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ASSISTED REPRODUCTION TECHNOLOGIES

Comparison of early pregnancy and neonatal outcomes after frozen and fresh embryo transfer in ART cycles

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Abstract
Purpose Frozen embryo transfer (FET) has no clear negative impact on neonatal outcome compared with fresh embryo transfer (ET) and appears to result in similar or even better neonatal outcome. The objective of this study was to compare early pregnancy outcome and neonatal health of children born after FET and fresh ET.
Methods In this study early pregnancy and neonatal outcomes after FET (n=200) and fresh ET (n=500) were compared.
Results For early pregnancy, biochemical pregnancy was comparable between FET and fresh ET groups. Spontaneous abortion was significantly higher in FET (14.5%) than fresh ET group (9%). Neonatal outcome was comparable between both groups except for lower live birth rate in FET (55%) versus fresh ET group (66%).
Conclusion FET has similar neonatal outcome in terms of prematurity, low birth weight, stillbirth, neonatal death and major malformation compared with fresh ET.

Capsule Frozen-thawed embryo transfer has no negative impact on neonatal outcome compared with fresh embryo.

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Keywords Frozen-thawed embryo transfer · Fresh embryo transfer · Pregnancy outcome · Neonatal outcome

Introduction

The first live birth following the transfer of cryopreserved embryos was reported in 1984 and this strategy has been progressively used in assisted reproductive technology (ART) [1]. Although during the last years the importance of embryo cryopreservation has increased and cryopreservation is one of the important IVF programmes, there are only limited studies that have been conducted to evaluate the obstetric and neonatal outcome of children born after replacement of cryopreserved embryos [2–7]. No increase in the incidence of prematurity, low birth weight (LBW), neonatal death were found in the FET group compared with the fresh ET group [5].

FET can provide several benefits in ART such as increasing cumulative pregnancy rate, decreasing risk of multiple pregnancy and hyper stimulation syndrome and increasing use of single embryo transfer (SET) [8]. A previous study reported that SET is a choice in FET which can be used in frozen cycles to reduce multiple delivery rates [9].

Many studies have shown that major malformation rates in children born after FET are comparable with those in children born after fresh ET and those in children after spontaneous conception [10–12].

A recent study revealed safety and cost-effectiveness of FET compared with fresh ET [13].

Preterm birth and perinatal mortality may have been significantly lower in children born after FET compared with fresh ET [6]. The low birth weight rate in children born after FET is lower than fresh ET [4, 6]. FET cycles

versus fresh ET cycles are being used in most other countries [14]. In most studies the reason for better outcome for children born after FET compared with those born after fresh ET is not clear. The selection of women and also embryos and adverse effect of hormone stimulation in fresh cycles might be a cause of this difference [15]. According to some studies, vitrification may increase the embryo survival rate and decrease the rate of cooling injury [16, 17].

Our hypothesis was to test the idea that FET has no negative impact on pregnancy outcome.

The purpose of this study was to evaluate early pregnancy and neonatal outcomes after FET and compare these outcomes with fresh ET.

Materials and methods

Study design and participants

In this follow up study, 500 pregnancies were obtained after the transfer of fresh ET and 200 pregnancies after FET from March 2006 to March 2008 were included and all these participants were followed up to the end of pregnancy outcome.

The results for singletons, twins and triples were compared separately.

The study was carried out in two infertility centers (Research and Clinical center for Infertility and Madar Hospital as a private assisted reproduction center) in Yazd.

This study was approved by the ethics committee of research and clinical center for infertility, Shahid Sadoughi University of Medical Science.

The specific questionnaire was filled out for each patient regarding her pregnancy or neonatal outcome obtained from the gynecologist, pediatricians and parents and sent back to our center. A nurse was responsible to receive them.

If pregnancy continued until delivery of the live birth, for all babies born in our city, a complete examination would be done at birth, looking for major malformations. For babies born in other cities, written reports were obtained from gynecologists or pediatricians and sent back to our centre. The children were followed up to the age of 3 months.

Ovarian stimulation and oocyte retrieval

All patients underwent stimulation using recombinant or urinary FSH or HMG combination with GnRH agonist, antagonist or microdose for ovarian stimulation. Oocyte retrieval was done 34–36 h after hCG injection and conventional IVF or ICSI was performed as clinically appropriate and luteal phase support was started on the same day with progesterone in oil 100 mg daily IM and continued until fetal heart activity was seen on ultrasound

Embryos for transfer and for cryopreservation

For fresh ET, it was performed on the day 2 after oocyte retrieval. The embryos were scored according to developmental stage and the presence of fragmentation on the day of embryo transfer. Excellent morphology showing 2–4 even size blastomeres with ≤10% fragmentation, good morphology embryos with 2–4 even or uneven size blastomeres with 10%–20% fragmentation, poor morphology embryos with un even few blastomeres with >20% fragmentation [16]. Depending on patients' embryos, 2 or 3 embryos with the best morphology (excellent or good quality) were selected and transferred. If on the day of embryo transfer excellent or good quality embryos were surplus to requirements, they were cryopreserved with vitrification protocol.

Protocol for vitrification

Embryos were first loaded with equilibrium solution containing 7.5% ethylene glycol (EG) (Sigma-Aldrich, Steinhem, Germany) and 7.5% dimethyl sulphoxide (DSMO) (Sigma-Aldrich) for 5–10 min at room temperature and then with vitrification solution containing 15% EG, 15% DSMO and 0.5 mol/L sucrose for 50–60 s at room temperature. After observed cellular shrinkage, embryos were loaded with a narrow capillary on the tip of cryotop. No more than four embryos were placed on each cryotop. The cryotops were quickly stored in liquid nitrogen (LN) for at least 2 months. At warming, cryotop was removed from LN and embryos were exposed to thawing solution containing sucrose at 37°C temperature for 50–60 s and then embryos were sequentially incubated in diluents solutions before being transferred [16].

Evaluation and transfer of thawed embryos

After thawing, each embryo was evaluated twice, once immediately for the number of surviving blastomeres and again after 18 h post-thaw in vitro culture for assessing of mitosis and number of blastomeres. Embryos were considered survived if >50% blastomeres were intact and selected for intrauterine transfer (Depending on patients' embryos, 2 or 3 embryos were transferred).

The evaluation criteria for survival of frozen-thawed embryos was described previously. Embryos were classified as fully intact or excellent morphology (100% cells survived with <10% fragmentation) or good morphology (100% cells survived with 10%–20% fragmentation), partially damaged or poor morphology (≥50% cells survived with or without any fragmentation) and Degenerated embryos (<50% cells survived) [16, 19]. Only fully intact and partially damaged embryos

were transferred. Degenerated or arrested embryos were not transferred.

For FET group, patients were prepared with oral Estradiol until endometrial thickness reached ≥8 mm and triple line in ultrasonography. In this time Progesterone in oil 100 mg IM was given daily and embryo transfer was performed after 3 days from beginning of injection of progesterone. Oral estradiol and progesterone were continued until the 10th gestational week.

Main outcome measures

Primary outcome measures were biochemical pregnancy, abortion and live birth rates and secondary outcome measures were preterm birth, low birth weight (LBW), stillbirth, neonatal death, major malformation, sex and multiple pregnancy rates.

If in the 7th gestational week a fetus with fetal heart activity was visualized by ultrasonography, pregnancy was considered Clinical. If there was no fetus, the pregnancy was considered Biochemical pregnancy. Spontaneous abortion: loss of fetus with gestational age before 20 weeks. Ectopic pregnancy: the diagnosis of extra uterine pregnancy confirmed by laparoscopy or ultrasound. Preterm birth: delivery before 37 completed weeks of gestation. Low birth weight : <2,500 at birth. Neonatal death: death of a child before day 7 until day 28. Stillbirth: death of child with a gestational age more than 20 weeks in intrauterine or intrapartum. Major malformation: malformations that cause functional impairment or need

Statistical analysis

Statistical analysis was performed using the statistical package for the social science version 15.0 for windows (SPSS Inc., Chicago, IL, USA). Differences among variables of the FET and fresh ET groups were analyzed using the Student's *t*-test for continuous variables which were normally distributed and Mann–Whitney *u* test for data not normally distributed and chi-squared test and Fisher exact tests for qualitative variables. $P<0.05$ was considered statistically significant. Comparisons of percentages among groups are presented as odds ratios (ORs) with corresponding 95% confidence intervals (95% CI) for each comparison made.

Results

Patients demographic and infertility characteristics of both groups were not significantly different (Table 1).

Data on early pregnancy outcome in terms of biochemical pregnancy, spontaneous abortion and ectopic pregnancy are detailed in Table 2. There were not any loss to follow up pregnancy in this study.

Biochemical pregnancy rate was 27% (54/200) In the FET group and 22.1% (122/500) in the fresh ET group. No higher incidence of biochemical pregnancy or ectopic pregnancy was found in the FET group compared with the fresh ET group.

Spontaneous abortion was 14.5% In the FET group and 9% in the fresh ET group. Significantly higher rate of spontaneous abortion was observed in the FET group compared with fresh ET group (OR 1.44; 95% CI 1.03–2.03).

During the study period, in the FET group, 200 pregnancies led to birth of 112 live born (56%), of which 84 were singletons (74.8%), 24 were twins (21.6%) and 4 were triples (3.6%).

In the fresh ET group, 500 pregnancy led to the birth of 330 live born (66%), of which 252 were singletons (76.8%), 64 were twins (18.9%) and 14 were triples (4.3%).

Significantly lower percentage of pregnancies led to the birth of a live child in FET group (55%) compared with fresh ET group (66%). (OR 1.49; 95% CI 1.06–2.03).

The rates of singleton and multiple pregnancies were comparable between both groups.

Mean gestational age and Birth weight for frozen and fresh live borns are listed in Table 3.

Birth weight of all children in the FET group compared with ET group was not found to be statistically different ($p=0.17$). No significant difference in Birth weight was found between both groups for singletons ($p=0.87$), twins ($p=0.15$) and triples ($p=0.12$).

Table 1 Characteristic and infertility characteristics of patients

	Frozen $N=200$	Fresh $N=500$	p-value
Age (mean±SD)	30.4±4.5	29.9±4.7	0.4
Duration of infertility[a] (years)	8 (IQ=6)	8 (IQ=5)	0.77
Causes of infertility*			
Male factor	229 (54.6%)	98 (49.5%)	
Ovary factor	124 (15.7%)	29 (14.5%)	
Tubal factor	79 (15.7%)	20 (10.1%)	
Unexplained	37 (7.4%)	17 (8.6%)	
Mixed	36 (7.2%)	7 (3.5%)	

[a] Median (Interquartile Range)

*P-value=0.19

Table 2 Early pregnancy outcome before 20 weeks of gestation and live birth rate in both groups

	Fresh N=500	Frozen N=196	Odds ratio (95% CI)	p-value
Biochemical pregnancy	122(22.1%)	54(27%)	1.08(0.74–1.58)	0.69
Abortion	42(9%)	29(14.5%)	1.44(1.03–2.03)	0.04
Ectopic pregnancy	6(1.2%)	1(0.5%)	2.31(0.27–3.36)	0.68
Live birth	330(66%)	112(56%)	1.49(1.06–2.03)	0.01
Singletons	252(76.8%)	84(74.8%)	1.56(0.38–6.41)	0.79
Twins	64(18.9%)	24(21.6%)	0.52(0.05–4.72)	0.80
Triples	14(4.3%)	4(3.6%)	3.5(0.17–6.33)	0.81

Gestational age of all children born in the FET compared with fresh ET was borderline significant (36.7±3.1 versus 37.2±2.4, p=0.06). No significant difference in gestational age was observed between both groups (singletons: p=0.13; twins: p=0.15; triples: p=0.12).

The preterm birth and neonatal death rates in the total FET and fresh ET groups were 18.9% versus 11% and 5.1% versus 3.9%, respectively.

For singletons, twins and triples, preterm birth and neonatal death rates were comparable between FET and fresh ET group (data shown in Table 4). Stillbirth was comparable between FET and fresh ET groups (data not shown).

No significant difference was observed regarding LBW (<2,500 g) comparing the total FET group with the fresh ET group (33.1% versus 35.1%, p=0.75). The frequency of LBW for singleton and multiple pregnancies was comparable between both groups. (singletons: p=0.09; twins: p=0.47; triples: p=1.000).

Total major malformations rate up to the age of 3 months was 3.6% in FET group and 3.1% in fresh ET group was not significantly different (p=0.78). All major malformations were considered as malformations of different organ systems.

In singletons, significantly higher male sex ratio was found in FET group compared with fresh ET group (p=0.03). In multiple pregnancies (twins and triples) this difference was not significant (Table 5). Although total number of boys was observed to be higher in the total FET group compared with fresh ET group but this difference was not significant (50.7% versus 43.1%, p=0.13).

Discussion

In this follow up study pregnancy outcome before 20 weeks of gestation and neonatal outcome of pregnancies after FET group were compared with those after fresh ET group.

Although women who were conceived by fresh ET were older than those obtained pregnancy by FET, this difference was not significant. However, in a previous study it was statistically significant [5].

In the present study early pregnancy outcome in terms of Biochemical pregnancy, spontaneous abortion and ectopic pregnancy showed that biochemical pregnancy rate was comparable between FET and Fresh ET. Belva et al. and Aytoz et al. reported that it was significantly higher in the FET group than fresh ET group [5, 18].

A recent study has shown a lower incidence of biochemical pregnancy with fully intact embryos (17%) compared to partially damage embryos (42.9%) after frozen embryo transfers [19], But salumets et al. found that there was no association between biochemical pregnancy rate and embryo quality before cryopreservation [20]. However biochemical pregnancy was similar in both groups in the present study (27% and 22%) according to morphological grading of embryos.

We found that spontaneous abortion rate in FET group was significantly higher than in fresh ET group and previous study showed the same [21] but it was similar between both groups in previous results [5]. Aytoz et al. reported spontaneous abortion in FET after ICSI procedure was significantly higher than in FET after IVF procedure.

Table 3 Mean gestational age and birth weight in both groups

	No	Fresh	No	Frozen	p-value
Gestational age[a]	330	37.7±2.4	112	36.7±3.1	0.06
Singletons	252	37.7±2.3	84	37.2±2.7	0.13
Twins	64	36.1±1.8	24	35.6±3.3	0.4
Triples	14	34.3±3.3	4	32.2±4.9	0.32
Birth weight[a]	419	2,614±747	131	2,715±756	0.17
Singletons	251	2,745±677	83	2,969±727	0.87
Twins	126	2,259±515	42	2,390±524	0.15
Triples	42	1,695±412	6	1,908±445	0.12

[a] Values are mean±SD

Table 4 Prematurity, low birth weight and Neonatal death in both groups

	Fresh	Frozen	Odds ratio (95% CI)	*P*-value
Preterm	37(11.3%)	20(17.9%)	1.71(0.94–3.09)	0.1
Singletons	18(7.2%)	11(13.3%)	1.85(.082–4.20)	0.11
Twins	11(17.5%)	7(29.2%)	1.90(0.63–5.70)	0.24
Triples	8(57.1%)	2(50%)	0.74(0.08–6.95)	1.00
Low birth weight	142(35.1%)	44(33.1%)	1.16(0.74–1.67	0.45
Singletons	40(19.2%)	16(19.3%)	0.79(0.41–1.50)	0.68
Twins	73(57.9%)	22(51.2%)	1.3(0.65–2.63)	0.42
Triples	41(97.6%)	6(100%)	0.87(0.78–0.97)	0.07
Neonatal death	15(3.9%)	7(5.1%)	0.62(0.27–1.45)	0.45
Singletons	7(2.8%)	1(1.2%)	0.67(.027–1.61)	0.68
Twins	5(4%)	3(6.8%)	0.56(0.12–2.46)	0.42
Triples	3(7.1%)	3(30%)	0.17(0.03–1.07)	0.07

They found that it was similar between fresh ET and FET groups, both for IVF and ICSI [18].

In this study, Live birth rate was significantly lower in FET group than fresh ET group. Two previous studies showed that with increased rate of early pregnancy loss the chance of live birth decreased after FET [5, 18]. A lower live birth rate in our results was a reflection of higher spontaneous abortion rate in the FET group compared with the fresh ET group. possible cause might be referred to freezing –thawing procedures could have a negative impact on embryos and cause the damage of embryo.

According to our results, a previous study in the UK showed that the mean gestational age and birth weight of singletons, twins and triple births were not significantly different between FET and fresh ET groups [2].

We concluded that preterm birth and LBW in singletons and multiple pregnancies were comparable between FET and fresh ET groups. some researchers showed that preterm birth and LBW in FET singletons were fewer than fresh ET singletons [4, 6, 7, 13, 23]. Wang et al. found fresh ET and female-factor infertility were associated with LBW and preterm birth for both singletons and twins after ART [4]. Two previous studies have shown patients who produce more and higher quality embryos, have better outcome of birth weight with frozen embryos [4, 22]. However no significant effect of average oocyte or embryo number or quality of embryos on birth weight was found in study by Shin et al. [6].

In the present study, except for singletons, we could not find any difference in male sex ratio in FET group compared with fresh ET group. A recent study [7] and some previous studies reported that the male sex ratio is significantly higher in Cryo-ICSI compared with fresh-ICSI in singleton [24, 25]. It is possible that increased use of ICSI in infertility and selection of X sperm cells could be the cause of this difference. One of the causes of reduced male sex ratio in fresh-ICSI group might be the fact that male and female embryos die differentially in the early stages of embryogenesis [25].

In this study, major malformation rate after FET seems comparable with fresh ET group. Some studies showed the same findings [6, 10, 18]. Although Wada et al. reported major malformation in cryopreserved group was significantly lower than in standard IVF group [2], a recent study showed, a significantly higher major malformation rate in FET group compared with fresh ET group. They concluded that the cause of this difference in FET group could be due to difference in cryopreservation protocols, freezing day, number or quality of frozen embryos transferred [5].

Our results showed that if the pregnancy reaches 20 weeks of gestation, FET does not adversely affect neonatal outcome in terms of Birth weight prematurity,

Table 5 Sex ratio in fresh and frozen groups

		Fresh	Frozen	Odds ratio (95% CI)	*p*-value
Sex	Boy	180(43.1%)	68(50.7%)	0.73(0.50–1.07)	0.13
	Girl	238(56.9%)	66(49.3%)		
Singletons	Boy	112(44.6%)	48(58.5%)	0.57(0.34–0.94)	0.03
	Girl	139(55.4%)	34(41.5%)		
Twins	Boy	53(42.4%)	16(38.1%)	1.19(0.58–2.44)	0.7
	Girl	72(57.6%)	26(61.9%)		
Triples	Boy	15(35.7%)	3(33.3%)	1.11(0.24–5.09)	1.00
	Girl	27(64.3%)	6(66.7%)		

LBW, stillbirth, neonatal death and major malformation compared with fresh ET.

In conclusion, early pregnancy and neonatal outcomes were comparable in fresh ET and FET except for abortion and live birth. Further studies are needed to assess the impact of freezing and thawing procedures and clinical factors on the development and implantation of embryo.

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References

1. Zeilmaker GH, Alberda AT, van Gent I, Rijkmans CM, Drogendijk AC. Two pregnancies following transfer of intact frozen-thawed embryo. Fertil Steril. 1984;42:293–6.
2. Wada I, Macnamee MC, Wick K, Bradfield JM, Brinsden PR. Birth characteristics and perinatal outcome of babies conceived from cryopreserved embryos. Hum Reprod. 1994;9:543–6.
3. Wennerholm WB. Cryopreservation of embryos and oocytes: obstetric outcome and health in children. Hum Reprod. 2000;15 Suppl 5:18–25.
4. Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. Fertil Steril. 2005;83:1650–8.
5. Belva F, Henriet S, Van den Abbeel E, Camus M, Devroey P, Van der Elst J, et al. Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. Hum Reprod. 2008;23:2227–38.
6. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. Hum Reprod. 2008;23:1644–53.
7. Pinborg A, Loft A, Aaris Henningsen AK, Rasmussen S, Nyboe Andersen A. Infant outcome of 957 singletons born after frozen embryo replacement: The Danish National Cohort Study 1995–2006. Fertil Steril. 2009 Jul 30.
8. Bergh C, Werner C, Nilsson L, Hamberger L. Cumulative birth rates following cryopreservation of all embryos in stimulated in vitro fertilization (IVF) cycles. J Assist Reprod Genet. 1995;12:191–4.
9. Hyden-Granskog C, Unkila-Kallio L, Halttunen M, Tiitinen A. Single embryo transfer is an option in frozen embryo transfer. Hum Reprod. 2005;20:2935–8.
10. Sutcliffe AG, D'Souza SW, Cadman J, Richards B, McKinlay IA, Lieberman B. Outcome in children from cryopreserved embryos. Arch Dis Child. 1995;72:290–3.
11. Wennerholm UB, Hamberger L, Nilsson L, Wennergren M, Wikland M, Bergh C. Obstetric and perinatal outcome of children conceived from cryopreserved embryos. Hum Reprod. 1997;12:1819–25.
12. Westergaard HB, Johansen AM, Erb K, Andersen AN. Danish National In-Vitro Fertilization Registry 1994 and 1995: a controlled study of births, malformations and cytogenetic findings. Hum Reprod. 1999;14:1896–902.
13. Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari AM, Hyden-Granskog C, et al. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. Hum Reprod. 2010;25:914–23.
14. Nyboe Andersen A, Goossens V, Bhattacharya S, Ferraretti AP, Kupka MS, de Mouzon J, et al. Assisted reproductive technology and intrauterine inseminations in Europe, 2005: results generated from European registers by ESHRE: ESHRE. The European IVF Monitoring Programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). Hum Reprod. 2009;24:1267–87.
15. Wennerholm UB, Soderstrom-Anttila V, Bergh C, Aittomaki K, Hazekamp J, Nygren KG, et al. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. Hum Reprod. 2009;24:2158–72.
16. Aflatoonian A, Oskouian H, Ahmadi S, Oskouian L. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. J Assist Reprod Genet. 2010 Apr 6.
17. Rezazadeh Valojerdi M, Eftekhari-Yazdi P, Karimian L, Hassani F, Movaghar B. Vitrification versus slow freezing gives excellent survival, post warming embryo morphology and pregnancy outcomes for human cleaved embryos. J Assist Reprod Genet. 2009;26:347–54.
18. Aytoz A, Van den Abbeel E, Bonduelle M, Camus M, Joris H, Van Steirteghem A, et al. Obstetric outcome of pregnancies after the transfer of cryopreserved and fresh embryos obtained by conventional in-vitro fertilization and intracytoplasmic sperm injection. Hum Reprod. 1999;14:2619–24.
19. Van den Abbeel E, Camus M, Van Waesberghe L, Devroey P, Van Steirteghem AC. Viability of partially damaged human embryos after cryopreservation. Hum Reprod. 1997;12(9):2006–10.
20. Salumets A, Suikkari AM, Makinen S, Karro H, Roos A, Tuuri T. Frozen embryo transfers: implications of clinical and embryological factors on the pregnancy outcome. Hum Reprod. 2006;21:2368–74.
21. Van Steirteghem AC, Van der Elst J, Van den Abbeel E, Joris H, Camus M, Devroey P. Cryopreservation of supernumerary multicellular human embryos obtained after intracytoplasmic sperm injection. Fertil Steril. 1994;62:775–80.
22. Schieve LA, Ferre C, Peterson HB, Macaluso M, Reynolds MA, Wright VC. Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. Obstet Gynecol. 2004;103:1144–53.
23. Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods. Fertil Steril. 2005;84:611–7.
24. Fedder J, Gabrielsen A, Humaidan P, Erb K, Ernst E, Loft A. Malformation rate and sex ratio in 412 children conceived with epididymal or testicular sperm. Hum Reprod. 2007;22:1080–5.
25. Luke B, Brown MB, Grainger DA, Baker VL, Ginsburg E, Stern JE. The sex ratio of singleton offspring in assisted-conception pregnancies. Fertil Steril. 2009;92:1579–85.