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COMPARISON OF INCIDENCE OF CONGENITAL- ANOMALIES IN DIFFERENT IVF-PROCEDURES

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ABSTRACT

BACKGROUND: A retrospective cohort study was conducted to evaluate and compare the incidence of congenital anomalies in babies and fetuses conceived after four procedures of assisted reproduction techniques (ART)- IVF, ICSI, IVF-FET and ICSI-FET.

MATERIAL AND METHODS The prevalence of congenital anomalies was compared retrospectively between ALL ART babies and fetuses conceived via all procedures of IVF-ICSI (ART) - in vitro fertilization with standard insemination (IVF), IVF with Intracytoplasmic sperm injection (ICSI), IVF with frozen embryo transfer (FET- IVF), and ICSI with frozen embryo transfer (FET-ICSI). Congenital anomalies were described according to European Surveillance of Congenital Anomalies (EUROCAT) classification. The parental backgrounds, biologic parameters, obstetric parameters, and perinatal outcomes were compared between babies and fetuses with and without congenital anomalies. Data were analyzed by the generalized estimating equation.

RESULTS: All IVF-ICSI-FET (ART) evolutionary pregnancies were included in the analysis. Our present study showed 04% babies and fetuses had a congenital anomaly . The major incidence found among the recorded anomalies were congenital heart defects, chromosomal anomalies, and urinary defects. However, the risk of congenital anomalies in babies and fetuses conceived after FET was not increased compared with babies and fetuses conceived after fresh embryo transfer, even when adjusted for confounding factors.

CONCLUSION: There is no increased risk of congenital anomalies in babies and fetuses conceived by fresh versus frozen embryo transfer after in vitro fertilization with and without micromanipulation. Indeed, distribution of congenital anomalies found in our study population is consistent with the high prevalence of congenital heart defects, chromosomal anomalies, and urinary defects that have been found by other authorsalso in children conceived by infertile couples when compared to children conceived spontaneously.

INTRODUCTION

Assisted reproduction technologies (ART) have been used increasingly worldwide since the birth of the first child conceived by in vitro fertilization with standard insemination (IVF) in 1978 [1]. This is a consequence of environment and social behavior changes and rising demand for male and female infertility treatment. The introduction of the in vitro fertilization with intracytoplasmic sperm injection (ICSI) in 1992 allowing the treatment of male infertility [2] and the substantial development of cryobiology have considerably contributed to ART efficiency improvement. A recent meta-analysis showed that the use of frozen embryo transfer (FET) significantly improved clinical and ongoing pregnancy rates [3]. Due to these late major evolutions in technical procedures, it remains essential to evaluate the safety of our practice regarding maternal outcomes and children health.

The evaluation of children health is a complex task due to the coexistence of multiple confounding factors such as technique procedure used to treat infertility, parental background [4,5], and the necessity to perform studies over a long-term period to measure the risk variation. Congenital anomalies are one of the main criteria used for assessing the safety of the procedure and children health. Their prevalence in general population is estimated to be 2.4% between 1998 and 2012 according to the European Surveillance of Congenital Anomalies (EUROCAT) Register [6].

The World Health Organization (WHO) defines them as structural or functional anomalies that occur during intrauterine life and can be identified prenatally, at birth or later in life. The EUROCAT oversees epidemiological surveillance of such anomalies by collecting data on major structural defects caused by abnormal morphogenesis (congenital malformations, deformation, disruptions, and dysplasia), chromosomal abnormalities, inborn errors of metabolism, and hereditary diseases [7].

MATERIALS AND METHODS

Study design/Type of study - This study was a retrospective analysis of fetuses and babies conceived after different ART procedures from ART Centre This study focused on congenital anomalies in four groups of infertility treatment: IVF, ICSI, FET-IVF, and FET-ICSI. These four groups were chosen because they correspond to the present different four techniques used in ART-IVF-ICSI Centre .

Sample size & Duration of study- . data collected during period of three years (JAN 2016 to DEC 2018) under department of Obstetrics and Gynaecology and department of Pediatrics in LN Medical College & JK Hospital, Bhopal.

Inclution & Exclution criteria: The details of treatment with ART were provided by the medical folder, used in the center for recording medical, clinical, and biological data of couples. The parental backgrounds and biologic parameters were filled in using this medical folder. The obstetric parameters were provided by the maternity hospitalization report.

The background characteristics of biologic and obstetric results comprise the following:

a. PARENTAL BACKGROUNDS: maternal and paternal age at conception (years), maternal and paternal body mass index (BMI) (kg/m2),
b. BIOLOGIC PARAMETERS: the day of embryo transfer (D3 or D5) and the number of embryos transferred
c. OBSTETRIC PARAMETERS: pregnancy complications (placenta-previa, high blood pressure induced by pregnancy and gestational diabetes), cesarean section, and the twin pregnancy)

Data collection ART procedures

Ovarian stimulation was performed with conventional protocols based on pituitary control with gonadotropin-releasing hormone (GnRH) agonist or antagonist; administration of follicle stimulating hormone (FSH) urinary

(u-FSH) or recombinant (r-FSH) was used to induce ovulation. In the IVF, oocyte-cumulous-complexes were inseminated with motile spermatozoa. In the case of ICSI, oocyte denudation was performed after oocyte-retrieval (OPU). Subsequently to denudation, oocytes were incubated in a Culture media before ICSI. Although D5 embryo transfer was privileged, depending on embryo quality, transfer on D3 is still punctually proposed to the couple. Poor-quality embryos were discarded [37]. For freezing, VITRIFICATION Technique was used. A maximum of two frozen/ thawed embryos were transferred in the spontaneous ovarian cycle (in normal ovulatory women) or using substitute hormonal treatment (in other cases) for endometrium preparation.

Pregnancies were assessed by blood human chorionic gonadotropin (hCG) detection (>50 UI/L) followed by ultrasound assessment of fetal heartbeat. Data concerning pregnancies from the beginning to the delivery were collected and evaluated accordingly.

Data Analysis :

Perinatal outcomes Data compiled for medical termination of pregnancy (MTP) and deliveries were collected from maternity hospitalization report or a copy of the personal child health record document. The information about the perinatal outcomes and congenital anomalies in the neonatal period provided by the auto- questionnaire filled by parents were verified and validated by a medical report. Perinatal outcomes were preterm birth (PTB) defined by delivery with less 37 weeks gestational age (WGA), low birth weight (LBW) defined by less of 2500 gm and the congenital anomalies.

Congenital anomalies_This study concerns only the congenital anomalies detected at birth or in the neonatal period (within 28 days after birth). The congenital anomalies were coded by a medical doctor using the International Classification of Diseases (ICD10). Only major congenital anomalies were classified according to the European Surveillance of Congenital Anomalies (EUROCAT) guidelines : nervous system; eyes; ears, face, and neck; congenital heart defects; respiratory; orofacial clefts; digestive system; abdominal wall defects; urinary; genital; limb; and other anomalies/syndromes and chromosomal. A child displaying several different malformations was counted in more than one class of malformation, except when a child showed a syndrome.

Population The full cohort included ART clinical pregnancies. This study comprised the MTP and the deliveries (live births, stillbirths after 22 WGA) from IVF, ICSI, FET-IVF, and FET-ICSI procedures . The evolution of pregnancies and neonatal data was collected prospectively.

Ethical approval :Taken

OBSERVATIONS

TABLE (01): BACK GROUND CHARACTERISTICS AND PERINATAL OUTCOMES ACCORDING TO ART PROCEDURES

BACKGROUND CHARACTERSTICS	IVF	ICSI	FET-IVF	FET-ICSI	pvalue
Parentalbackgrounds					
Maternalage(years)	32	33	33	32	0.06
Maternal BMI(kg/m2)	22	24	23	22	<0.01a
Paternalage(years)	34	35	35	35	0.03a
PaternalBMI(kg/m2)	21	22	21	22	0.21
Twin pregnancies	14%	13%	13%	12%	0.72

PERINATAL OUTCOMES	IVF	ICSI	FET-IVF	FET-ICSI	pvalue
Neonatal deaths	0.3%	0.2%	0.3%	0.5%	
Stillbirths	1.2%	0.7%	0.3%	0%	
MTP	1.3%	0.8%	0.7%	1.7%	
PTBc	17.1%	17.4%	16.7%	18.1%	0.12
LBWc	20.3%	20.5%	17.7%	15.7%	0.02a
All anomalies	5.0%	5.1%	3.6%	5.3%	0.29
Singleton	5%	4.3%	2.7%	6.1%	0.26
Twins	4.9%	8.2%	3.9%	1.7%	0.74

Values are *in* (%) or mean (standard deviation)

Invitrofertilization with standard insemination,ICSI invitrofertilization with intracytoplasmic sperminjection,FET frozen embryo transfer, BMI body mass index, DES daughter women exposed in uteroto diethylstilbestrol, MTP medical terminations of pregnancy, PTB preterm birth (<37weeks of gestational age), LBW low birth weight (<2500g) .Statistically significant result, p value<0.05

TABLE (02) DESCRIPTION OF THE INCIDENCE OF DIFFERENT CONGENITAL ANOMALIES

CONGENITALANOMALIESOF	IVF	ICSI	FET-IVF	FET-ICSI [95%CI]	
All anomalies	04%	4.2%	3.5%	3.5%	04
Congenital heart defects(CHD)	(0.3)	(0.8)	(0.6)	(0.1)	0.9
Severe CHD	(0.1)	(0.1)	0	0	
Ventricular septal defect	0	(0.1)	0	0	
Atrial septal defect	(0.1)	(0.3)	(0.01)	0	
Atrioventricular septal defect	0	(0.1)	0	(0.033)	
Tetralogy of Fallot	(0.033)		0	0	
Pulmonary valve atresia	(0.033)	0	0	0	
Mitral valve anomalies	(0.033)	0	0	0	
Chromosomal	(0.3)	(0.4)	0	(0.1)	0.5
Down syndrome	(0.1)	(0.2)	0	(0.1)	
Urinary	(0.3)	(0.5)	0	(0.01)	0.4
Congenital hydronephrosis	(0.26)	(0.5)	0	(0.01)	
Nervous system	(0.1)	(0.2)	(0.01)	(0.1)	0.2
Orofacial clefts	(0.1)	(0.01)	0	0	0.1
Digestive system	(0.1)	(0.1)	(0.01)	0	0.2
Genital	(0.2)	(0.3)	0	0	0.3
Limb	(0.3)	(0.2)	0	(0.01)	0.3

RESULTS

All IVF-ICSI-FET (ART) evolutionary pregnancies were included in the analysis. Our present study showed 04% babies and fetuses had a congenital anomaly . The major incidence found among the recorded anomalies were congenital heart defects, chromosomal anomalies, and urinary defects. However, the risk of congenital anomalies in babies and fetuses conceived after FET was not increased compared with babies and fetuses conceived after fresh embryo transfer, even when adjusted for confounding factors.

STATISTICAL ANALYSIS

All variables were described by proportions N (%) and mean (±standard deviation [±SD]). We then compared the four groups of procedures (IVF, ICSI, FET-IVF, and FET-ICSI) among background characteristics and perinatal outcomes. We used generalized estimating equations (GEE) for correlated data to compare the four groups of procedures (IVF, ICSI, FET-IVF, and FET-ICSI) among background characteristics and perinatal outcomes. The GEE allowed taking into account the correlation between subjects due to multiple pregnancies and siblings (same mother). To analyze if procedures have an impact on the risk of congenital anomalies, we performed an etiological analysis, modeling the congenital anomalies depending on risk factors including the group of procedures.

Firstly, we compared and described the two groups (babies and fetuses with congenital anomalies versus babies and fetuses without congenital anomalies).

Secondly, we performed a multivariate GEE to adjust for confounding factors. All variables with a p value <0.20 in the univariate analysis were included in the multivariate GEE. Using a backward selection method, we finally retained one variable, namely twins, in the final adjusted model. The groups (IVF, ICSI, FET-IVF, and FET-ICSI) were systematically added into the multivariate analysis. Statistical analyses were performed using Statistical Package for Social Sciences, version 17 (SPSS). A two-sided p value of <0.05 was considered as statistically significant.

DISCUSSION

The present study compared the prevalence of congenital anomalies found in four groups of fetuses/babies of different ART - Techniques (IVF, ICSI, FET-IVF, and FET-ICSI).

A supplementary analysis was performed to assess the differences in outcome between fresh embryos transfer compared to frozen embryos transfer. No significant difference was observed in prevalence of babies and fetuses with congenital anomalies versus babies and fetuses without congenital anomalies, even when adjusted for the same confounding factors.

These results also showed that micromanipulation (ICSI) and ovarian stimulation did not impact the malformation incidence. At the same time, our results suggested that there is no increased risk of congenital anomalies after blastocyst transfer (DAY 5) compared to cleavage-stage transfer (DAY 3).

In our indian population, the incidence of congenital anomaly in the four different art- procedures was 04% , which could support the fact that ART children are little more at risk to develop congenital anomalies [12, 40].

Seggers and colleagues have evaluated the prevalence in each subgroup of congenital anomalies, between a subfertility group (139 conceptions after IVF/ICSI and 201 natural conceptions) and a fertile group (4185 conceptions) [41]. Their results showed an increased risk to polydactyly hand in conception after IVF/ICSI versus the subfertility conceived naturally (OR 2.78, 95% CI 1.53– 5.07). Additionally, they found a higher risk of abdominal wall defects (OR 2.43, 95% CI 1.05–5.62), penoscrotal hypospadias (OR 9.83, 95% CI 3.58–27.04), and right ventricular outflow tract obstruction (OR 1.77, 95% CI 1.06–2.97) in the subfertility group versus fertile group.

The major incidence in our sample were found in congenital heart defects chromosomal anomalies and urinary defects. However it is crucial to compare our results with a control group before being able to draw any definitive conclusion. Our results were not representative of ART centers at the national level, particularly in terms of number of embryo transferred.

In the analysis of risk factors on congenital anomaly prevalence in the present study, it appeared that twins were more often observed in the group of babies and fetuses with congenital anomalies. This explains the higher prevalence in cesarean section, preterm, and low birth weight in this group. This observation provides a major argument to support our single embryo transfer policy. In fact, limiting multiple pregnancies minimizes complications during pregnancy and improves the health of children [43]. One of the strengths of this study was the coding of congenital anomalies in the EUROCAT classification. This classification was ensured by the medical doctor in charge of the children follow-up [34]. Embryological medical control and validation of the information were assessed in all the obtained reports or copies of the personal child health record document. Diagnosis of congenital anomalies was established after a medical examination to ensure a high quality of data.

Several independent studies have already described increased risks of congenital anomalies in children born after the ART [8–13]. A systematic review and a meta-analysis [14] of 45 studies allowed evaluating the risk of congenital anomalies in children born after ART versus non-ART children. When pooling the data of 45 studies, results indicated a significant 30% increased the risk of birth defects in children born after ART (risk ratio [RR] 1.32, 95% CI 1.24–1.42). This risk increased when they studied only major birth defects and the singletons (RR 1.36, 95% CI 1.30–1.43) [14]. Besides, a meta-analysis of 24 studies comparing children conceived by IVF and those born after ICSI failed to identify an increase in the risk of congenital anomalies in ICSI children compared with IVF (RR 1.05, 95% CI 0.91–1.20) [15]. Several studies have evaluated the health of children born after FET [16–20]. The latest publications suggested that these children have better perinatal outcomes compared with children born after fresh embryo transfer [21, 22] but have an increased risk of being born large for gestational age (LGA) [23]. The prevalence of congenital anomalies was not different in these children against prevalence in children born after fresh embryo transfer (odds ratio [OR] 0.95, 95% CI 0.71–1.27) [24, 30].

Our results suggested that there was no difference in congenital anomalies between these four procedures , even when adjusted for confounding factors.. Indeed, Pinborg and collaborators highlighted that FET children have a lower risk of LBW than children born after fresh embryo transfer [23].

Moreover, our study included all fetuses from the medical terminations that were made in most cases by a congenital anomaly. The inclusion of babies and fetuses allowed an exhaustive description of our population. However, some birth defects remain undiagnosed in the neonatal period. Our experience following up these children in the long term allowed verifying that the congenital anomalies included in the EUROCAT classification were diagnosed in the neonatal period and not at later ages [33]. Another strength of our study was the selection of confounding factors, which have been identified to have an effect on congenital anomalies or affect the results of the other studies [17, 44].

It would be more pertinent to compare our data with a group of naturally conceived children (NON-IVF), with the EUROCAT Register, and evaluate these anomalies during the childhood period and at later ages. Nevertheless, our preliminary results are in line with the epidemiological studies that have shown an increased risk of cardiovascular anomalies and urogenital and genetic defects for babies of infertile couples [40,].

CONCLUSION

To conclude our study suggest that the incidence of congenital anomalies was not different between fresh or frozen embryo transfer after IVF or ICSI. Consistently with the literature our data showed that the transfer of only one embryo improves the health of the offspring. The association of single embryo transfer policy and the development of cryobiology (vitrification) turns out to be an interesting way to limiting malformation prevalence in children conceived in different ART procedures.

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Ethical approval : Taken

WHAT THIS STUDY ADD TO EXISTING KNOWLEDGE :

The background characteristics and the perinatal outcomes in fetuses/babies conceived using one of the four studied ART procedures did not show significant statistical differences (except for maternal BMI, paternal age, the prevalence of primiparas, and LBW) . The rise of LBW in the IVF and ICSI procedures is in line with the literature

LIMITATION OF OUR STUDY

1. Small sample size
2. Chances of bias
3. Single center trial

REFERENCES

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet Lond. Engl. 1978;2:366.
2. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oo- cyte. Lancet Lond. Engl. 1992;340:17–8.
3. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertiliza- tion cycles: a systematic review and meta-analysis. FertilSteril. 2013;99:156–62.
4. Nygren K-G, Finnström O, Källén B, Olausson PO. Population- based Swedish studies of outcomes after in vitro fertilisation. ActaObstetGynecol Scand. 2007;86:774–82.
5. Brison DR, Roberts SA, Kimber SJ. How should we assess the safety of IVF technologies? ReprodBioMed Online. 2013;27:710–21.
6. EUROCAT—European Surveillance of Congenital Anomalies. Number of cases and prevalence per 10,000 births of all anomalies, for all full member countries, from 1980–2012. 2015. http://www.eurocat-network.eu/accessprevalencedata/prevalencetables. Accessed 25 Apr 2016
7. Boyd PA, Haeusler M, Barisic I, Loane M, Garne E, Dolk H. Paper 1: the EUROCAT network—organization and processes†. Birt Defects Res A ClinMolTeratol. 2011;91:S2–15.
8. Lancaster PA. Health registers for congenital malformations and in vitro fertilization. ClinReprodFertil. 1986;4:27–37.
9. Rimm AA, Katayama AC, Diaz M, Katayama KP. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. J Assist Reprod Genet. 2004;21:437–43.
10. Bonduelle M, Wennerholm U-B, Loft A, Tarlatzis BC, Peters C, Henriet S, et al. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injec- tion, in vitro fertilization and natural conception. Hum. Reprod. Oxf. Engl. 2005;20:413–9.
11. Olson CK, Keppler-Noreuil KM, Romitti PA, Budelier WT, Ryan G, Sparks AET, et al. In vitro fertilization is associated with an increase in major birth defects. FertilSteril. 2005;84:1308–15.
12. Källén B, Finnström O, Lindam A, Nilsson E, Nygren K-G, Otterblad PO. Congenital malformations in infants born after in vitro fertilization in Sweden. Birt Defects Res A ClinMolTeratol. 2010;88:137–43.
13. Wen SW, Leader A, White RR, Léveillé M-C, Wilkie V, Zhou J, et al. A comprehensive assessment of outcomes in pregnancies conceived by in vitro fertilization/intracytoplasmic sperm injection. Eur J ObstetGynecolReprod Biol. 2010;150:160–5.
14. Hansen M, Kurinczuk JJ, Milne E, de Klerk N, Bower C. Assisted reproductive technology and birth defects: a systematic review and meta-analysis. Hum Reprod Update. 2013;19:330–53.
15. Wen J, Jiang J, Ding C, Dai J, Liu Y, Xia Y, et al. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. FertilSteril. 2012;97:1331–7.4
16. Wennerholm UB, Albertsson-Wikland K, Bergh C, Hamberger L, Niklasson A, Nilsson L, et al. Postnatal growth and health in chil- dren born after cryopreservation as embryos. Lancet Lond Engl. 1998;351:1085–90.
17. Källén B, Finnström O, Nygren KG, Olausson PO. In vitro fertili- zation (IVF) in Sweden: risk for congenital malformations after different IVF methods. Birt. Defects Res. A. Clin. Mol. Teratol. 2005;73:162–9.
18. 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 compar- ison with outcome data of fresh ICSI and IVF cycles. Hum. Reprod. Oxf. Engl. 2008;23:2227–38.
19. Aflatoonian A, MansooriMoghaddam F, Mashayekhy M, Mohamadian F. Comparison of early pregnancy and neonatal out- comes after frozen and fresh embryo transfer in ART cycles. J Assist Reprod Genet. 2010;27:695–700.
20. Pinborg A, Loft A, AarisHenningsen A-K, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995-2006. FertilSteril. 2010;94:1320–7.
21. Wennerholm U-B, Henningsen A-KA, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, et al. Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. Hum. Reprod. Oxf. Engl. 2013;28:2545–53.
22. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. FertilSteril. 2012;98:368–77.9
23. Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? Hum. Reprod. Oxf. Engl. 2014;29:618–27.
24. Pelkonen S, Hartikainen A-L, Ritvanen A, Koivunen R, Martikainen H, Gissler M, et al. Major congenital anomalies in children born after frozen embryo transfer: a cohort study 1995- 2006. Hum. Reprod. Oxf. Engl. 2014;29:1552–7.
25. De Mouzon J, Bachelot A, Spira A. Establishing a national in vitro fertilization registry: methodological problems and analysis of suc- cess rates. Stat Med. 1993;12:39–50.
26. FIVNAT. Pregnancies and births resulting from in vitro fertilization: French national registry, analysis of data 1986 to 1990. FIVNAT (French In Vitro National). FertilSteril. 1995;64:746–56.
27. Olivennes F, Schneider Z, Remy V, Blanchet V, Kerbrat V, Fanchin R, et al. Perinatal outcome and follow-up of 82 children aged 1- 9 years old conceived from cryopreserved embryos. Hum. Reprod. Oxf. Engl. 1996;11:1565–8.
28. Olivennes F, Kerbrat V, Rufat P, Blanchet V, Fanchin R, Frydman R. Follow-up of a cohort of 422 children aged 6 to 13 years con- ceived by in vitro fertilization. FertilSteril. 1997;67:284–9.
29. Epelboin S. Children born of ICSI. J GynécologieObstétriqueBiolReprod. 2007;36(Suppl 3):S109–13.
30. Sagot P, Bechoua S, Ferdynus C, Facy A, Flamm X, Gouyon JB, et al. Similarly increased congenital anomaly rates after intrauterine insemination and IVF technologies: a retrospective cohort study. Hum. Reprod. Oxf. Engl. 2012;27:902–9.
31. Cassuto NG, Hazout A, Bouret D, Balet R, Larue L, Benifla JL, et al. Low birth defects by deselecting abnormal spermatozoa be- fore ICSI. ReprodBioMed Online. 2014;28:47–53.
32. Agence de la Biomédecine. Le rapport annuelmédicaletscientifique 2014. 2014a. http://www.agence-biomedecine.fr/annexes/bilan2014/donnees/sommaire-proc.htm. Accessed 16 Mar 2016
33. Boyer M, Meddeb L, Pauly V, Boyer P. Suivi des enfants de l'AMP: Expérience d'un centrefrançais. Physiol. Pathol. ThérapieReprod. Chez L'humain. Paris: Springer; 2011. p. 665–76. Meddeb L, Boyer M, Pauly V, Tourame P, Rossin B, Pfister B, et al. Procedure used to follow-up a cohort of IVF children. Interests and limits of tools performed to longitudinal follow up for a monocentric cohort. Rev Dépidémiologie Santé Publique. 2011;59:97–105.
34. Anzola AB, Pauly V, Geoffroy-Siraudin C, Gervoise-Boyer M-J, Montjean D, Boyer P. The first 50 live births after autologous oo- cytevitrification in France. J Assist Reprod Genet. 2015;32:1781– 7.
35. Merlet F. Regulatory framework in assisted reproductive technolo- gies, relevance and main issues. Folia HistochemCytobiol Pol AcadSci Pol HistochemCytochem Soc. 2009;47:S9–12.
36. Boyer P, Boyer M. Non invasive evaluation of the embryo: mor- phology of preimplantation embryos. GynécologieObstétriqueFertil. 2009;37:908–16.
37. Lie RT, Lyngstadaas A, Ørstavik KH, Bakketeig LS, Jacobsen G, Tanbo T. Birth defects in children conceived by ICSI compared with children conceived by other IVF-methods; a meta-analysis. Int J Epidemiol. 2005;34:696–701.
38. Wennerholm U-B, Söderström-Anttila V, Bergh C, Aittomäki K, Hazekamp J, Nygren K-G, et al. Children born after cryopreserva- tion of embryos or oocytes: a systematic review of outcome data. Hum. Reprod. Oxf. Engl. 2009;24:2158–72.
39. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012;366:1803–13.
40. Seggers J, de Walle HEK, Bergman JEH, Groen H, Hadders-Algra M, Bos ME, et al. Congenital anomalies in offspring of subfertile couples: a registry-based study in the northern Netherlands. FertilSteril. 2015;103:1001–10.e3
41. Agence de la Biomédecine. Le rapport annuelmédicaletscientifique 2014. 2014b. http://www.agence-biomedecine.fr/annexes/bilan2014/donnees/sommaire-proc.htm. Accessed 19 Jan 2017.
42. Sazonova A, Källen K, Thurin-Kjellberg A, Wennerholm U-B, Bergh C. Neonatal and maternal outcomes comparing women un- dergoing two in vitro fertilization (IVF) singleton pregnancies and women undergoing one IVF twin pregnancy. FertilSteril. 2013;99:731–7.
43. Glinianaia SV, Rankin J, Wright C. Congenital anomalies in twins: a register-based study. Hum ReprodOxf Engl. 2008;23:1306–11.
44. Kalfa N, Paris F, Soyer-Gobillard M-O, Daures J-P, Sultan C. Prevalence of hypospadias in grandsons of women exposed to di- ethylstilbestrol during pregnancy: a multigenerational national co- hort study. FertilSteril. 2011;95:2574–7.
45. Tournaire M, Epelboin S, Devouche E, Viot G, Le Bidois J, Cabau A, et al. Adverse health effects in children of women exposed in utero to diethylstilbestrol (DES). Therapie. 2016;71:395–404.