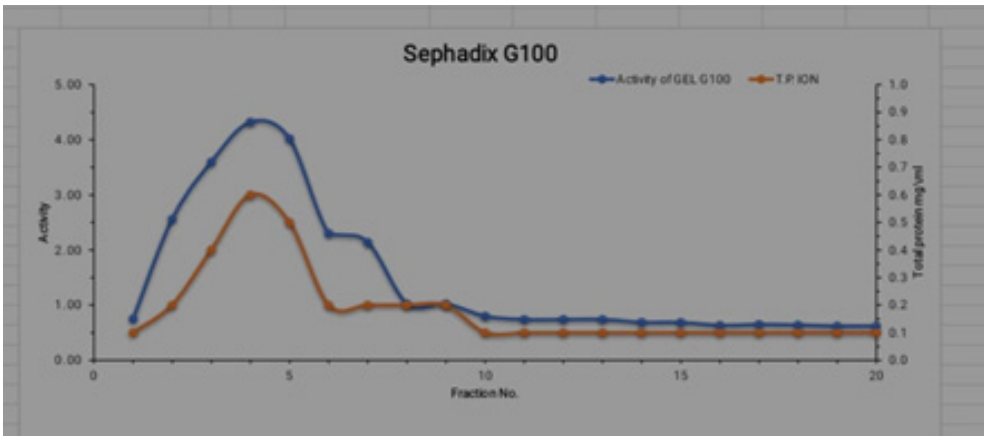

Figure (1) purification of prolidase by gel filtration chromatography

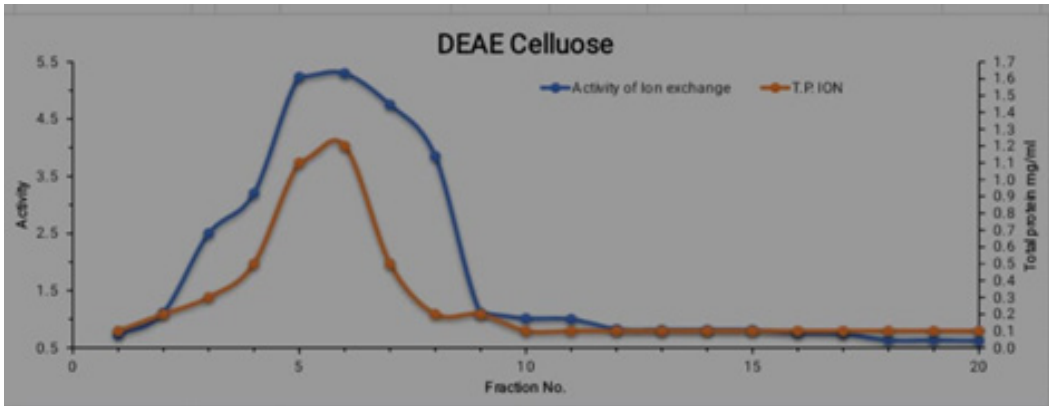

Figure (2) purification of prolidase using ion- exchange chromatography

Correlations between prolidase enzyme and biochemical variables

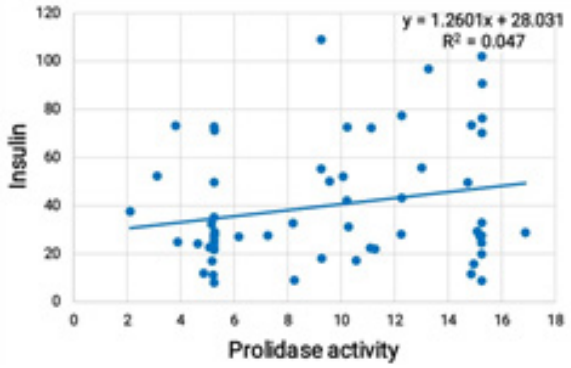

Fig (4)Association between HOMA-IR and and Prolidase activity

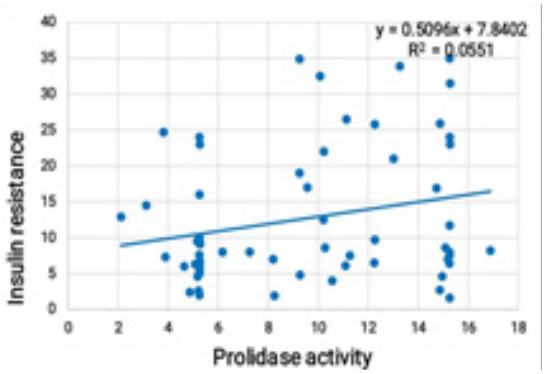

Fig (3) Association between IR prolidase activity

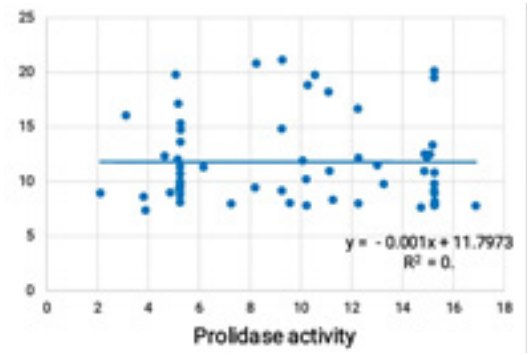

Fig (6) Association between vit E and Prolidase activity

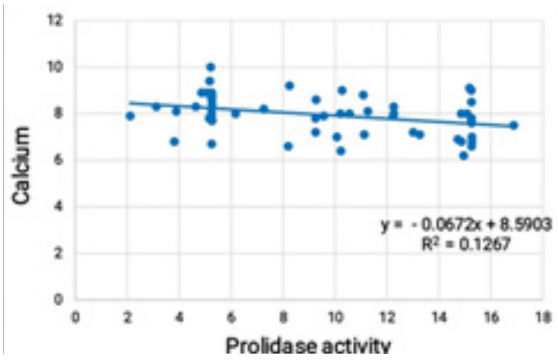

Fig (5) Association between Ca and prolidase activity

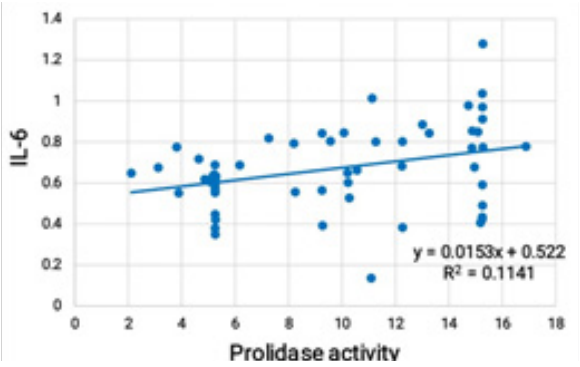

Fig (7) Association between IL6 and Prolidase activity

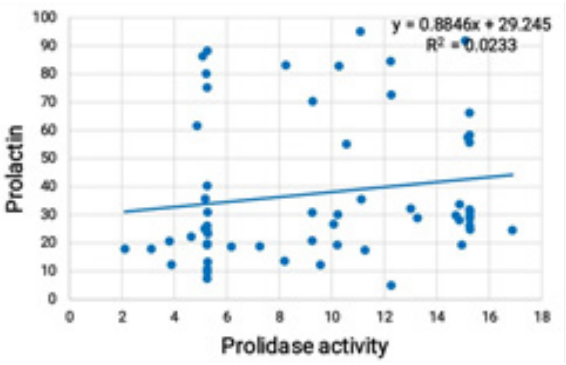

Fig (8) Association between prolactin and prolidase activity

Conclusion

The enzyme activity was evaluated and there was a significant increase in its activity compared to the healthy group at $p \leq 0.05$ The increase indicates a defect in collagen turnover that causes many pathological conditions and the development of miscarriage disease in women , as collagen turnover, matrix remodeling and cell growth play an effect on the activity of prolidase. It is known that the breakdown of tissue barriers is catalyzed by proteolytic enzymes that degrade the extracellular matrix, which forms collagen and glycoproteins. Kinetic results showed that the optimal concentration of the PEPD substrate was 0.1µM, and the enzyme showed maximum activity at 37°C, pH 7.2 with an incubation time of 5 minutes, and the Km 0.0259µM value of the enzyme was and Vmax 3.33µ mol.

$min^{-1}.mg^{-1}$. The enzyme level in the serum of Abortifacients patients was clearly associated with the quality of the blastocyst and the abnormal degradation of collagen significantly in the ovary and follicular collagen, which leads to a novulation. Prolidase plays a pivotal role in the immune response in patients with inflammatory conditions. The present study found that Prolactin levels were higher in women with PCOS compared to healthy controls, and Ca,Vit E levels were significantly lower, while insulin and insulin resistance were significantly higher, and interleukin 6 was significantly higher compared to healthy controls

References

1. Eni-Aganga I, Lanaghan ZM, Balasubramaniam M, Dash C, Pandhare J. PROLIDASE: A Review from Discovery to its Role in Health and Disease. Frontiers in molecular biosciences. 2021;8:723003.

2. Wilk P, Wątor E, Weiss MS. Prolidase–a protein with many faces .Biochimie. 2021;183:3-12.

3. Mermutluoğlu Ç, Tekin R, Tekin R, Tekin S, Erkan RC, Deveci Ö, et al. Evulation of prolidase enzyme, and galectin levels as a marker for fibrosis in patients with chronic hepatitis B. European Review for Medical and Pharmacological Sciences. 2022;26(24):9467-72.

4. Ara I, Maqbool M, Gani I. Reproductive Health of Women: implications and attributes. International Journal of Current Research in Physiology and Pharmacology. 2022:8-18.

5. Jabeen F, Khadija S, Daud S. Prevalence of primary and secondary infertility. Saudi Journal of Medicine. 2022;7(1):22-8.

6. Magdum M, Chowdhury MAT, Begum N, Riya S. Types of infertility and its risk factors among infertile women: A prospective study in dhaka city. Journal of Biosciences and Medicines. 2022;10(4):158-68.

7. Choi TY, Lee HM, Park WK, Jeong SY, Moon HS. Spontaneous abortion and recurrent miscarriage: A comparison of cytogenetic diagnosis in 250 cases. Obstetrics & gynecology science. 2014;57(6):518.

8. Dos Santos APV, Coelho EdAC, Gusmão MEN, da Silva DO, Marques PF, Almeida MS. Factors associated with abortion in women of reproductive age. Revista Brasileira de Gineco-

logia e Obstetrícia/RBGO Gynecology and Obstetrics. 2016;38(06):273-9.

9. Al-Turki HA. Prevalence of primary and secondary infertility from tertiary center in eastern Saudi Arabia. Middle East Fertility Society Journal. 2015;20(4):237-40.

10.Dural MD, Bıldırcın FD, Karlı P, Özdemir AZ. The importance of serum prolidase activity in endometriosis. Journal of Clinical and Experimental Investigations. 2019;10(4):em00729.

11. Toy H, Camuzcuoglu H, Camuzcuoglu A, Celik H, Aksoy N. Decreased serum prolidase activity and increased oxidative stress in early pregnancy loss. Gynecologic and obstetric investigation. 2010;69(2.122-7:(

12. Vural M, Toy H, Camuzcuoglu H, Aksoy N. Comparison of prolidase enzyme activities of maternal serum and placental tissue in patients with early pregnancy failure. Archives of gynecology and obstetrics. 2011;283:953-8.

13.Ando N, Gorai I, Hirabuki T, Onose R, Hirahara F, Minaguchi H. Prolactin disorders in patients with habitual abortion. Nihon Sanka Fujinka Gakkai Zasshi. 1992;44(6):650-6.

14.Gu F. Effect of serum prolactin levels on luteal function in patients with recurrent abortions. Zhonghua fu Chan ke za zhi. 1993;28(1):34-7, 60.

15.Tian L, Shen H, Lu Q, Norman RJ, Wang J. Insulin resistance increases the risk of spontaneous abortion after assisted reproduction technology treatment. The Journal of Clinical Endocrinology & Metabolism. 2007;92(4.1430-3:(

16.Creţu D, Cernea S, Onea CR, Pop R-M. Reproductive health in women with type 2 diabetes mellitus. Hormones. 2020;19(3):291-300.

17.Colamatteo A, Fusco C, Micillo T, D'Hooghe T, de Candia P, Alviggi C, et al. Immunobiology of pregnancy: from basic science to translational medicine. Trends in Molecular Medicine. 2023;29(9):711-25.

18.Wahid S, Khan RA, Feroz Z. Reduction in mortality and teratogenicity following simultaneous administration of folic acid and vitamin E with antiepileptic ,antihypertensive and anti-allergic drugs. Journal of Pharmacy and Bioallied Sciences. 2014;6(3):185-91.

19.Rey E, Jacob CE, Koolian M, Morin F. Hypercalcemia in pregnancy–a multifaceted challenge: case reports and literature review. Clinical Case Reports. 2016;4(10):1001.

20. Wueest S, Konrad D. The controversial role of IL-6 in adipose tissue on obesity-induced dysregulation of glucose metabolism. American Journal of Physiology-Endocrinology and Metabolism. 2020;319(3):E607-E13.