

# Serum Ischemia Modified Albumin Levels in Infertile Couples Underwent in Vitro Fertilization

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## ABSTRACT

**OBJECTIVE:** Ischemia-modified albumin (IMA) is a novel biomarker for diagnosis of distinct disease states including infectious diseases, coronary atherosclerotic disease, hypoxic fetal distress and diabetes mellitus. In vitro fertilization (IVF) is a medical process by which an egg is fertilized by sperm outside the body with variable success rates. It is important for the clinician to assess which women will get pregnant after IVF procedure. In this context we aimed to analyse the possible role of IMA in infertile couples underwent IVF.

**STUDY DESIGN:** The study group comprised 30 patients with IVF failure, 27 patients with a successful IVF cycle and 34 fertile controls. IVF failure is defined as IVF that does not result in a pregnancy. A rapid, spectrophotometric Albumin Cobalt Binding (ACB) test was used to determine serum IMA levels and results were presented by absorbance units (ABSU).

**RESULTS:** Serum IMA levels were 0.27 (0.07-0.58), 0.35(0.08-0.88), and 0.37(0.06-1.07) ABSU for patients with IVF cycle failure, successful IVF cycle and controls respectively. No statistically significant difference was observed between study participants.

**CONCLUSION:** Our results showed that ischemia modified albumin did not provide enough sensitivity or specificity to determine IVF success.

**Keywords:** In vitro fertilization, Infertility, Ischemia modified albumin  
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## Introduction

Current literature data demonstrates that serum albumin structure alters when ischemic conditions develops in body.<sup>1</sup> Although the exact mechanism of ischemia modified albumin (IMA) elevation is still not known, ischemia is the major arbiter of the final biochemical and histopathological findings. Ischemia induced oxygen deprivation in the affected body part induces a series of reactions involving the production of reactive oxygen species that leads a N-terminal structural change in albumin and finally produces IMA.<sup>2,3</sup> In this context IMA can be regard as a useful biomarker for evaluating patients with various disease conditions including ischemic events, in-

fectious diseases, type 2 diabetes mellitus, pulmonary embolism, chronic hepatitis c, and coronary bypass surgery.<sup>1,4-7</sup>

Oxidative stress is commonly associated in failed reproductive performance, including infertility, miscarriage, preeclampsia and diabetes-related congenital malformations.<sup>8</sup> It has been shown that pregnancies can normally develop in a relatively hypoxic intrauterine environment, and the subsequent reperfusion and oxidative stress is highly important for the development of physiological trophoblasts.<sup>9</sup> Previous studies reported an increased IMA levels in pre-eclamptic pregnancies, recurrent pregnancy losses and intrauterine growth restrictions.<sup>10,11</sup>

In vitro fertilization (IVF) is a medical process by which an egg is fertilized by sperm outside the body with a variable success rates. The success rate after treatment by IVF mainly depends on the characteristics of the couples being treated.<sup>12</sup> Success rates with IVF continues to increase depending on improved optimal culture conditions for gametes and embryos.<sup>13</sup> From this point of view it is not surprising to see growing number of studies focusing on a new marker for estimating which patient will develop a healthy pregnancy after an IVF cycle. Currently, no information exists on serum IMA levels and its relation with success of in-vitro fertilization (IVF) cycle in infertile women. This study is performed in order to answer the question that if IMA levels can help in predicting pregnancy after IVF.

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## Material and Method

### Patients

This study was performed in 57 infertile patients admitted to Dr. Zekai Tahir Burak Women's Health Training and Research Hospital infertility center. The control group consisted of 34 healthy fertile women. Patients undergoing controlled ovarian stimulation for IVF were invited with informed consents to enroll in the study after taking approval from the Institutional ethics committee. Etiology of infertility of study population was as follows; male factors (47.5%), unexplained (35.1%), tubal factors (8.7%), and others (8.7%). Ovarian stimulation was achieved with an initial gonadotropin dose that was individualized according to age of the patients, baseline serum FSH levels at third day, previous response to ovarian stimulation and body mass index. In first 3 days the starting regimen was fixed with a daily dose of 100-225 IU rec FSH (individualized for each patient). The gonadotropin dose following the first 3 days was adjusted according to patients ovarian response. Transvaginal ultrasonography with serum E2 were routinely measured every 2 or 3 days. Ovulation was triggered by administration of 250 µgr rec HCG when at least two follicles reached about a diameter of 18 mm. Thirty six hours later after HCG injection oocytes were retrieved. Intracytoplasmic sperm injection was done regardless of infertility origin. Embryo transfer was done on day 3 or 5.

In vitro fertilization failure is defined as IVF that does not result in a pregnancy. The control subjects, who had no clinical evidence of a major disease, were recruited from fertile women that underwent routine medical check-up. The exclusion criteria included; history of systemic diseases, ischemic events, diabetes mellitus, secondary inflammatory conditions, electrocardiogram abnormalities at the time of admission, hepatic, renal and cardiac failure.

### Biochemical analyses

Blood samples were drawn from infertile patients and healthy controls and dispensed into venipuncture in a fasting state. After obtaining plain tubes contained separation gels, the samples was allowed to clot for 30 minutes and centrifuged at 3000 rpm for 10 minutes before separating the serum. The samples stored at 20 C for a maximum of six

weeks before measuring IMA. The serum ischemia modified albumin levels were measured using a commercially available kit. Albumin cobalt binding test was analyzed according to the method defined by Bar-Or et al.<sup>14</sup>

### Statistical analyses

Statistical Package for Social Sciences (SPSS) for Windows 15.0 programme was used for statistical analysis. All data were entered into a database and were verified by a second independent person. The variables were investigated using visual (histograms, probability plots) and analytical methods to determine whether or not they are normally distributed. Data are presented as mean and  $\pm$ S.D. for normally distributed variables and as median, (min-max) for skew distributed continuous variables. Categorical variables are shown as frequencies. One-Way ANOVA for normally distributed variables and Kruskal-Wallis test for not normally distributed variables were used to compare variable in a three groups. Student's T-test for normally distributed variables and Mann-Whitney U test for not normally distributed variables were used to compare variable in a two groups. Two-sided values of  $p < 0.05$  were considered as statistically significant.

## Results

The baseline clinical characteristics of the subjects are shown in Table 1. A total of 57 infertile patients and 34 healthy controls included in the study. Mean age of study participants did not differ between study groups. Median IMA levels of infertile patients (n:57) and controls (n:34) was found to be 0.32 (0.07-0.88) and 0.37 (0.06-1.07) ABSU ( $p=0.259$ ). According to IVF results, infertile patients further divided into 2 groups. Thirty patients was evaluated as IVF failure and 27 patients were evaluated as having successful IVF cycle. Serum IMA levels were 0.27 (0.07-0.58), 0.35 (0.08-0.88), and 0.37(0.06-1.07) ABSU for patients with IVF cycle failure, successful IVF cycle and controls respectively (Figure 1). No statistically difference was observed between study groups according to IVF success. According to infertility etiologies the infertile patients further analysed for IMA levels (Table 2). No significant association was observed between infertility etiologies and serum IMA levels ( $p>0.005$ ).

Table 1: Clinical characteristics and laboratory values of study participants

	IVF Failure Group (n:30)	Successful IVF Group (n:27)	Controls (n:34)	p
Age (years)	29.73 $\pm$ 4.79	30.02 $\pm$ 5.32	33.08 $\pm$ 8.03	0.080
Body mass index (kg/m <sup>2</sup> )	25.37 $\pm$ 4.43	25.05 $\pm$ 4.28	25.67 $\pm$ 4.10	0.858
Duration of infertility (years)	7.98 $\pm$ 4.08	6.12 $\pm$ 3.67	-	0.084
FSH (mIU/ml)	6.31 $\pm$ 2.04	6.60 $\pm$ 1.57	-	0.554
LH (IU/l)	6.32 $\pm$ 5.44	6.01 $\pm$ 3.09	-	0.804
E2 (pg/ml)	45.18 $\pm$ 20.96	55.06 $\pm$ 30.06	-	0.155
IMA (ABSU)	0.27(0.07-0.58)	0.35(0.08-0.88)	0.37(0.06-1.07)	0.106

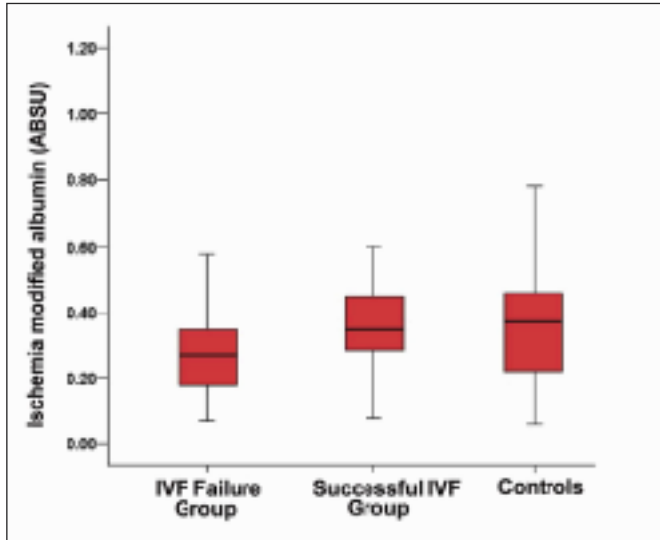


Figure 1: Box plot presentation of serum IMA levels of study participants

Table 2: Serum IMA levels of infertile patients according to infertility etiologies

	IVF Failure Group (n:30)	Successful IVF Group (n:27)	p
Unexplained	0.29 (0.10-0.54)	0.45 (0.15-0.79)	0.104
Male factors	0.26 (0.10-0.58)	0.34 (0.08-0.80)	0.466
Tubal factors	0.29 (0.26-0.32)	0.41 (0.35-0.50)	0.083
Other *	0.17 (0.07-0.28)	0.28 (0.20-0.45)	0.374

\*Endometriosis, pelvic adhesions etc.

## Discussion

In the present study we investigated the relationship between serum IMA levels of infertile women and IVF success. Serum IMA, which has been shown to be a valuable diagnostic and prognostic marker for various clinical situations especially in patients with ongoing ischemic events, lacks in estimating IVF success in infertile women. Moreover despite the findings that proposes ischemic events in infertility etiology, no significant association was demonstrated between infertile women and healthy controls.

The role of IMA in various clinical diseases has been a subject of intense research and controversy. Kanko et al.<sup>1</sup> in their studies on a group of patients that underwent coronary by-pass surgery due to ischemic heart disease within a specific period of time concluded that IMA is an early-rising marker of cardiac ischemia and enables providing a direction for the treatment at early phases of ischemic heart diseases. Moreover Zuwała-Jagiełło et al.<sup>4</sup> found a significant correlation between IMA and inflammation in chronic hepatitis C patients with diabetes. Contrary to this findings, in a case-control study by Ersoy et al.,<sup>15</sup> it was reported that serum IMA levels did not differ among patients with overt or subclinical hypothyroidism. IMA is produced during myocardial ischemia as a result of hy-

poxia, free radical injury, acidosis, ischemic conditions and energy dependent membrane disruption that modify the N-terminal end of albumin.<sup>16,17</sup> The definitive and exact pathophysiologic mechanism for IMA production is still unknown, direct and indirect evidence supports that it has to be related to the generation of radical oxygen species (ROS) that modifies metal binding domains of albumin.<sup>6</sup> Based on this evidence IMA can be regarded as non-specific and is elevated in subjects who undergo oxidative stress due to various clinical conditions.

In vitro fertilization treatment has led to a successful live pregnancies in this group of patients. Although IVF success rates have improved notably over the last ten years, the success rates are still under 35% in women aged less than 30 years, and under 10% in women aged between 40-44.<sup>18</sup> This low pregnancy rates with high costs of IVF cycles have emerged clinicians to search for factors that can predict IVF success before implying the procedure. In this context, we aimed to determine whether serum IMA levels could predict IVF success. Our hypothesis was that ischemia related oxidative stress leading IMA elevation might effect IVF success rates. Although difficult to explain, this lack of association may be associated with relatively low grades of ischemia in infertile women.

Similar to other organ systems, oxidative stress plays a crucial role in the functioning of female reproductive system. Moreover oxidative stress has an important role on many characteristics in infertile couples, including follicular fluid, embryo culture medium, endometrial implementation phase, and the continuity of the corpus luteum.<sup>17,19</sup> It is clearly demonstrated that reactive oxygen species, which are markers of oxidative stress, in follicular fluid, are important factors in the development and quality of embryos.<sup>20,21</sup> In a study by Prefumo et al.<sup>22</sup> it was shown that these supraphysiologically elevated serum IMA levels were related to the hypoxic intrauterine environment. Despite the increased risk of oxidative stress and ischemia in infertile couples, IMA concentrations in serum of infertile women were not found to be higher than controls.

In conclusion, serum IMA levels of infertile women did not differ from healthy controls. Although our study must not be used alone for to achieve a definitive outcome, further studies are therefore warranted to reveal the role of IMA in infertile couples especially in the era of IVF treatment.

## In Vitro Fertilizasyon İşlemi Uygulanan İnfertil Çiftlerde Serum İskemi Modifiye Albümin Seviyeleri

**AMAÇ:** İskemi modifiye albümin (IMA) enfeksiyon hastalıkları, koroner arter hastalıkları, hipoksik fetal stres ve diabetes mellitus gibi değişik hastalık durumlarında tanı amacıyla kullanılan yeni bir belirteçtir. In vitro fertilizasyon (IVF) ise vücut dışında

bir yumurta ile spermin döllmesi ile sağlanan ve değişik başarı oranlarına sahip bir tıbbi işlemdir. Bir klinisyen için IVF sonrası hangi gebeliğin başarı ile sonuçlanacağını önceden tahmin edebilmek önemlidir. Biz bu çalışmamızda IMA'nın IVF uygulanan hastalarda muhtemel rolünü incelemeyi amaçladık.

**GEREÇ VE YÖNTEM:** Çalışma grubumuz IVF başarısızlığı olarak değerlendirilen 30 hasta ile 27 başarılı IVF siklusu ve 34 doğurgan kontrol grubunu içermekteydi. IVF başarısızlığı gebelik ile sonlanmayan IVF uygulaması olarak kabul edildi. Spektrofotometrik albümin kobalt bağlanma testi serum IMA seviyelerini hesaplamak için kullanıldı ve sonuçlar absorbans ünitesi (ABSU) şeklinde sunuldu.

**BULGULAR:** Serum IMA seviyeleri IVF başarısızlığı grubunda, başarılı IVF siklus ve kontrollerde sırasıyla 0.27 (0.07-0.58), 0.35(0.08-0.88) ve 0.37(0.06-1.07) ABSU idi. Çalışma grupları arasında belirgin farklılık gözlenmedi.

**SONUÇ:** Çalışma sonucumuzda iskemi modifiye albüminin IVF başarısını belirlemede yeterli sensitivite ve spesiviteye sahip olmadığı saptanmıştır.

**Anahtar Kelimeler:** In vitro fertilizasyon, İnfertilite, İskemi modifiye albümin

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