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Perinatal Outcomes of Babies Born with Fresh or Frozen-Thawed Testicular Sperm in Patients with Azoospermia

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ABSTRACT

Aim: *To compare the perinatal outcomes of pregnancies obtained with fresh or frozen-thawed sperm in patients who underwent surgical sperm extraction for the diagnosis of azoospermia.*

Materials and Methods: *In this retrospective study, data were collected on couples who conceived following Intracytoplasmic Sperm Injection using surgically retrieved fresh or frozen-thawed sperm. Participants were divided into two equal groups as follows. Group 1 (n = 100) consisted of patients who underwent ICSI and subsequent embryo transfer using fresh testicular sperm and Group 2 (n = 100) consisted of patients who underwent ICSI by using frozen-thawed testicular sperm. Perinatal outcome was compared according to the use of fresh or frozen-thawed sperm. Primary outcome measures included clinical pregancy, miscarriage, live birth, congenital abnormality, birthweight, gestational age at delivery, stillbirth and neonatal death.*

Results: *Live birth and clinical pregnancy rates were found to be significantly higher in patients who underwent ICSI/ET with frozen-thawed testicular sperm compared to fresh sperm group. The miscarriage rates were significantly lower in the frozen-thawed sperm group compared to the fresh testicular sperm group. Clinical pregnancy was detected in 18 cases, while no pregnancy was detected in 82 cases undergoing ICSI with fresh sperm. In the group where ICSI/ET was applied with frozen sperm, clinical pregnancy was detected in 51 cases, whereas pregnancy was not detected in 49 cases. In the frozen sperm group, in addition to C/S and multiple pregnancy rates, the number of babies with a birth weight below 2500 g was significantly higher than in the fresh sperm group. There was no significant difference between the groups in terms of minor and major congenital anomalies, birth weight, gestational age at delivery, stillbirth and neonatal death.*

Conclusion: *Using fresh or frozen testicular sperm does not have a significant effect on perintal outcome in patients with azoospermia.*

Keywords

Fresh sperm, Frozen sperm, ICSI, Azoospermia, Perinatal outcome, Fertility outcome.

Introduction

Azopermia, which is seen with a frequency of 10-20% in the infertile population and characterized by lack of sperm in the ejaculate, is a serious cause of male infertility that can be treated using testicular sperm [1]. It is divided into two subgroups as obstructive (OA) and non-obstructive (NOA). Since there is no sperm in the ejaculate, the possibility of finding sperm thanks to different surgical interventions such as microdissection testicular sperm extraction (micro-TESE) has given these couples the opportunity to have children from their own genetic structures [1-4]. However, despite repeated mTESE, the sperm retrieval rate does not exceed 50%. For this reason, it is vital that the remaining sperm in mTESE be frozen after they are used for ICSI.

Surgically obtained sperms in NOA cases are unhealthy in terms of chromosomal content and organization compared to testicular sperm of OA cases and ejaculate sperm of healthy individuals [1-4]. Since there is increasing evidence that in men with NOA spermatozoa have an increased chromosomal aneuploidy rate [5,6], follow-up of the perinatal and maternal outcome after the use of testicular sperm from NOA patients is crtical. It has been reported that the use of frozen-thawed testicular sperm or fresh testicular sperm did not have a significant effect on fertilization rate, implantation rate, and clinical pregnancy rate [1]. On the other hand, there are no clinical studies investigating the effect of using fresh or frozen-thawed testicular sperm on maternal morbidity and perinatal outcome in NOA cases. The aim of this study is to analyse the maternal and pertinatal outcome outcome of children born after ICSI with fresh or frozen-thawed testicular sperm in patients with non-obstructive azoospermia.

Materials and Methods

In this retrospective study, data were collected on couples who conceived following Intracytoplasmic Sperm Injection using surgically retrieved fresh or frozen-thawed sperm from 2017 to 2020. Two hundred participants were divided into two equal groups as follows. Group 1 (n = 100) consisted of patients who underwent ICSI and subsequent embryo transfer using fresh testicular sperm and Group 2 (n = 100) consisted of patients who underwent ICSI by using frozen-thawed testicular sperm. We extracted all data of the study from electronic medical record system. Two semen analysis was performed in the male partners at least 3 weeks apart and upon 3 to 7 days of abstinence. Azoospermia was defined as the absence of sperm cells in the seminal fluid. The main criteria for inclusion in the study were presence of sperm in mTESE. Participants with karyotype anomalies were not included in the study. Similarly, cases with no sperm in TESE were excluded from the study. The total number of patients meeting these criteria was determined to be 200. All patients with a diagnosis of azoospermia who underwent mTESE and had sperm were included in the study. While 95 of 200 azoospermia cases were obstructive azoospermia, the remaining 105 cases were diagnosed as non onbstructive azoospermia. Standard antagonist protocol was applied to both groups of participants. Controlled ovarian stimulation was performed with gonadotropin dose determined according to the patients' age, clinical findings and BMI evaluation.

Outcome measures included clinical pregancy, miscarriage, live birth, birth weight, gestational age at delivery, stillbirth and neonatal death. Maternal events such as preeclampsia, eclampsia, gestational diabetes, and cesarean delivery were also recorded. Preterm delivery, minor and major birth defects, fetal growth restriction, and the need for intensive care unit and the gender of newborn babies were also recorded from database. Perinatal outcome was compared according to the use of fresh or frozen-thawed sperm. The definitions of the primary outcome measures we evaluated in the study are as follows; Clinical pregnancy rate defined as evidence of a gestational sac, confirmed by ultrasound examination. Live birth rate defined as delivery of a live fetus after 24 completed weeks of gestational age. Miscarriage loss of a pregnancy before 24 weeks' gestation and congenital abnormalities defined as a physical defect present at birth. Stillbirth defined as a baby born with no signs of life at or beyond 24 weeks' gestation and neonatal death defined as death of the baby in the first 28 days of life. Preterm birth was defined as birth at a gestational age <32 weeks, and fetal growth restriction was defined as a birth weight under the third percentile. This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the local Ethics Committee. Verbal informed consent was obtained from all participants at the time of enrollment.

Statistical analysis

The sample size was calculated with the GPower 3.1 (http://www.gpower.hhu.de/) program. The total mean of two groups compared based on the Mann-Whitney test with the effect size of 40%, power of 90% and 0.05 type 1 error, was found to be at least 93 patients. The results of Kolmogorov test show that not all quantitative variables have a normal distribution. Mann-Whitney test is used to examine the relationship between quantitative variables in the two Groups. Chi-square test and Fisher's exact test are used to examine qualitative variables. p-value ≤ 0.05 was considered significant. Statistical Package for Social Sciences version 26.0 (SPSS Inc., Chicago, IL, USA) was used to perform data analysis.

Results

The mean age of the participants in fresh and frozen sperm group was 27.4 (± 2.3) and 28.5 (± 3.2) respectively (Table 1). The mean age of fresh sperm group was significantly lower than the frozen-thawed sperm group (p<.01). While the mean infertility duration of fresh sperm group was 5.9 it was 6.7 in frozen-thawed sperm group. The mean infertility duration of fresh sperm group was significantly lower than the frozen-thawed sperm group (p<.02). The mean BMI of each group was found similar 26.1 vs 26.5. The total oocyte, M II oocyte and 2 PN embryo counts of the patients in the fresh sperm group were found to be significantly higher than the frozen sperm group (p <.001, p<.02, and p<.001).

As shown in Table 2, in the group that underwent ICSI/ET with fresh sperm, clinical pregnancy was detected in 18 cases (18%), while no pregnancy was detected in 82 cases (80 %). In the group where ICSI/ET was applied with frozen sperm, clinical pregnancy was detected in 51 cases (51%), whereas pregnancy was not detected in 49 cases (49%). Clinical pregnancy rates were found to be significantly higher in patients who underwent ICSI/ET with frozen sperm compared to fresh sperm group (51% vs. 18%, p<.001). Similarly, live birth rates were found to be significantly higher in the frozen-thawed sperm group compared to the fresh sperm group (2% vs. 46%, p<.002). Miscarriage rates were found to be significantly higher in patients who underwent ICSI/ET with fresh sperm compared to frozen-thawed sperm group (13% vs. 8%, p<.02). In the frozen sperm group, in addition to C/S and multiple pregnancy rates, the number of babies with a birth weight below 2500 g was significantly higher than in the fresh sperm group. While stillbirth was detected in one case and neonatal death in one

Table 1: Demographic and laboratory parameters of fresh and frozen-thawed sperm groups.

Variable	Groups		z	P-value
	ICSI with fresh sperm (n=100)	ICSI with frozen sperm (n=100)		
Age (yrs)	27.4 (2.3)	28.5 (3.2)	-1,32	0.01
Infertility duration (yrs)	5.9 (2.1)	6.7 (1.6)	-3.50	0.02
BMI (kg/m^2)	26.1 (2.5)	26.5 (1.6)	-0.03	0.7
Day 2 estradiol	41.2 (50.3)	40.4 (1.54)	-0.07	0.4
Day 2 progesterone	0.1 (0.3)	0.4 (0.2)	-0.01	0.4
Total rFSH dose	2204.4 (654.3)	2390.3 (780.2)	-1.13	0.03
The number of total oocyte	21.3 (5.7)	16.3 (1.0)	-6.5	0.001
M II oocyte	17.3 (6.4)	12.4 (4.9)	-5.8	0.02
2 PN	12.3 (5.9)	8.54 (4.1)	-5.9	0.001

Table 2: Perinatal outcomes of 200 couples undergoing IVF/ICSI with fresh or frozen-thawed sperm.

	ICSI with fresh sperm (n=100)	ICSI with frozen sperm (n=100)	*P* value
CPR, n (%)	18 (18%)	51 (51%)	0.001
Miscarriage, n (%)	13 (13%)	8 (8%)	0.02
LBR, n (%)	2 (2%)	46 (46%)	0.002
Female/male, n	2/0	28/18	
Cesarean section, n	2 (2%)	43 (43%)	0.001
Multiple pregnancy, n	0	3 (3%)	0.02
High-order pregnancy with fetal reduction, n	0	0	NA
Stillbirth, n (%)	0	0	NA
Neonatal death, n (%)	0	1 (1%)	0.24
Preeclampsia/eclampsia, n (%)	0	1 (1%)	0.30
Gestational diabetes, n (%)	0	2 (2%)	0.22
Birth parameters			
Gestational age (wk)	36.3 ± 1.4	37.1 ± 1.3	0.20
Birth weight (g)	2915 ± 423.4	3021 ± 677.4	0.42
Prematurity (gestational age 37< week), n (%)	0	2 (2%)	0.56
Birth weight <2500 g, n (%)	0	6 (6%)	0.03
Need for intensive care unit, n (%)	0	2 (2%)	0.30
Minor congenital anomalies, n (%)	0	1	0.44
Major congenital anomalies, n (%)	0	0	NA

case in the frozen sperm group, no stilbirth or neonatal death was found in the fresh sperm group. Prematurity was observed in two cases in the frozen sperm group, but not in the fresh sperm group. There was no significant difference between the groups in terms of minor and major congenital anomalies. Two newborns in the frozen sperm group needed intensive care. There was no difference between the groups in terms of birth weights and birth weeks. In the frozen sperm group, gestational diabetes was found in two cases and preeclampsia in one case, while no maternal morbidity was observed in the fresh sperm group.

Discussion

So far, there are no clinical studies investigating the effect of using fresh or frozen-thawed testicular sperm on perinatal outcome in NOA cases**.** In the present study, we investigated the impact of ICSI with fresh versus cryopreserved testicular sperm on perinatal outcomes in patients with NOA. Although the negative effect of sperm freezing-thawing processes on sperm quality is a known fact [7], the clinical outcomes and development of children born after ICSI with fresh or frozen-thawed testicular spermatozoa recovered from men with NOA have not been thoroughly assessed or compared. We observed comparable obstetric and perinatal outcomes in newborns conceived after ICSI with fresh testicular sperm or fozen-thawed testicular sperm from men with NOA. Our patient group consisted of only NOA cases. OA cases were not included in the study. The reason for this was that the rate of aneuploid embryos in NOA cases was significantly higher than in OA cases [8]. Since this fact will prevent the groups from being homogeneous, our study group was formed only from NOA cases.

When the patients who underwent ICSI/ET with fresh or frozen sperm were compared in terms of reproductive and preinatal outcome, we encountered two different results. The first result was that both clinical pregnancy and live birth rates were significantly higher in the group who underwent ICSI with frozen testicular sperm compared to the fresh sperm group. Consistent with our results, Park et al. [9] showed that the implantation and clinical pregnancy rates after ICSI with frozen sperm in azoopermia cases were significantly higher than fresh sperm-ICSI cycles.

Abortion rates were found to be significantly higher in fresh sperm group. The second important result of our study is that no

significant difference was found between perinatal mortality and morbidity of patients who underwent ICSI with fresh versus frozen-thawed testicular sperm. As the only exception to this, we recorded a significant increase in the rate of low birth weight babies in the frozen sperm group. When the literature was reviewed, in fact, no significant difference was found between ICSI babies and babies born naturally in terms of perinatal mortality [10-13]. However, it has been reported that the rate of birth defects is higher in ICSI pregnancies [14-16]. When the ejaculate sperm obtained from oligoasthenotratozoospermia cases was compared with the cases who underwent ICSI with testicular sperm, it has been reported that perinatal outcoms are similar [7].

There is no study in the literature investigating the effect of using fresh versus frozen testicular sperm on perinatal outcome. Most of the studies evaluated the NOA and OA groups in terms of clinical pregnancy rates and pointed to the presence of perinatal problems that were not significant in the NOA group but showed an increasing trend [7,8,11]. The reason for this increase is attributed to the fact that testicular sperm in NOA cases is less qualified than both ejaculate sperm and OA sperm. Performing fresh or frozen embryo transfer after ICSI with ejaculate sperm in healthy individuals does not affect clinical pregnancy rates and perinatal outcome. In the preent study, we clearly showed that using fresh or frozen-thawed testicular sperm for ICSI in NOA cases did not have a significant effect on perinatal oucome. There was an increase in the risk of low birth weight babies only in the frozen-thawed testicular sperm group. Moreover, there was no difference between the groups in terms of other perinatal findings and causes of maternal morbidity. The rates of minor and major congenital anomalies were also recorded similarly between the two groups. While minor congenital malformation was detected in only one case in the frozen sperm group, no anomaly was found in the fresh sperm group. Our results were consistent with a study by Ludwig et al. [17]. They showed that the risk of congenital malformation does not increase in babies born after the use of testicular spermatozoa for ICSI.

To our knowledge, perinatal outcomes in couples who underwent ICSI using fresh or frozen sperm due to azoospermia have not been compared until now. We conclude that there is no evidence of differences in perinatal outcomes except low birth weight between azoospermia couples undergoing ICSI with fresh or frozen-thawed testicular sperm.

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