ORIGINAL RESEARCH ARTICLE

Pregnancy results after intracytoplasmic sperm injection-embryo transfer in patients with infertility due to non-obstructıve azoospermia and oligoasthenoteratozoospermia

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Abstract

This study evaluated pregnancy results after fresh and frozen embryo transfer in males with infertility due to non-obstructive azoospermia and oligoasthenoteratozoospermia. In this retrospective study, a total of 801 embryo transfer cycles were followed up, including 423 fresh embryo transfers and 378 frozen embryo transfers in which intracytoplasmic sperm injection (ICSI) was performed because of male infertility. This study included females aged 28-38 years without uterine, endometrial, ovarian and tubal abnormalities and with regular menstrual cycles (n=801), and males aged 28-38 years with non-obstructive azoospermia and oligoasthenoteratozoospermia. Descriptive statistical methods and the independent t-test were used in the comparison of two groups with normal distribution, the Mann-Whitney U test was used in the comparison of two groups without normal distribution, and the Chi-square test was used to compare categorical variables. There were no statistically significant differences between the fresh embryo transfer group and frozen embryo transfer group in terms of rates of pregnancy, biochemical pregnancy, clinical pregnancy, live birth rate, and abortion rate. There was no difference between fresh embryo transfer and frozen embryo transfer in terms of pregnancy results in couples with non-obstructive azoospermia and oligoasthenoteratozoospermia as male infertility factor. *(Afr J Reprod Health 2021; 25[1]: 122-128).*

Keywords: Non-obstructive azoospermia, oligoasthenoteratozoospermia, clinical pregnancy, intracytoplasmic sperm injection, frozen embryo transfers

Résumé

Cette étude a évalué les résultats de grossesse après transfert d'embryons frais et congelés chez des hommes souffrant d'infertilité due à une azoospermie non obstructive et une oligoasthénotératozoospermie. Dans cette étude rétrospective, un total de 801 cycles de transfert d'embryons ont été suivis, dont 423 transferts d'embryons frais et 378 transferts d'embryons congelés dans lesquels une injection intracytoplasmique de spermatozoïdes (ICSI) a été réalisée en raison de l'infertilité masculine. Cette étude comprenait des femmes âgées de 28 à 38 ans sans anomalies utérines, endométriales, ovariennes et tubaires et avec des cycles menstruels réguliers (n = 801), et des hommes âgés de 28 à 38 ans souffrant d'azoospermie non obstructive et d'oligoasthénotératozoospermie. Des méthodes statistiques descriptives et le test t indépendant ont été utilisés dans la comparaison de deux groupes avec une distribution normale, le test U de Mann-Whitney a été utilisé dans la comparaison de deux groupes sans distribution normale et le test du chi carré a été utilisé pour comparer des catégories catégoriques. variables. Il n'y avait aucune différence statistiquement significative entre le groupe de transfert d'embryons frais et le groupe de transfert d'embryons congelés en termes de taux de grossesse, de grossesse biochimique, de grossesse clinique, de taux de naissances vivantes et de taux d'avortement. Il n'y avait aucune différence entre le transfert d'embryons frais et le transfert d'embryons congelés en termes de résultats de grossesse chez les couples présentant une azoospermie non obstructive et une oligoasthénotératozoospermie comme facteur d'infertilité masculine. *(Afr J Reprod Health 2021; 25[1]: 122-128).*

Mots-clés: Azoospermie non obstructive, oligoasthénotératozoospermie, grossesse clinique, injection intracytoplasmique de spermatozoïdes, transferts d'embryons congelés

Introduction

In recent years, advances in Assisted Reproductive Technology (ART) have been proven in reproductive medicine for its effectiveness and safety. As a result of these developments, there is growing evidence that frozen embryo transfer (FET) achieves the same or better results compared

to fresh embryo transfer (ET) in different infertile populations. In a recent randomized controlled study, FET has been shown to significantly increase live birth rate compared to ET[1]. In the case of ovarian hyperstimulation, in order to increase estrogen replacement in patients with a thin endometrium thickness and recurrent pregnancy loss and failures, FET is now recommended. This approach increases pregnancy outcomes compared to ET[2-5].

Intracytoplasmic sperm injection (ICSI) has significantly improved pregnancy outcomes in cycles, and has become the preferred treatment in male and female infertility regardless of the severity of the condition[6]. Although ICSI is the first choice in male infertility, which is defined by traditional parameters and allows fertilization, there are still limitations in its use in clinical practice[7]. Infertile men with severe oligoasthenoteratozoospermia (OAT) or non-obstructive azoospermia (NOA) are frequently seen in today's infertile patient population. Most of these men are healthy and the cause of impaired spermatogenesis is usually not determined[8]. In males with no sperm in their ejaculate (azoospermia), success in treatment is achieved using ICSI[9]. Male factor infertility such as OAT was found associated with decreased fertilization rates, poor embryo quality, and poor pregnancy results in many publications[10-11]. In contrast, clinical results (fertilization rates, embryo quality, implantation and/or pregnancy rates) in ICSI cycles with poor sperm quality such as non-obstructive azoospermia (NOA) and OAT were not affected in many studies[12-13].

The aim of this study was to evaluate pregnancy results after fresh embryo transfer (ET) and frozen embryo transfer (FET) in males with infertility due to non-obstructive azoospermia and oligoasthenoteratozoospermia.

Methods

Study design and participants

This retrospective study was performed in Sisli Kolan International Hospital of Fertility Unit (Istanbul, Turkey) between June 1st, 2014, and June 31st, 2018. Clinical follow-up and treatment of patients were performed by a single physician. A total of 801 embryo transfer cycles, including 423 embryo transfers and 378 frozen embryo transfers were followed up in which ICSI was performed for the first time because of male infertility with NOA and OAT.

Females aged 28-38 years without uterine, endometrial, ovarian and tubal abnormalities and with regular menstrual cycles were included in the study. Patients with uterine factors, endometrial abnormalities, endometrium thickness below 7 mm during embryo transfer, ovarian dysfunction, patients in which controlled ovarian stimulation (COH) cycles was underwent with agonist protocol, patients who needed oocyte donors, patients with endocrine disorders (hypothyroidism, hyperthyroidism, diabetes mellitus, hyperprolactinemia), with previous chemotherapy and radiotherapy, patients without good quality (Grade A) 5th day blastocyst embryos, and patients with chromosome abnormalities detected in preimplantation genetic screening (PGS) or in next-generation genetic sequencing (NGS) were excluded from the study. The patients who underwent COH with long agonist protocol were excluded from the study because we use this kind of protocol solely in severe endometriosis patients and we thought that this will change the homogeneity of the study population.

Males with NOA or OAT were included in the study and males with other male factors, with Klinefelter syndrome and Y-microdeletion or with normal sperm parameters were excluded. In the study, 801 ICSI cycles were included, 360 of which were performed in males with NOA using testicular sperm extraction (TESE), and 441 were performed in males with OAT using fresh ejaculate. According to the guidelines of the World Health Organization[14], ejaculates were examined at least twice at different times to diagnose NOA and OAT. OAT was diagnosed if sperm concentration was < 5 million/mL, < 4% normal forms in morphology, and reduced sperm motility were detected in semen analysis, and azoospermia was diagnosed if no sperm was found in the ejaculate. After urologic and hormonal examinations (serum follicle-stimulating hormone (FSH) and total testosterone measurements), pre-treatment genetic analysis was performed in males with azoospermia. FSH levels normal or high, testicular volumes were medium or low (9 - 15 mL) and diagnosis was based on testicular biopsy results in males with NOA. A formal scrotal exploration was performed before surgical extraction.

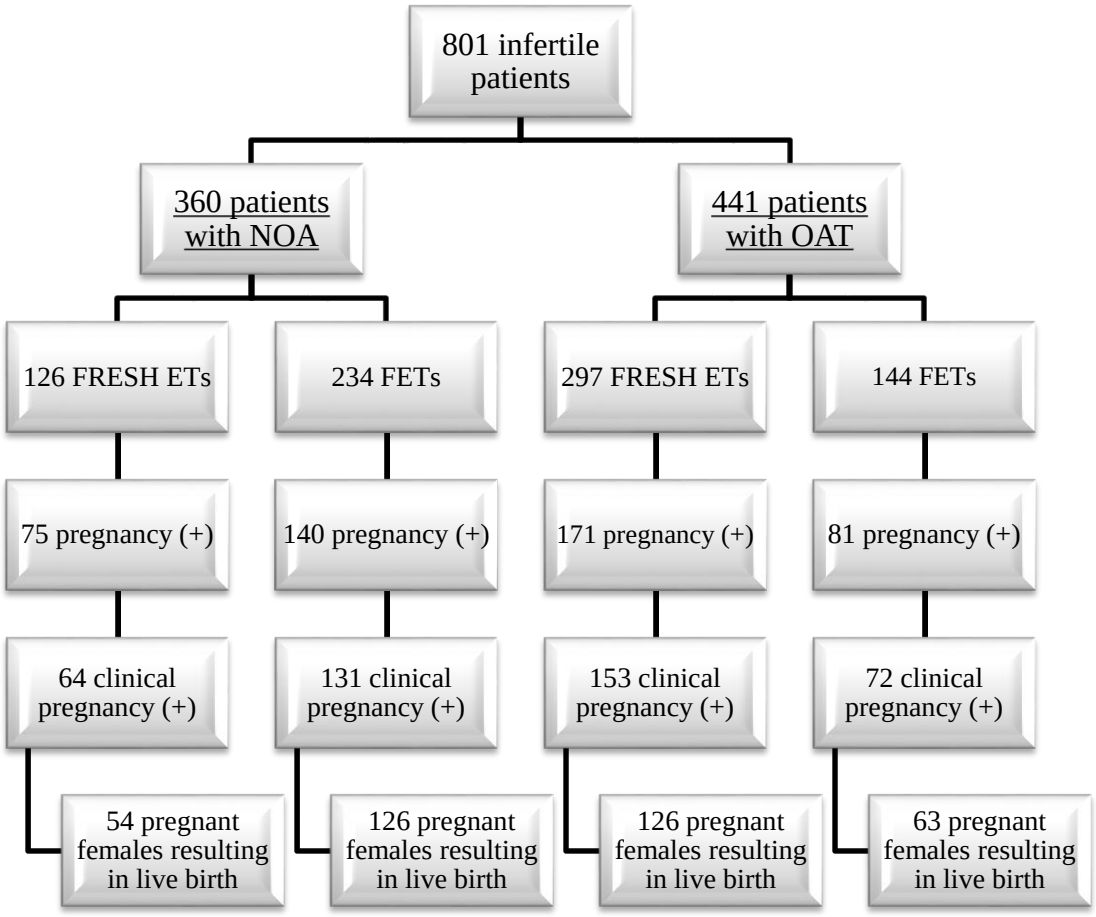

Figure 1: Consort diagram

Table 1: Clinical features of the patients

Clinical features		Whole group n=801	FRESH ET group n=423	FET group n=378	P
Female Age	**Mean±SD**	33.38±4.47	33.4±4.5	33.36±4.49	0.961*
Male Age	**Mean±SD**	36.44±5.52	36.17±5.69	36.74±5.37	0.631*
BMI	**Mean±SD**	23.84±3.99	23.86±4.37	23.83±3.57	0.235*
	Mean±SD	5.35±3.63	4.91±3.01	5.83±4.19	
Infertility duration	**Median (IQR)**	5 (3-7)	4 (3-7)	5 (3-7.25)	0.334ǂ
	Mean±SD	8.05±3.56	8.03±3.44	8.08±3.73	
D_3 FSH	**Median (IQR)**	6.77 (5.55-10)	6.77 (5.4-10.2)	6.79 (5.63-9.9)	0.882ǂ
	Mean±SD	8.79±4.22	9±4.49	8.54±3.93	
D_3 LH	**Median (IQR)**	7.56 (5.95-10.9)	7.3 (5.9-10.9)	7.95 (5.9-10.93)	0.967ǂ
	Mean±SD	49.29±27.67	54.37±32.86	43.62±19.21	
D_3 Estradiol	**Median (IQR)**	38.2 (33.5-55)	41 (35-57)	38 (33-47.5)	0.132ǂ
	Mean±SD	10.74±2.15	10.64±1.62	10.86±2.63	
Stimulation Day	**Median (IQR)**	10 (10-11)	11 (10-11)	10 (9-11)	0.565ǂ
Number of Oocytes	**Mean±SD**	11.16±6.78	9.77±6.09	12.71±7.23	
Collected	**Median (IQR)**	11 (6-16)	9 (4-14)	11 (6.75-18.25)	0.054ǂ
Number of Transferred Embryos	**1 Embryo**	243 (30.34%)	126 (29.79%)	117 (30.95%)	0.905+
	2 Embryos	558 (69.66%)	297 (70.21%)	261 (69.05%)	
Male Factor	**NOA**	360 (44.94%)	126 (29.79%)	234 (61.90%)	**0.002+**
	OAT	441 (55.06%)	297 (70.21%)	144 (38.10%)	

*Independent t-test, ǂMann-Whitney U test , +Chi Square test, BMI: Body Mass Index, Mean±SD: mean±standard deviation, D_3:third day of the cycle, NOA; Non-obstructive azoospermia, OAT; oligoasthenoteratozoospermia, FET: frozen embryo transfer

Table 2: Pregnancy results of fresh ET and FET groups

Pregnancy results		Whole group n=801 n	%	Fresh ET group n=423 n	%	FET group n=378 n	%	p+
	No	334	41.70	177	41.84	157	41.54	
Pregnancy test result	**Yes**	467	58.30	246	58.16	221	58.46	0.752
	No	754	94.13	392	92.68	362	95.77	
Biochemical pregnancy	**Yes**	47	5.87	31	7.32	16	4.23	0.813
	No	381	47.57	206	48.70	175	46.30	
Clinical pregnancy	**Yes**	420	52.43	217	51.30	203	53.70	0.833
	No	432	53.93	243	57.46	189	50.00	
Live birth	**Yes**	369	46.07	180	42.54	189	50.00	0.525
	No	750	93.63	386	91.26	364	96.30	
Abortion	**Yes**	51	6.37	37	8.74	14	3.70	0.379

+Chi square test, ET: Embryo transfer, FET: Frozen embryo transfer

Table 3: Pregnancy results in the NOA and OAT groups in patients who underwent fresh ET

Pregnancy results		Fresh ET group NOA n=126 n	%	OAT n=297 n	%	p+
	No	51	40.48	126	42.42	
Pregnancy test result	**Yes**	75	59.52	171	57.58	0.687
	No	115	91.27	277	93.27	
Biochemical pregnancy	**Yes**	11	8.73	20	6.73	0.980
	No	62	49.21	144	48.48	
Clinical pregnancy	**Yes**	64	50.79	153	51.52	0.250
	No	72	57.14	171	57.58	
Live birth	**Yes**	54	42.86	126	42.42	0.878
	No	116	92.06	270	90.91	
Abortion	**Yes**	10	7.94	27	9.09	0.772

+Chi Square test, NOA: Non-obstructive azoospermia, OAT: Oligoasthenoteratozoospermia

Table 4: Pregnancy results in the NOA and OAT groups in patents who underwent FET

Pregnancy results		FET group NOA n=234 n	%	OAT n=144 n	%	p+
	No	94	40.17	63	43.75	
Pregnancy result	**Yes**	140	59.83	81	56.25	0.234
	No	225	96.15	137	95.14	
Biochemical pregnancy	**Yes**	9	3.85	7	4.86	0.365
	No	103	44.02	72	50.00	
Clinical pregnancy	**Yes**	131	55.98	72	50.00	0.544
	No	108	46.15	81	56.25	
Live birth	**Yes**	126	53.85	63	43.75	0.056
	No	229	97.86	136	94.44	
Abortion	**Yes**	5	2.14	9	5.56	0.211

+Chi Square test, NOA: Non-obstructive azoospermia, OAT: Oligoasthenoteratozoospermia, FET: Fresh embryo transfer

The following protocols were used in ovarian stimulation in fresh ET and FET cycles, oocyte retrieval, and in the evaluation of clinical results in all participants. The pregnancy test was performed 12 days after the embryo transfer by measuring β-hCG in the blood. β-hCG values > 5 U/mL were accepted as a positive pregnancy test. Biochemical pregnancy was defined as a positive β-hCG value 12 days after the embryo transfer without viable gestational sac inside the endometrium excluding the ectopic pregnancies in ultrasonographic examination during the first visit after the pregnancy test (5th day). Clinical pregnancy was defined as the presence of a live fetus and/or a

gestational sac in USG 4 weeks after embryo transfer. Gestational age >22 weeks was accepted as a live birth. After fetal heart beats were heard in USG, infants born before 22 gestational weeks were considered as a miscarriage. The treatment process of the patients is shown in the flow diagram in Figure 1.

Statistical analysis

In this study, statistical analysis was performed using the NCSS (Number Cruncher Statistical System) 2007 Statistical software (Utah, USA) package program. Descriptive statistical methods (mean, standard deviation, median and interquartile range) and the independent t-test were used in the comparison of two groups with normal distribution, the Mann-Whitney U test was used in the comparison of two groups without normal distribution, and the Chi-square test was used to compare categorical variables. The results were evaluated at $p<0.05$ for statistical significance.

Results

The results of a total of 801 embryo transfer cycles, including 423 fresh ETs and 378 FETs in which intracytoplasmic sperm injection (ICSI) was performed because of male infertility, were evaluated. 360 (44.94%) males had NOA and 441 (55.06%) had OAT. In 423 fresh ETs, 126 males had NOA and 297 had OAT. In 378 FETs, 234 males had NOA and 144 had OAT. The clinical features of the patients are summarized in Table 1. There were no statistically significant differences between the fresh ET and FET groups in terms existence of pregnancy, biochemical pregnancy, clinical pregnancy, live birth, and abortion (Table 2). There were no statistically significant differences between the NOA and OAT groups in patients who underwent fresh ET and FET in terms of total pregnancy rate, biochemical pregnancy, clinical pregnancy, live birth and abortion (Table 3) (Table 4).

Discussion

Today, ICSI is widely used in the treatment of couples with severe male infertility, and in addition to patients with limited number of sperms in ejaculate, it also creates a chance of pregnancy for patients with azoospermia, which is known as the most important form of male infertility. According to another study comparing the effects of ejaculatory sperm and surgically extracted sperm on ICSI results in severe male infertility, this problem has not been fully resolved[15].

Impaired spermatogenesis causes a decrease in sperm count, as well as increases the risk of carrying some sperm defects to the embryo. This has the potential to affect the fertilization of ovum by sperm, the implantation of the developing embryo, and to increase the risk of passing of karyotypic abnormalities to the next generations. In a study, sperm DNA fragmentations and chromosomal aneuploidy were found to be higher in the sperms of patients with OAT or NOA[16]. It was reported that high sperm DNA fragmentation rates were not associated with fertilization problems, which was an early paternal effect, but were associated with low pregnancy rates, which was a late paternal effect[17]. Whether there is a difference between ejaculatory and testicular sperms in terms of sperm DNA damage has been questioned. Greco et al. hypothesized that the sperms obtained from the testicle instead of ejaculate could not be affected by this pathologic condition because most of the DNA damage in sperm occurred in post-testicular level, after their release from Sertoli cells, and reported that pregnancy rates were higher in the testicular sperm group[18].

The effect of testicular and ejaculatory sperm on ICSI results is still being discussed. In the literature, similar implantation and clinical pregnancy rates were found in testicular/epididymal and ejaculatory sperm specimens in a study that examined the effect of sperm source on treatment results in FET cycles[19]. In some studies comparing sperm sources in fresh embryo transfer cycles, it was observed that fertilization and pregnancy rates were lower in patients with NOA in whom testicular sperms were used[13,20,21]. In another study, it was found that the fertilization rate was lower in the testicular group, but embryo quality and implantation rate were not different between the testicular and ejaculatory sperm groups[22]. In our study, there were no statistically significant differences between the NOA and OAT groups in terms of existence of pregnancy, biochemical pregnancy, clinical pregnancy, live birth, and abortion. With the development of ART, embryo-freezing technology has become an important part of in vitro fertilization (IVF) and ICSI treatment[23]. Fresh embryo transfer has yielded same or better

results compared with FET in different infertile patient populations because cryopreservation and vitrification technology has greatly improved conventional freezing technology[1,24-26].

In a study, it was found that the rate of clinical pregnancy was significantly higher in the DET group compared with the fresh ET group[27]. As a result, we strongly recommend that patients with impaired endometrial receptivity undergo FET cycles[27]. In a recent similar study, it was shown that pregnancy results were better in the FET group than in the fresh ET group[28]. Another observational study that included 1209 patients who underwent IVF showed that the DET and fresh ET groups had equivalent rates of live birth[5]. A recent meta-analysis of four randomized clinical trials involving 1892 patients compared FET and fresh ET[29]. In this meta-analysis, fewer abortions but more pregnancy complications were observed in the FET group. However, there was no difference between the two groups in cumulative living birth rates[29].

In our study, couples that had male infertility due to NOA or OAT underwent FET and fresh ET cycles. There were no differences between the FET and fresh ET groups in terms of existence of pregnancy, biochemical pregnancy, clinical pregnancy, live birth, and abortion. Our study is the only study with this high number of patients with severe male factor which includes NOA and OAT and evaluates the pregnancy results of such infertile population with FET after Freeze -All strategy and Fresh ET. On the other hand, the effect of ejaculatory and surgically extracted sperms on congenital abnormalities and neonatal development is still being discussed. Belva et al. compared neonatal prognoses in ICSI cycles using non-ejaculatory and ejaculatory sperms in their large case series and found no differences[30]. In our study, no congenital abnormalities were observed. However, the most important limitation of this study is that the frozen and fresh embryo transfer numbers in the NOA and OAT group are significantly different.

Conclusion

This study showed that there was no difference between fresh ET and FET following ICSI in terms of pregnancy results in couples with NOA and OAT as a male infertility factor.

References

1. Chen ZJ, Shi Y, Sun Y, Zhang B, et. al. Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. N Engl J Med, 2016; 375: 523–33.
2. Devroey P, Polyzos NP and Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. Hum Reprod, 2011; 26: 2593–97.
3. Mahajan N and Sharma S. The endometrium in assisted reproductive technology: How thin is thin? J Hum Reprod Sci, 2016; 9: 3–8.
4. Geraedts J and Sermon K. Preimplantation genetic screening 2.0: The theory. Mol Hum Reprod, 2016; 22: 839–44.
5. Roy TK, Bradley CK, Bowman MC and McArthur SJ. Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. Fertil Steril, 2014; 101: 1294–301.
6. Palermo GD, Cohen J, Alikani M, Adler A and Rosenwaks Z. Intracytoplasmic sperm injection: a novel treatment for all forms of male factor infertility. Fertil Steril 1995; 63:1231–40.
7. Tesarik J, Mendoza C and Greco E. Paternal effects acting during the first cell cycle of human preimplantation development after ICSI. HumReprod 2002; 17:184–9.
8. Matzuk MM and Lamb DJ. The biology of infertility: research advances and clinical challenges. Nat Med 2008; 14:1197–213.
9. Palermo G, Joris H, Devroey P and Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17–8.
10. Shoukir Y, Chardonnens D, Campana A and Sakkas D. Blastocyst development from supernumerary embryos after intracytoplasmic sperm injection: a paternal influence? Hum Reprod 1998;13:1632–7.
11. Sanchez R, Stalf T, Khanaga O, Turley H, Gips H and Schill WB. Sperm selection methods for intracytoplasmic sperm injection (ICSI) in andrological patients. J Assist Reprod Genet 1996;13:228–33.
12. Bukulmez O, Yucel A, Yarali H, Bildirici I and Gurgan T. The origin of spermatozoa does not affect intracytoplasmic sperm injection outcome. Eur J Obstet Gynecol Reprod Biol 2001;94:250–5.
13. Goker EN, Sendag F, Levi R, Sendag H and Tavmergen E. Comparison of the ICSI outcome of ejaculated sperm with normal, abnormal parameters and testicular sperm. Eur J Obstet Gynecol Reprod Biol 2002;104: 129–36.
14. World Health Organization. Laboratory manual for the examination of human semen and sperm–cervical mucus interaction. Cambridge, UK: Cambridge University Press; 199213. Hauser R, Bibi G, Yogev L, Carmon A, Azem F, Botchan A, et al. Virtual azoospermia and cryptozoospermia--fresh/frozen testicular or ejaculate sperm for better IVF outcome? J Androl 2011;32(5):484-90.
15. Guo HB, Zhang YH, Zhang CL, Li HS, Gu BX, Yin BL,

Song X and Xie J. Outcomes of ICSI with sperm from different sources: a retrospective study of 431 cycles. Zhonghua Nan Ke Xue 2009; 15(10):925-8.
16. Burrello N, Vicari E and Calogero AE. Chromosome abnormalities in spermatozoa of patients with azoospermia and normal somatic karyotype. Cytogenet Genome Res 2005; 111(3-4):363-5.
17. Henkel R, Kierspel E, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, Menkveld R, Schill W and Kruger TF. DNA fragmentation of spermatozoa and assisted reproduction technology. Reprod Biomed Online 2003;7(4):477-84.
18. Greco E, Scarselli F, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Franco G, Anniballo N, Mendoza C and Tesarik J. Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod 2005;20(1):226-30.
19. Figueira DR, Madaschi C, Nichi M, Rodrigues D, Pasqualotto FF, Iaconelli A Jr and Borges EA comparison of post-thaw results between embryos arising from intracytoplasmic sperm injection using surgically retrieved or ejaculated spermatozoa. Fertil Steril 2009;91 (3):727-32.
20. Verza S Jr and Esteves SC. Sperm defect severity rather than sperm source is associated with lower fertilization rates after intracytoplasmic sperm injection. Int Braz J Urol 2008;34:49–56.
21. Aboulghar MA, Mansour RT, Serour GI, Fahmy I, Kamal A, Tawab NA and Amin YA. Fertilization and pregnancy rates after intracytoplasmic sperm injection using ejaculate semen and surgically retrieved sperm. Fertil Steril 1997;68(1):108-11.
22. Nagy Z, Liu J, Cecile J, Silber S, Devroey P and Van Steirteghem A. Using ejaculated, fresh, and frozen-thawed epididymal and testicular spermatozoa gives rise to comparable results after intracytoplasmic sperm injection. Fertil Steril 1995;63(4):808-15.
23. Wong KM, Mastenbroek S and Repping S. Cryopreservation of human embryos and its contribution to *in vitro* fertilization success rates. Fertil Steril, 2014; 102: 19–26.
24. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, Vanderpoel S and Racowsky C. Oocyte, embryo and blastocyst cryopreservation in ART: Systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update, 2017; 23: 139–55.
25. Mahajan N and Sharma S. The endometrium in assisted reproductive technology: How thin is thin? J Hum Reprod Sci, 2016; 9: 3–8.
26. Pereira N, Petrini AC, Lekovich JP, Schattman GL and Rosenwaks Z. Comparison of perinatal outcomes following fresh and frozen-thawed blastocyst transfer. Int J Gynaecol Obstet, 2016; 135: 96–100.
27. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C and Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for *in vitro* fertilization: A prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril, 2011; 96: 344–48.
28. Roque M, Valle M, Guimarães F, Sampaio M and Geber S. Freeze-all policy: Fresh *vs.* frozen-thawed embryo transfer. Fertil Steril, 2015; 103: 1190–93.
29. Wong KM, Van Wely M, Mol F, Repping S and Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database Syst Rev, 2017; 3: CD011184.
30. Belva F, De Schrijver F, Tournaye H, Liebaers I, Devroey P, Haentjens P and Bonduelle M. Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. Hum Reprod 2011;26 (7):1752-8.