

# Comparison of Antioxidant Status Levels and the Impact of Oxidative Stress in Spontaneous Inevitable Abortion With Normal Pregnancy and Healthy Non Pregnant Women

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## Abstract

**Introduction:** One major cause for spontaneous and threatened abortion is oxidative stress (OS). Spontaneous abortion might be associated with OS in the maternal circulation.

**Purpose:** This study is conducted to note the effect of OS during pregnancy and to explore the OS and antioxidant status in the maternal circulation in spontaneous inevitable abortion and in normal pregnancy.

**Materials and Methods:** Ferric reducing antioxidant power (FRAP), serum uric acid, total protein (TP), albumin, glucose, and cholesterol were measured among 126. Woman presenting with spontaneous inevitable abortion (No. 42, Group IA), Group IA after 48 h of abortion (No. 42 Group IB), age-matched normal pregnant woman (No. 42, Group II), and normal non-pregnant woman (No. 42, Group III).

**Results:** The mean age of the subjects in Groups I-III was 26.5, 27.09 and 26.76 years. FRAP value and uric acid levels are much lower among the women presenting with spontaneous inevitable abortion ( $P < 0.001$ ). FRAP value and uric acid level were much higher among the healthy pregnant woman controls and non-pregnant woman controls ( $P = 0.01$ ).

**Conclusion:** When and uric FRAP acid value compared between, non-pregnant, normal pregnancy and spontaneous inevitable abortion by the student *t*-test, there was a significant difference in the mean value ( $P < 0.01$ ). Improving the antioxidant status by supplementation during pregnancy might be useful in preventing OS related disorders.

**Key words:** Ferric reducing ability of plasma, Oxidative stress, Spontaneous abortion, Total antioxidant status, Pregnancy

## INTRODUCTION

The ability to propagate life by the process of reproduction is a boon to mankind as well as to all life forms. However, disorders of pregnancy such as placental-related disorders, miscarriage, and preeclampsia affect around a third of human pregnancies causing termination of the

pregnancy. Before the end of the first trimester, 30-50% of conceptions end in spontaneous abortion (SAb). Oxidative stress (OS)-induced damage had played a major role in SAb, idiopathic recurrent pregnancy loss, hydatidiform mole, defective embryogenesis, and drug-induced teratogenicity. <sup>1</sup> OS-induced placental dysfunction is a common cause of polygenic etiologies of abortion, recurrent pregnancy loss, defective embryogenesis, hydatidiform mole, and drug-induced teratogenic effect. The aim of this study was to look at the association between OS and spontaneous inevitable abortion. Hence, the study “comparison of antioxidant status levels and the impact of oxidative stress in spontaneous inevitable abortion with normal pregnancy and healthy non pregnant women” was taken up to determine the total antioxidant (TAO) status of plasma

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by FRAP and uric acid level. (Halliwell and Gutteridge, 1985; Berlett and Stadtman, 1997 (Smith and Lawing, 1983; Berlett and Stadtman, 1997; Nyyssonen *et al.*, 1997) state that uric acid conceivably acts as a powerful antioxidant in plasma. The antioxidant in highest concentration in human blood is uric acid, which provides about half of the TAO capacity of human serum.

## REVIEW OF LITERATURE

### SAb

SAb, also known as miscarriage, refers to a pregnancy that ends spontaneously before the fetus has reached a viable gestational age.<sup>1</sup> The loss of a pregnancy before 20 weeks was known as early pregnancy loss. Recurrent pregnancy loss was defined as three or more consecutive pregnancy losses before 20 weeks of gestation.<sup>2</sup>

### Spontaneous Inevitable Abortion

Gross rupture of membranes evidenced by leaking of amniotic fluid, in the presence of cervical dilatation signals almost certain abortion. If, however, the gush of vaginal discharge was followed by bleeding, pain, or fever, then the abortion considered as inevitable. Spontaneous inevitable abortion SAb is the most common complication of early pregnancy.<sup>3</sup> The frequency decreases with increasing gestational age. Around 80% of clinically recognized pregnancies will undergo SAb within the first 12 weeks of gestation.<sup>4-6</sup>

### Pathogenesis

Miscarriage and pre-eclampsia are the most common disorders of human pregnancy. Ultrasound imaging has enabled the events during early pregnancy visualized *in vivo*. As a result, a new understanding of the early maternofetal relationship has emerged and a new insight into the pathogenesis of these disorders. In miscarriage, there is rapid and generalized placental tissue degeneration, in preeclampsia, the placental damage is progressive and it was compensated for some time depending on the severity of the initial placental defect and the intrinsic placental antioxidant capacity.<sup>7,8</sup>

### Pregnancy - A State of OS

Pregnancy is a state of OS arising from increased placental mitochondrial activity and production of reactive oxygen species (ROS). The placenta produces ROS includes nitric oxide, carbon monoxide, superoxide anion, and peroxynitrite, which have pronounced effects on the placental function including trophoblast proliferation and vascular reactivity. In the first trimester, the establishment of blood flow into the intervillous space was associated with a burst of OS. Evidence for this OS includes decreased

activity of antioxidants, increased lipid peroxides and isoprostanes.

### OS in Early Pregnancy

Early anatomical and histopathological studies have exclusively focused on defective villous development in early pregnancy loss.<sup>9</sup> There is now clear evidence that miscarriages are placentation disorders and that the villous changes described previously are the consequences, rather than the cause.<sup>10</sup> In about two-thirds of early pregnancy failures, there is anatomical evidence of abnormal placentation is by a thinner and fragmented trophoblast shell, incomplete plugging of the lumen at the tips of the spiral arteries and decreased cytotrophoblast invasion of the endometrium.<sup>11,12</sup> This is associated with the absence of physiological changes in most spiral arteries and leads to a premature onset of the maternal circulation throughout the entire placenta. Entry of excessive maternal blood into the intervillous space has two effects: (i) A direct mechanical effect on the villous tissue, which becomes progressively entangled inside the large intervillous blood thrombi and (ii) a widespread and indirect O<sub>2</sub>-mediated trophoblastic damage and increased apoptosis.<sup>13-15</sup>

The consequences are placental degeneration with complete loss of function of syncytiotrophoblast and detachment of the placenta from the uterine wall. Any factor that causes abnormally high and fluctuating concentrations of O<sub>2</sub> will have a harmful on the early villous tissue. In this review, we have presented the current evidence regarding the role of OS in pregnancy and SAb, assessment of OS by assaying TAO status by FRAP assay and uric acid levels.

## MATERIALS AND METHODS

Following the approval of the Institutional ethical committee, this study was carried out over a period of 6-12 months in a tertiary care hospital. Where 126 adult (21-25 years) women gave their consent to take part in the study. The participants are divided into four subgroups. (i) Those patients presenting with SAb ( $n = 42$  Group IA), (ii) Group IA patients after 48 h of SAb ( $n = 42$  Group IB), (iii) healthy age-matched normal pregnant woman ( $n = 42$  Group II), and (iv) healthy age-matched non-pregnant woman ( $n = 42$  Group III). All subjects with H/O DM, HT, thyroid disorders and prior abortions was eliminated from this study. Blood samples are collected under aseptic conditions from the anterior cubital vein, 3 ml was kept for estimation of FRAP. The remaining sample was placed in a sterile vial without any anticoagulant at 37°C for clot formation. After clot retraction serum was separated. Uric acid (UA, ref. 3.5-7 mg/dl), albumin (Al, ref. 3.5-5 g/dl), TP (TP, ref.

6.0-8.00 g/dl), glucose (GLU, ref. 80-110 mg/dl), and total cholesterol (CHO, ref. 150-200 mg/dl) levels were measured by commercially available kits on automated analyzer XL300.

**Measurement of Plasma TAO Status-FRAP Assay**

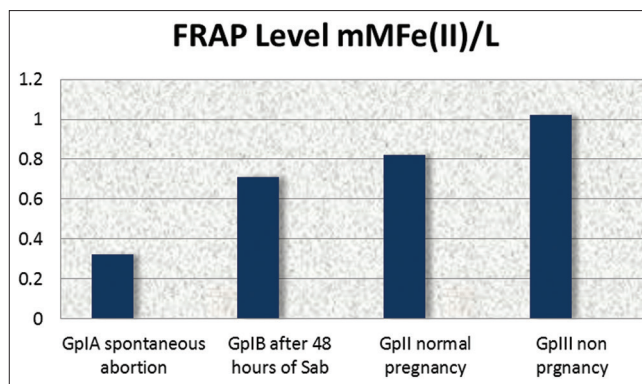
An imbalance of oxidants and antioxidants within the human body, in which either high oxidants or antioxidant protection is low, it will lead to a state of “OS.” This is associated with a variety of chronic degenerative diseases. With the standard FRAP assay, an increase in antioxidants should result in an increase FRAP value. FRAP assay, one of the most sensitive and specific assay. Tests which measure the combined antioxidant effect of the non-enzymatic defense in biological fluids will be useful in providing an index of ability to resist oxidative damage. TAO status was measured by FRAP assay of Benzie and Strain (1996).<sup>16,17</sup> FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. The relative activity of individual serum antioxidants and their estimated contribution to plasma FRAP: Uric acid - 61.7%, albumin - 7.26%, tocopherol - 5.84%, ascorbic acid - 10.1%, bilirubin - 4.34%, and others - 10.76.

**Statistical Analysis**

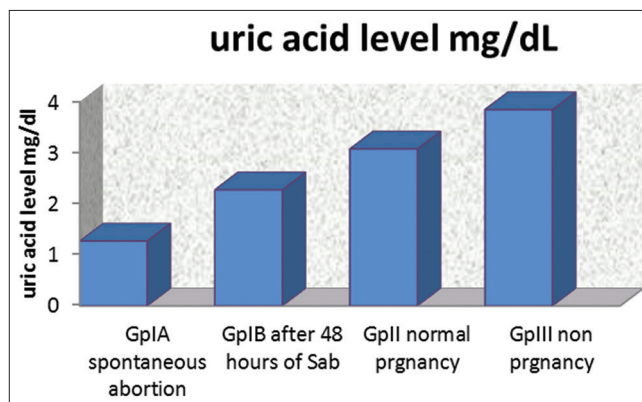
Demographic and clinical variables were given in frequencies with their percentages. Mean and standard deviations for them was calculated. FRAP and uric acid was analyzed using Student’s independent *t*-test. Maternal age, gestational age, and weight were analyzed using one-way analysis of variables F-test and multiple comparisons were analyzed using the Bonferroni *t*-test. Pearson correlation (*r*) is used to assess the degree of association between variables in different groups. *P* < 0.05 was considered statistically significant.

**RESULTS**

Comparison of observed biochemical parameters among all the subjects was presented in Tables 1-5. Comparison of mean FRAP value and mean uric acid level among various groups were plotted in Figures 1 and 2. Demographic and clinical variables were presented in Table 6. The mean age of the subjects among Groups I-III was 26.5, 27.09 and 26.76 years. From the study, we find that the mean value of FRAP assay for the various study groups as Group IA (women with spontaneous inevitable abortion) mean  $0.322 \pm 0.688$  mMFe (II)/L, Group IB (after 48 h of abortion) mean  $0.706 \pm 0.670$  mMFe (II)/L, Group II (normal pregnant women) mean  $0.829 \pm 0.129$  mMFe (II)/L, and Group III (normal non-pregnant women) mean  $1.020 \pm 0.195$  mMFe (II)/L. Comparison of the mean level of FRAP among women with spontaneous inevitable abortion (Group IA), after



**Figure 1: Comparison of mean plasma ferric reducing antioxidant power value among study groups**



**Figure 2: Comparison of mean serum uric acid value among study groups**

48 h of abortion (Group IB), normal pregnant women (Group II), and normal non-pregnant women (Group III). FRAP value is expressed as mMFe (II)/L. On comparison FRAP mean level of Group IA ( $0.322 \pm 0.688$ ) with Group IB ( $0.706 \pm 0.670$ ), it was found that FRAP value was very low at the time of abortion, and it increases after 48 h of expulsion of the abortus, it is represented by, a significant increase in FRAP value (*P* = 0.001). When the mean FRAP value of Group IA was compared with Groups II and III it was much higher in Group II, highest in Group III and the difference was highly significant (*P* = 0.001). FRAP mean of Group II ( $0.829 \pm 0.129$ ) is compared with Group III ( $1.020 \pm 0.195$ ) it was higher in Group III, and the difference was significant (*P* = 0.001).

The mean uric acid levels in the study groups were found as Group IA ( $1.277 \pm 0.260$  mg/dl), Group IB ( $2.578 \pm 0.398$  mg/dl), Group II ( $3.073 \pm 0.605$  mg/dl), and Group III ( $3.843 \pm 0.737$  mg/dl). The mean uric acid level of Group IA compared with Group IB ( $2.578 \pm 0.398$ ). It was very low at the time of the abortion and it increases after 48 h of the expulsion of the abortus (*P* = 0.001). When the mean uric acid level of Group IA compared with Groups II and III it was much higher in the Group II, and highest in

**Table 1: Comparison of mean plasma FRAP value among study groups**

Groups	n	Mean FRAP value mM/L	Standard deviation	Group IA versus Group IB	Group IA versus Group II	Group IA versus Group III	Group II versus Group III
Group IA	42	0.32150	0.068824	t=25.97	t=22.48	t=21.88	t=5.29
Group IB	42	0.70648	0.067026	P=0.001	P=0.001	P=0.001	P=0.001
Group II	42	0.82881	0.128997				
Group III	42	1.02002	0.195045				

FRAP: Ferric reducing antioxidant power

**Table 2: Comparison of mean serum uric acid level among study groups**

Groups	n	Mean uric acid mg/dl	SD	Group IA versus Group IB	Group IA versus Group II	Group IA versus Group III	Group II versus Group III
Group IA	42	1.2771	0.26055	t=17.73	t=17.68	t=21.27	t=5.23
Group IB	42	2.5779	0.39764	P=0.001	P=0.001	P=0.001	P=0.001
Group II	42	3.0729	0.60460				
Group III	42	3.8431	0.73710				

SD: Standard deviation

**Table 3: Comparison of mean values of Group-IA versus Group-IB**

Group and means	FRAP mM/L	Uric acid mg/dl	Total protein gm/dl	Albumin gm/dl	Glucose mg/dl	Cholesterol mg/dl
Group IA Mean±SD	0.32±0.07	1.28±0.26	5.95±0.35	3.17±0.19	86.26±5.59	181.66±34.06
Group IB Mean±SD	0.71±0.07	2.57±0.40	6.40±0.44	3.62±0.18	83.55±5.54	181.19±32.96
P	0.001 HS	0.001 HS	0.001 HS	0.001 HS	0.03 S	0.03 S

P-value: HS: Highly significant, S: Significant, NS: Not significant, FRAP: Ferric reducing antioxidant power, SD: Standard deviation

**Table 4: Comparison of mean values of Group-IA versus Group-II**

Group and means	FRAP mM/L	Uric acid mg/dl	Total protein gm/dl	Albumin gm/dl	Glucose mg/dl	Cholesterol mg/dl
Group IA Mean±SD	0.32±0.07	1.28±0.26	5.95±0.35	3.17±0.19	86.26±5.59	181.66±34.06
Group II Mean±SD	0.82±0.12	3.07±0.60	6.45±0.47	3.47±0.20	87.02±4.78	158.88±24.031
P	0.001 HS	0.001 HS	0.001 HS	0.001 HS	0.50 NS	0.95 NS

FRAP: Ferric reducing antioxidant power

Group III and the differences were significant ( $P = 0.001$ ). Similarly, when the mean of Group II was compared with Group III it was higher in the Group III, and the difference was significant ( $P = 0.001$ ). This suggests that the women with SAb were initially having severe OS, which is reflected by a reduction in the level of FRAP and uric acid. On removal of the stress (expulsion of the abortus), the antioxidant status improves, as observed by the rise of FRAP and uric acid level after 48 h. Tables 1 and 2 show the mean values of FRAP and uric acid of the various groups. Tables 3-5 show the comparison of mean values of FRAP, uric acid, TP, albumin, and cholesterol in Groups IA, IB, II, and III. Demographic and clinical data of the subjects were shown in Table 6. There were no differences in mean age, gestational age, gravidity, and mean weight between patients with SAb and controls. As seen in Table 1, plasma TAO levels of patients with SAb was found significantly

lower than those of healthy pregnant women and healthy non-pregnant women of the same reproductive age group. OS was significantly higher in the patients with spontaneous inevitable abortion, which is reflected as a reduction in FRAP value ( $0.32 \pm 0.07$ ) mMFe (II)/L, the healthy pregnant women had lower values of FRAP ( $0.83 \pm 0.13$ ) mMFe (II)/L than non-pregnant women ( $1.02 \pm 0.19$ ) mMFe (II)/L. When FRAP value compared among normal pregnancy and abortion by Student's t-test, there was a significant difference in the mean ( $P < 0.01$ ) showing that there is an association of OS in SAb.

## DISCUSSION

OS is the imbalance among pro-oxidants and antioxidants it is been shown in several female reproductive pathologies.



**Table 5: Comparison of mean values of Group-II versus Group-III**

Group and means	FRAP mM/L	Uric acid mg/dl	Total protein gm/dl	Albumin gm/dl	Glucose mg/dl	Cholesterol mg/dl
Group II						
Mean±SD	0.82±0.12	3.07±0.60	6.45±0.47	3.47±0.20	87.02±4.78	158.88±24.031
Group III						
Mean±SD	1.02±0.19	3.84±0.73	6.20±0.40	3.43±0.52	87.11±7.19	161.26±35.04
P	0.001 HS	0.001 HS	0.01 S	0.63 NS	0.94 NS	0.71 NS

FRAP: Ferric reducing antioxidant power

**Table 6: Demographic and clinical variables**

Basis	Group I (n=42) pregnant women at the time of abortion	Group II (n=42) normal pregnant women	Group III (n=42) normal non-pregnant women
Mean maternal age (years)	26.5	27.09	26.76
Mean gestational age (weeks)	12.90	16.33	-
Primigravida	20	22	-
Multigravida	22	20	-
Mean weight (kg)	49.23	49.54	48.73

Various literatures have documented that significant rise in OS during pregnancy. Adequate uteroplacental circulation is one prime need for a successful pregnancy. Placenta-related disorders of pregnancy affect around a third of human pregnancies and primarily include miscarriage and pre-eclampsia. This study was up to look at the association among OS and spontaneous inevitable abortion. From the study, we found that the FRAP value, as an indicator of TAO status, as well as the uric acid value both are much decreased in the women with SAb initially. After the expulsion of abortus, the OS is removed and the antioxidant status was improved an increase shows that in the FRAP as well as that of uric acid. Increases in these two parameters are highly significant. As seen in Table 1, plasma TAO level of patients with SAb is much lower than that of healthy pregnant women and healthy non-pregnant women of the same reproductive age group. This suggests that OS plays an important role in spontaneous inevitable abortion. This finding correlates with the findings of studies conducted by Barrington *et al.*<sup>18</sup> The decrease in the uric acid levels at the time of abortion and its rise after 48 h suggests that the antioxidants are used at the time of OS. The TAO status as measured by FRAP also shows a similar pattern. This finding correlates with the studies of Jauniaux *et al.* 2000.<sup>19</sup> It is known that during pregnancy, there is a lot of stress on the women, both physical and emotional. Metabolically this reflects as OS and there is a need for an increase in need of antioxidants during pregnancy. TP and Albumin values found to increase in SAb cases after 48 h of expulsion. This increase is probably due to the removal of OS (The products of conception and the toxic metabolites associated). We did not observe any statistical difference in the values of the other parameters (glucose and cholesterol) among all the study groups.

## CONCLUSION

The antioxidant status of women with spontaneous inevitable abortion compared with healthy pregnant women and non-pregnant women. TAO status measured by FRAP in the plasma and the uric acid level were found much lower in women with spontaneous inevitable abortion than the normal pregnant women. After 48 h of abortion, both FRAP and uric acid level rose significantly. This suggests that the OS was to a certain extent; however, the values are still lower than that of normal pregnant as well as healthy non-pregnant women. The normal pregnant women also had a lower level of antioxidants than non-pregnant women. This suggests pregnancy itself is a stressful condition for the women. The role of OS is becoming increasingly important as there is new cumulative evidence suggesting that OS is involved in conditions such as abortions, preeclampsia, hydatidiform mole, fetal teratogenicity, preterm labor, and intrauterine growth retardation. OS is involved in causing an adverse effect on natural fertility, with a definite role in varied aspects of assisted conception. Optimizing various techniques in the ART laboratory may be an effective strategy to intercept OS in IVF/ICSI and IVM settings.

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