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Predictive effect of thiol/disulfide homeostasis dynamics on early pregnancy viability: A case-control study

Erken gebelik viabilitesi üzerinde tiyol/disülfit homeostazı: Bir olgu-kontrol çalışması

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Abstract

Objective: The main aim of this study was to investigate the differences in maternal serum thiol/disulfide homeostasis among women with abortion imminens (AI), missed abortion (MA), and healthy pregnancies during the first trimester.

Materials and Methods: This was a prospective case-control study. This study was conducted on pregnant women who visited the Obstetrics Clinic at University of Health Sciences Turkey, Etlik Zübeyde Hanım Gynecology Training and Research Hospital and were diagnosed with either AI or MA during the 6th to 14th weeks of pregnancy. The participants had a normal pregnancy follow-up, no chronic illnesses, and did not take any multivitamin or antioxidant supplements except for folic acid. The study incorporated 33 pregnant women with AI, 36 with MA, and 40 with normal pregnancies. Age, and body mass index were matched across the three groups. This study used a recently developed automated spectrophotometric technique to quantify thiol/disulfide concentrations.

Results: The AI group had considerably elevated levels of total thiol and native thiol (SH) compared with the MA group. Nevertheless, there was no notable disparity observed between the group of healthy pregnancies and the other two groups. Serum disulfide (SS) levels did not exhibit any significant variations among the three groups. Similarly, the ratios of SS/SH, SS/total thiol, and SH/total thiol did not show any significant differences between the groups ($p>0.05$).

Conclusion: Patients with MA had decreased levels of total thiol and SH, which possess antioxidant capabilities, compared to the AI group. A decrease in antioxidant levels in the body may contribute to the etiology of MA. When considering our findings alongside existing literature, it remains inconclusive whether the serum thiol-disulfide ratio can predict a healthy pregnancy or MA following AI. Therefore, it is not yet seen as a promising diagnostic tool for assessing pregnancy viability. Additional investigation is required to establish the influence of dynamic thiol/disulfide homeostasis on early pregnancy loss.

Keywords: Early pregnancy loss, antioxidants, disulfide, oxidative stress, thiol

Öz

Amaç: Bu çalışmanın temel amacı, ilk trimester abortus imminens (AI), düşük ve sağlıklı gebeliği olan kadınlarda anne serumu tiyol/disülfit dengesindeki farklılıkları incelemektir.

Gereç ve Yöntemler: Bu çalışma, Sağlık Bilimleri Üniversitesi, Etlik Zübeyde Hanım Kadın Hastalıkları Eğitim ve Araştırma Hastanesi Kadın Doğum Kliniği'ne başvuran, 6 ile 14. haftalar arasında AI veya düşük tanısı almış, normal gebelik süreci geçiren, kronik hastalığı bulunmayan ve folik asit dışında

PRECIS: This study investigates the role of thiol/disulfide homeostasis in early pregnancy outcomes, suggesting potential new biochemical markers for predicting pregnancy viability.

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herhangi bir multivitamin veya antioksidan takviyesi kullanmayan hamile kadınları içeren prospektif bir çalışmadır. Çalışma, 33 AI, 36 düşük tanısı konmuş ve 40 normal gebelik olgusunu kapsamaktadır. Katılımcıların yaş, ve vücut kitle indeksi her üç grup arasında eşitlenmiştir. Tiyol/disülfit seviyeleri, yeni geliştirilmiş bir otomatik spektrofotometrik yöntemle ölçülmüştür.

Bulgular: AI grubunda, düşük grubuna göre anlamlı derecede yüksek olan toplam tiyol ve doğal tiyol (SH) seviyeleri saptanmıştır. Ancak, sağlıklı gebelik gösteren grup ile diğer iki grup arasında belirgin bir fark bulunmamıştır. Serum disülfit (SS) düzeyleri üç grup arasında istatistiksel olarak anlamlı bir farklılık göstermemiştir. Ayrıca, SS/SH, SS/toplam tiyol ve SH/toplam tiyol oranları gruplar arasında önemli bir fark göstermemiştir (p>0,05).

Sonuç: Düşük olgularında AI grubuna kıyasla düşük toplam tiyol ve SH seviyeleri gözlemlenmiştir; her ikisi de antioksidan özelliklere sahiptir. Vücuttaki antioksidan seviyelerindeki azalma, düşüğün etiyolojisine katkıda bulunabilir. Bulgularımızı mevcut literatürle birleştirdiğimizde, serum tiyol-disülfit oranının AI sonrasında sağlıklı bir gebeliği ya da düşüğü öngörüp öngöremeyeceği konusunda kesin bir sonuca varılamamıştır. Dolayısıyla, gebeliğin sağlıklı ilerleyip ilerlemeyeceğini değerlendirmede umut verici bir tanı aracı olarak görülmemektedir. Erken gebelik kaybında dinamik tiyol/disülfit homeostazının etkisini göstermek için ek araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Erken gebelik kaybı, antioksidanlar, disülfit, oksidatif stres, tiyol

Introduction

In the first half of pregnancy, the threat of abortion imminens (AI) is characterized by the presence of fetal heartbeat within the intrauterine cavity, without rupture of fetal tissue or membrane, accompanied by vaginal bleeding, cramping, or pain. The situation where non-viable pregnancy products are found in the uterus while the cervix is closed is called missed abortion (MA)[1]. MA occurs in situations where, despite the absence of a fetal heartbeat, the pregnancy does not result in bleeding or miscarriage[2]. AI is observed in approximately 20% of all pregnancies[3]. The diagnosis must be confirmed by ultrasonographic imaging of the gestational sac (GS) and the embryo or fetus with a beating heart[4]. The diagnosis and occurrence of MA are gradually increasing (3.89-14.1%) because of the progress in ultrasound imaging technologies[5]. Although certain fetomaternal factors play a role in the etiology, the pathophysiology remains largely unclear. Nevertheless, it is accepted as a multifactorial phenomenon[6]. Spontaneous abortions are most frequently observed before the 8th week of pregnancy, whereas bleeding episodes during pregnancy typically occur between the 8th and 10th weeks of pregnancy[7]. Oxidative stress (OS) is characterized by an imbalance in the excessive production of free radicals and their neutralization, leading to the accumulation of oxidative damage. Human cells are equipped with both enzymatic and non-enzymatic antioxidant defense systems to maintain vital redox balance[8]. Studies examining the role of OS in initiating various diseases and syndromes highlight the significance of this subject[9]. Thiols, organic molecules containing a sulfhydryl group, play a crucial role in redox homeostasis through their oxidation and reduction. Major plasma thiols, including albumin, cysteine, glutathione, thioredoxin, and homocysteine, can undergo reversible oxidation in the presence of oxygen to form disulfide (SS) bonds, thus maintaining a balance between reduced and oxidized states[10]. Under antioxidant protection, the dynamic conversion between SH (thiol) and SS groups establishes a thiol-disulfide equilibrium, playing a role in cellular signaling, enzyme activities, detoxification, and apoptosis[11]. Previous studies have linked abnormal thiol/disulfide homeostasis (TDH) to hypoxia and reperfusion injury, various liver, heart, and neurological diseases, diabetes, cancer, aging, and complications in pregnancy[12].

The successful development of the embryo heavily relies on the implantation of trophoblast cells into the maternal decidua during the first trimester. A mildly hypoxic environment is essential for the proliferation and differentiation of trophoblasts in the early stages of pregnancy, facilitating the formation of a healthy maternofetal circulation[13]. Although less pronounced than in the initial stages, the continuation of pregnancy involves an ongoing hypoxia-reperfusion state and endothelial dysfunction in the maternofetal bed[14]. Previous studies have associated prolonged OS and diminished protective mechanisms with adverse pregnancy outcomes[15]. In the past, the identification of the SH component in TDH could only be accomplished using complex techniques. Nevertheless, a recently uncovered approach developed by Erel and Neselioglu[16] enables the feasible determination of both constituents of this equilibrium. TDH demonstrates the qualities of being dynamic, reversible, and bidirectional[16]. This study aimed to establish the correlation between different groups by analyzing serum TDH levels in women with AI, MA, and healthy pregnancies.

Materials and Methods

This study adhered to the Helsinki Declaration on Human Subject Research and was authorized by the Ethics Committee of Ankara Yıldırım Beyazıt University (date: 15/06/2016, approval no: 169). A total of 109 women aged over 18 years who were hospitalized in the early pregnancy unit or attending the outpatient clinic of University of Health Sciences Turkey, Etlik Zübeyde Hanım Gynecology Training and Research Hospital between 2017 and 2018 were enrolled. Each participant provided their signature on a written informed consent document. The study comprised 109 patients, including 33 with AI diagnosis (group 1), 36 with MA (group 2), and 40 in the healthy pregnancy control group (group 3). AI was diagnosed in those showing a GS, embryo, or fetus on ultrasonography, exhibiting vaginal bleeding without cervical dilation, and without gynecological pathologies such as cervical polyps and cervicitis that could cause bleeding. Pain and hematoma identified by ultrasound were not considered diagnostic criteria.

The defining features of MA in the first trimester are the GS is unhealthy and there is no fetal heartbeat, the cervical os is closed, and there is little or no vaginal bleeding. Forty randomly selected patients, attending routine checkups in our antenatal clinic during the same period and with no artificial insemination history in the ongoing pregnancy, formed a control group representing healthy pregnancies. Demographic details and test results of each expectant mother were prospectively collected. Excluded from the study were those with endocrine disorders (such as diabetes and thyroid disease), viral diseases, and other immunological, rheumatological, or thrombophilic conditions (such as antiphospholipid syndrome). Included were pregnant women between the ages of 18 and 43, in singleton pregnancies of 6 to $13^{6/7}$ weeks, non-smokers, not on any antioxidant or multivitamin supplements except folic acid. Pelvic examinations of the cases were performed and age, birth and personal history, relevant laboratory values and body mass indexes (BMI) were documented. Pregnancy weeks were calculated according to the last menstrual period. Initially, all cases underwent biometric measurements with a transvaginal transducer (7.5 MHz) ultrasound machine in the lithotomy position, assessing pregnancy viability, GS shape, yolk sac, and crown-rump length. Patients diagnosed by ultrasonography underwent blood sample collection. Venous blood samples of 10 cm^3 were drawn into tubes containing ethylenediaminetetraacetic acid immediately post-ultrasonographic diagnosis. Plasma samples were separated from cells by centrifugation at 1500 × g for 10 min and stored at -80 °C until the day of analysis.

Thiol Analysis Method: Plasma SH, total thiol, and SS levels were determined using an innovative and fully automated technique developed by Erel and Neselioglu[16]. This method relies on the conversion of dynamic SS bonds into functional SH groups using sodium borohydride ($NaBH_4$). In their research, they described this method as practical, cost-effective, straightforward, rapid (with an average processing time of about 10 minutes), and completely automated. To prevent excessive reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and the SS bond formed during the DTNB reaction, all residual $NaBH_4$ was removed using formaldehyde. The total thiol content of the samples was measured using a modified Ellman's method. The measured total thiol content was then divided by the SH content, and the resultant difference was halved.

Statistical Analysis

The collected data were analyzed using the 23rd version of SPSS developed by IBM Corp. The Kolmogorov-Smirnov test was applied to determine the distribution pattern of continuous variables. In cases where the numerical variables in the study groups exhibited a normal distribution, statistically significant differences were identified using One-Way ANOVA, followed by Tukey's test. For non-normally distributed data, the Kruskal-Wallis test was employed. Descriptive statistics are presented as mean and standard deviations or medians and interquartile ranges for continuous variables and as frequencies and percentages for categorical variables. A threshold of $p<0.05$ was set for statistical significance.

Results

In this study, 109 women were enrolled, comprising 33 with AI, 36 with MA, and 40 with normal pregnancies. No significant differences were observed in age and BMI among the groups. Normal pregnant women differed significantly from those in the AI and MA groups in terms of number of pregnancies, number of births, and duration of pregnancy ($p<0.05$ for each) (Table 1). Spontaneous abortion occurred in 3 women from the AI group and 2 women from the normal pregnancy group, with other pregnancies continuing until at least the 20th week. Ongoing pregnancy rates were calculated to be 90.9% in the AI group and 95% in the normal pregnancy group.

No significant differences were found between the groups in terms of white blood cell count, neutrophil, lymphocyte, hemoglobin, hematocrit, red cell distribution width, platelet count, total protein, and albumin levels. However, the mean corpuscular volume and mean platelet volume were found to be higher in the normal pregnancy group than in the AI and MA groups ($p=0.004$ and $p=0.003$, respectively) (Table 2).

Total thiol levels and SH values in the AI group were significantly higher than those in the MA group ($p=0.001$ for both). However, no significant difference was observed between the healthy group and the other two groups. In addition, there were no notable variations observed between the groups in relation to SS, SS/SH, SS/total thiol, and SH/total thiol ratios (Table 3).

Discussion

The objective of this study was to evaluate the viability of early pregnancy in the first trimester by measuring the TDH levels of the patients. Our results indicated significantly reduced levels of serum SH and total thiol, both known for their antioxidant properties, in patients with MA compared with those receiving AI treatment. No notable differences were found between the three groups regarding SS levels, SS/SH ratio, SS/total thiol

Table 1. Demographic characteristics of the groups are illustrated

Variables	Abortus imminens (n=33)	Missed abortus (n=36)	Healthy pregnant (n=40)
Age (year)	25.0 (7)	27.0 (11)	28.0 (10)
BMI (kg/m^2)	24.24 (6.02)	24.20 (2.92)	24.14 (5.31)
Gravida	1.00 (1)	1.00 (2)	2.00 (2)*
Parity	0.00 (1)	0.00 (2)	1.00 (0)*
Gestational age (week)	8.4 (4.1)	8.2 (3.0)	10.7 (4.7)♯

BMI: Body mass index
Statistical values are expressed as medians and interquartile ranges.
*: $p<0.05$ as compared to abortus imminens
♯: $p<0.05$ as compared to missed abortus.

Table 2. Comparison of the laboratory parameters of the groups

Parameters	Abortus imminens (n=33)	Missed abortus (n=36)	Healthy pregnant (n=40)	P1	P2	P3	P4
WBC (X 10^3/μL) (mean ± SD)[a]	8.38±2.31	7.71±1.86	7.82±1.86	0.334	0.463	0.968	0.348
Neutrophil, (X 10^3/μL) [median (IQR)][k]	5.76 (2.76)	5.36 (1.88)	4.87 (1.48)	0.305	0.258	0.892	0.114
Lymphocyte (X 10^3/μL) [median (IQR)][k]	1.55 (0.98)	1.70 (0.65)	1.51 (0.56)	0.310	0.458	0.114	0.548
MCV (fL) [median (IQR)][k]	85.20 (6.30)	82.65 (6.73)	86.80 (5.75)	**0.004**	**0.009**	**0.003**	0.475
Hb (g/dL) [median (IQR)][k]	12.80 (1.65)	12.20 (1.10)	12.55 (1.40)	0.126	0.833	0.091	0.068
Hct (%) [median (IQR)][k]	37.50 (4.25)	36.70 (1.85)	37.35 (4.90)	0.306	0.731	0.123	0.319
RDW (%) [median (IQR)][k]	14.50 (0.90)	14.45 (1.13)	14.15 (0.98)	0.300	0.107	0.364	0.678
Platelet, (X 10^3/μL) (mean ± SD)[a]	245.78±58.36	229.02±40.50	245.35±65.38	0.354	0.999	0.417	0.432
MPV (fL) [median (IQR)][k]	7.40 (0.95)	7.60 (0.77)	8.05 (1.37)	**0.003**	**0.002**	**0.011**	0.364
Total protein (g/dL) [median (IQR)][k]	6.80 (0.55)	6.90 (0.40)	6.95 (0.58)	0.525	0.283	0.594	0.477
Albumin (g/dL) [median (IQR)][k]	4.30 (0.40)	4.30 (0.40)	4.10 (0.50)	0.305	0.152	0.247	0.754

[a]: One-Way ANOVA, Post-hoc test: Tukey HSD
[k]: Kruskal-Wallis test and Mann-Whitney U test
p values in bold are statistically significant (p<0.05).
IQR: Interquartile range, Hb: Hemoglobin, Hct: Hematocrit, WBC: White blood cell, MCV: Mean corpuscular volume, RDW: Red cell distrubution width, MPV: Mean platelet volume
P1: Comparison of all three groups, P2: Comparison of AI with healthy pregnant, P3: Comparison of MA with healthy pregnant, P4: Comparison of AI with MA, SD: Standard deviation

Table 3. Dynamic thiol/disulphide homeostasis parameters of the patients

Parameters	Abortus imminens (n=33)	Missed abortus (n=36)	Healthy pregnant (n=40)	P1	P2	P3	P4
Total thiol (μmol/L) (mean ± SD)[a]	416.76±54.18	372.91±52.81	392.77±37.48	**0.001**	0.091	0.177	**0.001**
Native thiol (μmol/L) (mean ± SD)[a]	373.75±52.31	332.73±53.07	353.56±35.32	**0.002**	0.167	0.136	**0.001**
Native thiol/Total thiolx100 [median (IQR)][k]	90.55 (1.24)	90.37 (2.35)	90.36 (1.37)	0.882	0.626	0.954	0.701
Disulphide/Total thiolx100 [median (IQR)][k]	4.73 (0.62)	4.81 (1.17)	4.81 (0.68)	0.880	0.622	0.950	0.701
Disulphide (μmol/L) [median (IQR)][k]	20.49 (3.64)	18.23 (6.44)	18.26 (5.24)	0.222	0.175	0.938	0.094
Disulphide/Native thiolx100 [median (IQR)][k]	5.22 (0.76)	5.32 (1.45)	5.33 (0.85)	0.885	0.626	0.954	0.710

[a]: One-Way ANOVA, Post-hoc test: Tukey HSD
[k]: Kruskal-Wallis test and Mann-Whitney U test
p values in bold are statistically significant (p<0.05).
IQR: Interquartile range
P1: Comparison of all three groups, P2: Comparison of AI with healthy pregnant, P3: Comparison of MA with healthy pregnant, P4: Comparison of AI with MA, SD: Standard deviation

ratio, and SH/total thiol ratio, which are markers of OS. The diminished levels of SH and total thiol in the MA group suggest a potential role of these antioxidants in the pathophysiology of miscarriage. Studies focusing on serum TDH in AI, MA, and healthy pregnancies are limited.

The precise etiology of MA remains elusive in about half of the cases, underscoring the necessity for additional research to understand the underlying causes and to prevent this condition. Several studies hypothesize that an imbalance in the antioxidant/oxidant equilibrium may contribute to the etiology of miscarriage. Although pregnancy naturally involves an elevated level of OS, the development of systems that defend against oxidative damage typically offers protection against such complications. However, our analysis of serum TDH in MA patients indicated a significant increase in these levels, suggesting an excessive rise in OS beyond the capacity of antioxidant defenses as a potential significant etiological component contributing to the development of MA[17]. An examination is conducted on the initial phases of the pathophysiology of spontaneous abortion, and novel viewpoints are introduced[18]. Due to the intervillous circulation in the pregnant woman, the oxygen level increases in the placenta[19]. OS is likely to have a significant impact on the process of distinguishing the placenta. A study conducted by Sebire et al.[20] provided evidence that pregnancy losses that occur between the 7th and 12th weeks of pregnancy may be linked to heightened blood circulation between the villi

during this period. Additionally, Hempstock et al.[21] studied placental OS in early pregnancy loss, assessing placental tissues visually for tissue damage and immunohistochemically for OS. Their findings indicated that patients who experienced MA had substantially higher levels of OS and tissue damage. As observed in our study, patients with MA demonstrated a notable decrease in antioxidant serum SH and total thiol levels.

In organisms, dynamic TDH plays a pivotal role as a fundamental component of antioxidant protection, detoxification, regulation of enzymatic activities, and cellular signaling processes. The maintenance of cellular balance is dependent on alterations in the SH-SS equilibrium. It is possible for cells to produce free oxygen radicals during metabolism or as a reaction to the body's defense systems. It is possible for free radicals to damage cells if there is an imbalance of antioxidants. The controlled production of free radicals under suitable conditions is essential for maintaining cellular homeostasis[22].

Patients experiencing recurrent abortions have been observed to lose antioxidant defenses because of increased consumption. An imbalance between oxidants and antioxidants is believed to be associated with pregnancy loss[23].

Oxygen free radicals are naturally produced during cellular metabolism or as a part of the body's defense requirements. However, without a proper antioxidant equilibrium, uncontrolled generation of these radicals can result in pathogenic changes in cells. The controlled production of free radicals is crucial for sustaining cellular homeostasis[22]. Additionally, patients with recurrent abortions have reported a decrease in antioxidant defenses due to their heightened consumption. An imbalance between oxidants and antioxidants has been suggested to be associated with pregnancy loss[23]. Low levels of plasma ascorbic acid, a-tocopherol, total thiols, and erythrocyte reduced glutathione in individuals with unexplained recurrent pregnancy loss or autoimmune or luteal phase insufficiency suggest an increase in OS[24]. Reduced antioxidant levels may exacerbate pro-oxidant damage to endothelial cells, leading to an imbalance between prostacyclin and thromboxane, potentially resulting in pre-eclampsia or miscarriage[24]. A study by Korkmaz et al.[25] looked at how severe pre-eclampsia affected TDH and found a strong link between the level of TDH decline and the severity of pre-eclampsia. In our study, we supported with statistical analysis that there is a significant reduction in antioxidant levels in individuals with MA compared to those with AI.

During the first trimester of pregnancy, vaginal bleeding is frequently observed and is frequently interpreted as an early indication of placental malfunction. It has been proposed that OS has a significant role in the development of pregnancy problems, such as abortion, and contributes to endothelial dysfunction during aberrant placentation[26]. OS significantly affects the physiology and development of pregnancy. Inadequate trophoblast invasion in the placenta may lead to various conditions, such as early- and late-onset preeclampsia, MA, and miscarriages[27].

In our study, the MA group consisted of pregnant women unclassified as high risk. We utilized this innovative methodology to more quickly and easily confirm the diagnosis and accelerate the follow-up process. In our study, which we planned considering this situation, we attempted to understand the importance of whether it makes any contribution to the early diagnosis or treatment of this disease by measuring serum TDH values in patients with suspected MA.

Dalle-Donne et al.[28] reported an increase in lipid peroxidation in the placenta during pregnancy; however, in healthy pregnancies, there was a simultaneous increase in the antioxidant defense mechanism in response to this OS. Gubaljević and Čaušević[29] measured serum 8-isoprostane levels as a potential indicator of OS during pregnancy. They found that healthy pregnant women had higher levels of 8-isoprostane compared to non-pregnant women. When comparing these levels between the two trimesters, second-trimester pregnant women exhibited significantly higher 8-isoprostane levels than those in the first trimester. In another study[30], a small group of pregnant women experiencing vaginal bleeding before the 10th week of gestation and a control group exhibiting comparable features in healthy pregnancies were evaluated for various OS markers to investigate the role of OS in vaginal bleeding during the first trimester of pregnancy. The specific causes disrupting the oxidant/antioxidant balance in first-trimester vaginal hemorrhage remain unclear. In our current study, no significant differences were observed in serum SS levels, a marker of OS, among the groups of AI, MA, and healthy pregnant women. However, there was a decrease in levels of SS and total thiol, both known for their antioxidant properties, particularly in the MA group. This could indicate a reduction in antioxidant levels in cases of MA, although the absence of significant differences in disulfide and other OS markers might be due to the early gestational weeks of the participants. The cross-sectional nature of the study limits the establishment of a causal relationship. Furthermore, the serum levels of OS markers may not directly reflect the levels in tissues, and future molecular or immunohistochemical studies could provide additional insights.

Study Limitations

There are several limitations to this study. First, the sample size is limited, necessitating more comprehensive prospective studies. Second, due to cost-effectiveness issues, genetic analysis was not performed in the abortion group, which might have led to the overlooking of genetic anomalies. The strength of this study lies in being one of the few prospective studies investigating the impact of oxidation on AI and MA.

Conclusion

The etiology of first-trimester vaginal bleeding remains largely undetermined, necessitating further research to elucidate its causes and develop effective treatment approaches.

Several studies suggest that an imbalance in antioxidant/oxidant equilibrium may play a role in the etiology of miscarriages. Although an increase in OS is expected during pregnancy, the development of antioxidant defense systems usually helps prevent potential complications. We noticed a notable reduction in antioxidant levels during serum TDH assessment in patients with MA. The insufficiency of antioxidant defenses in response to physiological OS during pregnancy may be a significant etiological factor in the development of MA. Consequently, additional prospective studies are required to explore the potential role of antioxidant therapy as a preventive approach in both AI and MA cases.

In conclusion, based on the findings of our study and existing literature, it appears that the serum SH/SS ratio cannot yet be considered a reliable diagnostic tool to predict a healthy pregnancy or MA following AI, nor can it be effectively used as a test tool for assessing pregnancy viability.

Ethics

Ethics Committee Approval: This study adhered to the Helsinki Declaration on Human Subject Research and was authorized by the Ethics Committee of Ankara Yıldırım Beyazıt University (date: 15/06/2016, approval no: 169).

Informed Consent: Each participant provided their signature on a written informed consent document.

Authorship Contributions

Surgical and Medical Practices: B.D.Ç., G.D., M.M.A., Concept: S.Ş.A., M.M.A., Design: S.Ş.A., Ö.E., Data Collection or Processing: B.D.Ç., Analysis or Interpretation: G.D., M.A., Ö.E., Literature Search: B.D.Ç., S.Ş.A., M.A., M.M.A., Ö.E., Writing: B.D.Ç., S.Ş.A., G.D.

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