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Comparison of Amniotic Fluid Oxidative Stress Index in IVF/ICSI Pregnancies and Spontaneous Natural Conception

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ABSTRACT

Objective: *To compare amniotic fluid total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) values of spontaneous pregnancies and IVF/ICSI pregnancies.*

Materials and Methods*: A total of 50 patients who became pregnant spontaneously or by IVF/ICSI were included in the study. Twenty of the fifty participants consisted of patients who became pregnant by IVF/ICSI (n=20). The remaining 30 patients consisted of patients who became pregnant spontaneously (n= 30). Following the classical lower segment incision during C/S delivery, amniotic fluid sample was taken with a 5 cc syringe before the fetal membranes were cut. TAS and TOS levels were studied in an autoanalyzer by using TAS or TOS kits. OSI value was obtained by dividing TOS by TAS (TOS/TAS=OSI). OSI results are presented as percentages.*

Results: *The amniotic fluid TOS levels of the patients in the ICSI group were found to be significantly higher than the pregnant women in the control group (23.11 ± 4.55 vs. 20.30 ± 6.05, p<0.01). On the other hand, amniotic fluid TAS levels of the pregnant women in the ICSI group were found to be significantly lower than the patients with spontaneous pregnancy (0.84 ± 0.10 vs. 0.96 ± 0.32, p<0.03). The OSI values (TOS/TAS) of the patients who became pregnant with ICSI were found to be significantly higher than the patients with spontaneous pregnancy (27.51 ± 6.33 vs. 21.1 ± 8.20, p<0.02). There was no significant correlation between OSI values and obstetric and neonatal parameters. There was no significant correlation between OSI and age, gravidity, parity, birth weight and gestational age at birth.*

Conclusions: *The amniotic fluid oxidant/antioxidant balance of IVF/ICSI pregnancies was pathologically increased compared to natural conceptions. This increase in oxidative stress may negatively affect the long-term well-being of ICSI babies.*

Keywords

IVF/ICSI, TAS, TOS, OSI, Fetal well being.

Introduction

Thanks to the rapid developments in ART technology in the last four decades, it has led to remarkable increases in both pregnancy rates and healthy birth rates. However, from the first day, it has always been questioned whether there is a difference in the antenatal, neonatal and childhood development parameters of IVF/ICSI babies compared to spontaneous natural pregnancies. In terms of fetal growth kinetics, it was stated that the second trimester growth parameters of ICSI pregnancies differ from those of natural conceptions, although it varies depending on the protocol applied [1]. Although no significant changes were observed in the antenatal and early neonatal periods, it has been reported that the stages of trophoblastic invasion and placentation differ in ICSI babies [1]. These changes may cause long-term health problems in fetal growth and development of ICSI babies

[2-4]. Gestational diabetes mellitus, preterm premature rupture of membranes, preterm birth and placentation anomalies are among the most frequently encountered problems in the antenatal period [5]. However, the biggest problem here is the question of whether the feto-maternal pathologies that occur in ICSI pregnancies are due to the technology applied or to keep the indications too broad when making the ICSI decision [3,4].

ART technique used in sperm retrieval, pre-ICSI induction protocol, chemical agents used in the laboratory, embryo-sperm manipulations, diagnostic embryo biopsies, hatching methods, some physical processes during egg collection may cause short and long-term stress pathologies in embryo development [2-4]. Oxidative stress caused by increased reactive oxygen species (ROS) production during ICSI procedure and failed activation of antioxidant defense system plays a critical role in embryo implantation and grwoth [6,7]. Antioxidant defense systems originating from Thiol or sulphydryl (–SH) are involved in the neutralization of the harmful effects of ROS during embryognesis [8,9]. Measuring oxidative stress markers one by one in biological compartments such as blood, amnion, and cerebrospinal fluid is not only technically difficult, but also not cost effective. For this reason, in order to evaluate the oxidant/antioxidant balance in routine clinical practice, information about oxidative stress is obtained by measuring total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (TAS/TAS=OSI) [8,9]. The oxidant/antioxidant balance in the amniotic fluid of /ICSI infants has not been evaluated to date. This study was designed to compare the amniotic fluid TAS, TOS and OSI values of patients with a singleton pregnancy who conceived with ICSI and reached the term with natural pregnancies.

Materials and Methods

A total of 50 patients who became pregnant spontaneously or by IVF/ICSI were included in the study. Twenty of the fifty participants consisted of patients who became pregnant by IVF/ICSI (n=20). The remaining 30 patients consisted of patients who became pregnant spontaneously (n= 30). IVF/ICSI indications were tubal, ovulatory, or male factor, or combinations of all three. GnRH antagonist protocol was applied to IVFICSI patients. Recombinant follicle stimulating hormone was started as the initial dose on the third day of the menstrual cycle. Vaginal ultrasonography was used for monitoring follicle development.GnRH antagonist was added daily when the leading follicle reached a diameter of 14 mm. When the mean diameter of three leading follicles reached 17 mm a single dose of recombinant hCG was used to trigger ovulation. The oocyte pick-up was carried out at a minimum of 35 hours after hCG administration. A fifth day embryo transfer was performed for each patient. The gestational week was determined according to the last menstrual period or according to the registered ultrasonography examination performed in the first 14 weeks of pregnancy.

Inclusion criteria for the study; She conceived spontaneously or by IVF/ICSI, had a single pregnancy, did not give birth before, planned her current birth as C/S, her gestational age was compatible with term, first trimester screening test was normal, cordocentesis and/or amniocentesis was not performed, no placentation anomaly, and Patients without a history of labor induction with PGE1. Those who had preterm birth or ruptured membranes, had a history of antenatal bleeding, had problems in at least one of the first or second trimester screening tests, and had meconium in the amniotic fluid were excluded from the study. Those who had SARS-COV-2 during pregnancy or were vaccinated two weeks before the cesarean section were not included in the study. Those with a history of pregnancy-induced hypertension or preeclampsia, diagnosed with gestational diabetes or taken to C/S due to antenatal bleeding were excluded from the study. Those with acute or systemic inflammatory disease or those using anti-inflammatory drugs were not included in the study. Following the classical lower segment incision during C/S delivery, amniotic fluid sample was taken with a 5 cc syringe before the fetal membranes were cut. Subsequently, the fetus and its appendages were removed. Patients with amniotic fluid contaminated with blood or meconium were not included in the evaluation. The Local Ethics Committee approved this study (Date: 6/2021-Decision No: 132). All procedures conducted in studies, including human participants, conformed to the national or institutional research committee's ethical standards and the 1964 Helsinki Declaration and its later amendments or other ethical standards.

TOS, TAS and OSI (TOS/TAS) Analysis

The centrifugation of amniotic fluid samples was carried out at 3500 rpm for 5 minutes, and aliquoted amniotic fluid samples were stored at -20°C until analyses. TAS and TOS levels were studied in an autoanalyzer by using TAS or TOS kits (Rel Assay, Mega Medicine Industry, Gaziantep, Turkey). While TAS results were presented as mmol Trolox Equivalent/L the TOS results were presented as μmol H_2O_2 Equivalent/L. OSI value was obtained by dividing TOS data by TAS data (TOS/TAS=OSI). OSI results are presented as percentages [9].

Statistical Analysis

All analyses were carried out using SPSS for Windows version 21 (SPSS Inc., Chicago, IL, USA). Normality of distribution was assessed with the Kolmogorov-Smirnov test. The independent samples t-test was used to assess inter-group differences. The Mann-Whitney U test was used for inter-group differences, and the Wilcoxon signed-rank test was used for intra-group comparisons. The correlations between quantitative data were assessed by calculating Spearman's correlation coefficient. Data were presented as with mean and standard deviation (SD) and *P* value <0.05 was accepted as a significant.

Results

Maternal age, fetal weight, birth weeks, parity and gravida were found to be similar in IVF/ICSI and spontaneous pregnancy groups. The amniotic fluid TOS levels of the patients in the ICSI group were found to be significantly higher than the pregnant women in the control group (23.11 ± 4.55 vs. 20.30 ± 6.05, p<0.01). On

the other hand, amniotic fluid TAS levels of the pregnant women in the ICSI group were found to be significantly lower than the patients with spontaneous pregnancy (0.84 ± 0.10 vs. 0.96 ± 0.32, p<0.03). The OSI values (TOS/TAS) of the patients who became pregnant with ICSI were found to be significantly higher than the patients with spontaneous pregnancy (27.51 ± 6.33 vs. 21.1 ± 8.20, p<0.02). There was no significant correlation between OSI values and obstetric and neonatal parameters. There was no significant correlation between OSI and age, gravidity, parity, birth weight and week of birth.

Table 1: Demographic parameters, TAS, TOS, and OSI values of IVF/ICSI and spontaneous pregnancy groups.

	IVF/ICSI (n=20)	Spontaneus pregnancy (n=30)	*p* value
Infertility duration (years)	2.65 ± 0.44	0	*NA*
IVF protocol	Antagonist	0	*NA*
Embryo transfer	Single ET	0	*NA*
Age (years)	25.3 ± 6.22	24.7 ± 3.10	0.54
Gestational age	37.1 ± 4.50	36.7 ± 6.10	0.41
Mode of delivery	C/S	C/S	NA
Fetal weight (gr)	2659.6 ± 221.2	2790.6 ± 133.5	0.20
TAS (mmol Trolox Equivalent/L)	0.84 ± 0.10	0.96 ± 0.32	0.03
TOS (μmol H_2O_2 Equivalent/L)	23.11 ± 4.55	20.30 ± 6.05	0.01
OSI (TOS/TAS) (arbitrary unit)	27.51 ± 6.33	21.1 ± 8.20	0.02

Discussion

Data on whether oxidative stress increases in IVF/ICSI pregnancies are limited. In order for all living things to continue their vital functions at the cell level, oxidant/antioxidant balance must be provided. Fetal defense mechanisms and immune-modulatory effect alone are not sufficient to provide oxidant/antioxidant balance. Both the maternal compartment and the placento-vascular bed support the fetus to maintain fetal well-being and protect fetal cells from the destructive effects of oxidative stress. Maintaining the oxidant/antioxidant balance during pregnancy allows fetal development to continue until term and a healthy baby is born. Disruption in oxidant/antioxidant balance may lead to early pregnancy loss, fetal growth retardation, premature membrane rupture or preterm delivery. In addition, unbalanced fetal oxidative stress can lead to intrauterine death of the fetus. Babies who reach term under oxidative stress may suffer from some metabolic and inflammatory diseases in newborn and adulthood [10,11].

Since physical and chemical laboratory processes applied to oocyte and sperm during ICSI may cause oxidative stress in embryos, oxidative stress may continue in pregnancies after embryo transfer [12-14]. To test this hypothesis, we evaluated the change in oxidant/antioxidant balance in amniotic fluid samples taken during cesarean delivery from patients who became pregnant with IVF/ICSI and reached term, by means of TOS and TAS. TOS is an indicator of the total effect of all oxidants in biological fluids, including the amnion. TAS shows the total effect of antioxidants in all biological compartments. The ratio of TOS/TAS to each other is defined as the oxidative stress index (OSI), which is a quantitative but relative value of the oxidant/antioxidant balance in that biological fluid [8,9]. The reason why we call it relative is that there may be differences between the measurements as the OSI can be subject to instantaneous change. In this study, amniotic fluid OSI indexes of term pregnancies formed by IVF/ICSI were evaluated. Spontaneous pregnancies were taken as the control group. When compared with the amniotic fluid OSI values of the spontaneously conceived patients, the OSI values of the IVF/ICSI group were found to be significantly higher. While TOS values increased significantly in IVF/ICSI group compared to patients with spontaneous pregnancy, a significant decrease was found in TAS values. Since the OSI value was TOS/TAS, the OSI ratio in IVF/ICSI patients showed a significant increase compared to controls.

We can briefly summarize the possible reasons for the decrease in TAS while TOS increases in the amniotic fluids of patients who became pregnant with IVF/ICSI. rFSH or clomiphene citrate preparations administered to patients before ICSI may increase OSI rates by causing rapid follicle development [15]. Similarly, metal needles entering the follicle during oocyte collection, pressurized aspiration of intrafollicular fluid, flushing, and puncture of the oocyte membrane with an ICSI needle in the laboratory may cause oxidative stress. Sperm retrieval methods, mediums used for sperm and oocyte development, PVP, and methods such as assisted hatching are also oxidative stress stimuli [16-19]. Because of all these reasons, it is a logical result that the OSI values of IVF/ICSI pregnancies are higher than spontaneous pregnancies. However, the question that needs to be answered here is how the fetuses reach term in a healthy way despite the increase in OSI. Possibly, the fetus and its appendages tolerate oxidative stress until term with the support of the maternal compartment. High OSI indicates that the fetus is not troubled in terms of development. The similarity of fetal birth weights and APGAR scores suggests that fetal prognosis is good in the early neonatal period. However, these babies should be kept under close follow-up in terms of the development of a disease related to the increase in OSI in the later developmental stages and childhood [2].

Rererences

1. Ginod P, Choux C, Barberet J, et al. Singleton fetal growth kinetics depend on the mode of conception. Fertil Steril. 2018; 110: 1109-1117.e2.
2. Gungor K, Dokuzeylul Gungor N. Antithyroid antibodies may predict serum beta HCG levels and biochemical pregnancy losses in euthyroid women with IVF single embryo transfer. Gynecol Endocrinol. 2021; 37: 702-705.
3. Dokuzeylül Güngör N, Güngör K, Kavrut M, et al. A retrospective series of homologous intracytoplasmic sperm injection cycle results of 99 women with mosaic Turner Syndrome. Clin Exp Obstet Gynecol. 2021; 48: 942-948.
4. Jin L, Li Z, Gu L, et al. Neonatal outcome of children born after ICSI with epididymal or testicular sperm: A 10-year study in China. Sci Rep. 2020; 10: 5145.

5. Jiang F, Gao J, He J, et al. Obstetric outcomes for twins from different conception methods - A multicenter cross-sectional study from China. Acta Obstet Gynecol Scand. 2021; 100: 1061-1067.
6. Celik O, Celik N, Ugur K, et al. Nppc/Npr2/cGMP signaling cascade maintains oocyte developmental capacity. Cell Mol Biol (Noisy-le-grand). 2019; 65: 83-89.
7. Celik O, Celik N, Gungor S, et al. Selective Regulation of Oocyte Meiotic Events Enhances Progress in Fertility Preservation Methods. Biochem Insights. 2015; 8: 11-21.
8. Cakirca G, Nas C, Yilmaz K, et al. Evaluation of dynamic thiol-disulfide balance in children with stage 3–5 chronic kidney disease. Ann Med Res. 2019; 26: 1565-1568.
9. Erel O. A new automated colorimetric method for measuring total oxidant status. Clini Biochem. 2005; 38: 1103-1111.
10. Celik N, Aydin S, Ugur K, et al. Patatin-like phospholipase domain containing 3-gene (adiponutrin), preptin, kisspeptin and amylin regulates oocyte developmental capacity in PCOS. Cell Mol Biol (Noisy-le-grand). 2018; 64: 7-12.
11. Hussain T, Murtaza G, Metwally E, et al. The Role of Oxidative Stress and Antioxidant Balance in Pregnancy. Mediators Inflamm. 2021.
12. Duhig K, Chappell LC, Shennan AH. Oxidative stress in pregnancy and reproduction. Obstet Med. 2016; 9: 113-116.
13. Sultana Z, Maiti K, Aitken J, et al. Oxidative stress, placental ageing-related pathologies and adverse pregnancy outcomes. Am J Reprod Immunol. 2017; 77.
14. Celik O, Celik E, Yilmaz E, et al. Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin-releasing hormone agonist and antagonists, on follicular fluid stem cell factor and serum urocortin 1 levels on the day of oocyte retrieval. Arch Gynecol Obstet. 2013; 288: 1417-1422.
15. Dokuzeylül Güngör N, Güngör K. Ovarian stimulation drugs alter the metabolite content of the growing follicle: in vivo spectroscopic evaluation of follicle fluid. J Turk Ger Gynecol Assoc. 2021; 22: 132-138.
16. Güngör K, Güngör ND, Başar MM, et al. Relationship between serum vitamin D levels semen parameters and sperm DNA damage in men with unexplained infertility. Eur Rev Med Pharmacol Sci. 2022; 26: 499-505.
17. Dokuzeylül Güngör N, Güngör K, Yurci A, et al. Ovarian drilling down-regulates endometrial nuclear factor-κB p65 expression in women with PCOS: A prospective case-control study. Turk J Obstet Gynecol. 2022; 19: 45-50.
18. Çetin C, Güngör ND, Yavuz M. First trimester glycosylated hemoglobin for gestational diabetes mellitus screening. Taiwan J Obstet Gynecol. 2021; 60: 899-902.
19. Yurci A, Dokuzeylul Gungor N, Gurbuz T. Spectroscopy analysis of endometrial metabolites is a powerful predictor of success of embryo transfer in women with implantation failure: a preliminary study. Gynecol Endocrinol. 2021; 37: 415-421.