J Assist Reprod Genet (2016) 33:711–717
DOI 10.1007/s10815-016-0714-4

ASSISTED REPRODUCTION TECHNOLOGIES

Obstetric outcome and incidence of congenital anomalies in 2351 IVF/ICSI babies

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Received: 17 January 2016 / Accepted: 30 March 2016 / Published online: 26 April 2016

Abstract
Purpose The aim of this study was to provide a comprehensive follow-up of fetal and perinatal outcome and the incidence of congenital anomalies in babies born after fresh embryo transfers compared to those conceived spontaneously in infertile couples.
Methods Retrospective comparative analysis of all clinical pregnancies from fresh cleavage-stage embryo transfer cycles (IVF and ICSI) compared with infertile patients who conceived spontaneously in the same time period (control). Congenital anomalies were classified following the European Surveillance of Congenital Anomalies (EUROCAT) classification.
Results A total of 2414 assisted reproductive technology (ART) pregnancies were compared to 582 spontaneous conceptions in the control infertile group representing 2306 deliveries. No significant differences were found in pregnancy outcome between the two groups (delivery rate, abortion rate, ectopic pregnancies, medical abortions for fetal anomalies, single and twins mean gestational age, and weight at delivery). A significant difference ($p < 0.001$) was found in the twin (21.3 vs 2.3 %) and triplet rates (2.3 vs 0 %). A total of 2351 babies were delivered in the ART group and 449 in the control group. A total of 90 babies (3.8 %) were diagnosed with a major congenital anomaly in the ART group and 15 (3.3 %) in the control group (p = ns). The overall rate of major congenital anomalies (105/2800) in ART and spontaneous pregnancies in infertile couples was significantly higher when compared to the EUROCAT 2.0 versus 3.75 % ($p = 0.0002$).
Discussion Babies born after ART treatments and from spontaneous conception in infertile couples had rates of congenital anomalies higher than those recorded by the EUROCAT. However, the rates of anomalies were not different within the infertile population whether conceived by ART or spontaneously. These data suggest that the diagnosis of infertility in itself is the common denominator for the increase in the rates of anomalies seen in both ART and spontaneous conceptions.

Capsule The rates of congenital anomalies in children born from infertile couples either with ART or spontaneously are higher than those reported in non-infertile couples. These data suggest that the diagnosis of infertility in itself is the common denominator for the increase in the rates of anomalies seen in both ART and spontaneous conceptions.

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Keywords ART · Pregnancy outcome · Neonatal anomalies · IVF · ICSI

Introduction

Assisted reproductive technologies (ART), including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [1, 2], represent the mainstay of treatment for many infertile couples. Since 1978, the year of the first baby born after IVF in the UK, the number of pregnancies and births after ART has been increasing exponentially. The last summary report estimated that more than five million children have been born after IVF or ICSI cycles [3]. According to the last report

(2012) of the Italian National Assisted Reproduction Register, 15,670 pregnancies were obtained with ART and 11,974 births were recorded, while 1667 (13.2 %) pregnancies were lost to follow-up [4].

Congenital birth defects and early/premature births are common and complex conditions related to perinatal/infant mortality and morbidity throughout the world. Particularly, babies born from infertile couples after ART treatments represents a major clinical and epidemiological issue, especially considering that 1 to 2 % of babies born annually are conceived after ART procedures. The evaluation of risk for congenital defects and/or premature delivery is a fundamental step for an adequate pre-conception counseling. Recent advances in fetal/neonatal care have improved clinical outcomes for these babies; however, major congenital defects and the associated disabilities have a big impact on children and families' lives with social and ethical implications.

Several outcome studies have reported increased complication rates in infants conceived with ART compared with the general population [5–7]. Some of the complications have been attributed to a higher frequency of multiple births and to the increased risk of premature delivery; however, other reports indicate that also singleton IVF children have a greater risk of low birth weight and of congenital anomalies [8–16].

The aim of this study is twofold: (a) to describe the obstetrical outcomes and the rate of congenital anomalies in babies born after transfer of fresh, cleavage-stage embryos obtained with IVF/ICSI in infertile couples referred to a single large tertiary care ART center [17] and (b) to compare these rates to those observed in babies born from infertile couples conceiving spontaneously and to those reported in the general population.

Materials and methods

All pregnancies obtained after the transfer of at least one fresh cleavage stage embryo obtained by IVF or ICSI at Humanitas Fertility Center (Milan, Italy) from January 1996 to May 2009 were included in the study. During the study period, blastocyst culture was not yet routinely offered due to legal restrictions allowing the insemination of a maximum of three mature oocytes, thus limiting prolonged embryo culture [18, 19].

The control group included spontaneous conceptions achieved in infertile couples during the same time period. These infertile couples were selected from spontaneously pregnant patients who were evaluated in our unit and had either failed to achieve a pregnancy after at least 12 months of unprotected intercourse, or had a previous IVF/ICSI or IUI/OI treatment in our or other units at least 3 months prior and were waiting to undergo another treatment cycle (control group inclusion criteria). No treatment was performed in the control group. Pregnancies ≥24 weeks were considered deliveries, pregnancies ending in spontaneous abortions were divided in miscarriages prior to 12 and >12 weeks. Pregnancies concluded with a therapeutic abortion for fetal anomaly and ectopic pregnancies were also considered in the study, as well as anomalies diagnosed in the perinatal and post-natal periods.

In addition to obstetricians, a team of clinical psychologists assessed the course and the outcome of the pregnancies through a structured questionnaire administered by phone calls and direct contact at 1 month (perinatal period) after delivery. The length of time for the follow-up period was in line with data presented in the National and International Registers. Direct consultations with family doctors and pediatricians were sought in all cases where an anomaly was suspected.

The questionnaire was divided into three main headings: history of the conception and pregnancy, birth defects, and chromosome or other genetic analysis if performed.

Major congenital anomalies were classified according to the European Surveillance of Congenital Anomalies (EUROCAT) guidelines, a registry that reports major but not minor anomalies [20]. This classification was selected because it is the largest European registry and it is utilized by the Italian ART Registry [4] as well as by the ESHRE European Registry [21]. The incidences of anomalies after the transfer of cleavage-stage fresh embryo cycles (ART) [17] were compared to those observed in the control group of spontaneous pregnancies in infertile couples and to those observed in the general population during the years of study.

Major defects of the central nervous system or neural tube, cardiovascular system, gastrointestinal tract, genital and urinary tract, muscular and skeletal system, cleft, chromosomal, metabolic (phenylketonuria), and genetic defects (like epidermolysis bullosa, multiple bone exostoses and cystic fibrosis), and complex malformative syndromes were included in the classification of major anomalies. Robertsonian translocations, Down syndrome, and Klinefelter syndrome were among the chromosome structural and numerical defects encountered.

Data were analyzed with Stata 13.0; chi-Square with Fisher exact test was performed to analyze ART vs. control group conceptions. $P < 0.05$ was considered statistically significant.

Results

A total of 10,706 ART cycles were started during the years of analysis leading to 8744 transfers of at least one cleavage stage embryo and resulting in 2414 pregnancies; 688 (28.5) were IVF cycles, 1489 (61.7 %) ICSI cycles, and 237 (9.8 %) ICSI-TESE (using frozen testicular sperm). The mean number of cycles was 1.8 ± 1.1, and the mean number of embryos transferred was 2.7 ± 0.7. Mean women's age at the cycle start was 34.5 ± 3.8 years, male age was 37.7 ± 5.0, basal FSH

7.2 ± 2.7, female body mass index (BMI) was 21.9 ± 3.4, primary infertility 1823 (75 %), and the mean years of infertility 3.5 ± 2.3 (Table 1). The main indications for ART treatment were tubal diseases in 514 (21.2 %), endometriosis in 160 (6.6 %), reduced ovarian reserve in 7 (0.3), ovulatory dysfunction in 32 (1.3 %), unexplained 195 (8.1 %), male factor in 733 (30.4 %), severe male factor, defined a total motile count (TMC) less than one million, or testicular frozen sperm in 757 (31.4 %) mixed male and female factor in 16 (0.7 %). In the period considered (1996–2009), out of 13,489 infertile couples assessed, there were 582 spontaneous conceptions used as control group (no fertility treatment at all). The mean women's age at conception was 34.7 ± 3.7 years, the male age was 37.2 ± 4.6, the BMI was 22.7 ± 2.3, primary infertility incidence was 34.5 (201), and the years of infertility were 3.0 ± 1.9, and basal FSH 7.4 ± 2.9 (Table 1). The main indications for couples seeking treatment but conceived spontaneously were tubal diseases in 20 (3.4 %), endometriosis in 12 (2.1 %), reduced ovarian reserve in 1 (0.2 %), ovulatory dysfunction in 32 (5.5 %), unexplained 331 (8.1 %), male factor in 158 (27.1 %), and mixed male and female factor in 28 (4.8 %). These results are presented in Table 1.

No significant differences were found in female, male mean age, and basal FSH level at conception between ART and infertile couples who conceived spontaneously except for statistically significant difference in female BMI. Primary infertility and years of infertility were significantly higher in ART pregnancies. A significant difference was found between most diagnosis of infertility in the spontaneous pregnancy group and the main indications for ART treatment. A significant lower rate of tubal diseases, endometriosis, male factor, and severe male factor was found in the spontaneous pregnancy control group, while in this group, there were statistically significant higher rates of ovulatory dysfunction or unexplained diagnosis (Table 1).

In ART cycles, a total of 1867 (77.3 %) deliveries were recorded, of which 418 (17.3 %) ended in spontaneous abortions ≤12 weeks and 34 (1.4 %) >12 weeks, 23 (1 %) were therapeutic abortions, 34 (2.4 %) ectopic pregnancies, and 7 (0.3 %) pregnancies were lost to the follow-up.

In the spontaneous conception group, a total of 582 pregnancies were recorded of which 439 (76.0 %) resulted in deliveries while 103 (17.8 %) ended in spontaneous abortions ≤12 weeks and 8 (1.4 %) >12 weeks. In addition, 4 (0.7 %) were therapeutic abortions, 15 (2.6 %) were ectopic pregnancies, and 13 (2.2 %) pregnancies were lost to follow-up.

No significant differences were found in pregnancy outcome (delivery, abortion, therapeutic abortion, and ectopic rate in comparison with ART pregnancies. Pregnancy outcomes are summarized in Table 2.

Out of 1867 ART deliveries, 1424 (76.4 %) were singleton pregnancies, 398 (21.3 %) twins, and 43 (2.3 %) triplets. A total of 2351 babies were delivered, 1214 females (51.6 %) and 1137 males (48.4 %). Mean gestational age at the delivery was 37.2 ± 3 weeks, with differences between singleton pregnancies (38.6 ± 2.3 weeks), twins (35.5 ± 2.6 weeks), and triplets (32.6 ± 2.2 weeks). Mean birth weight at delivery was 2712.6 ± 744.5 g, with significant differences between singletons (3078.0 ± 609.9 g), twins (2228.9 ± 534.2 g), and triplets (1639.1 ± 420.6 g) (Table 3).

Out of 439 spontaneous conceptions deliveries, 429 (97.7 %) were singleton pregnancies, 10 (2.3 %) twins and no triplets. A total of 449 babies were delivered: 221 females (49.2 %) and 228 males (50.8 %). Mean gestational age at the delivery was 37.1 ± 2.8 weeks, with a significant difference

Table 1 Characteristics of patients in the ART and in the spontaneous conception groups

	ART	Percent	Spontaneous	Percent	*p*
Pregnancies	2414		582		
Female age	34.5 ± 3.8		34.7 ± 3.7		0.208
Male age	37.7 ± 5.0		37.2 ± 4.6		0.028
Female basal FSH (mU/ml)	7.2 ± 2.7		7.4 ± 2.9		0.114
BMI	21.9 ± 3.4		22.7 ± 2.3		<0.001
Primary infertility	1823	75.5	201	34.5	<0.001
Years of infertility	3.5 ± 2.3		3.0 ± 1.9		<0.001
Tubal diseases	514	21.3	20	3.4	<0.001
Endometriosis	160	6.6	12	2.1	<0.001
Reduced ovarian reserve	7	0.3	1	0.2	0.338
Irregular ovulation	32	1.3	32	5.5	<0.001
Unexplained	195	8.1	331	56.9	<0.001
Male factor	733	30.4	158	27.1	0.051
Severe male factor	757	31.4	0	0	<0.001
Mixed male and female factor	16	0.7	28	4.8	<0.001

Table 2 Pregnancy outcomes in ART and spontaneous conception in infertile patients

	ART	Percent	Spontaneous	Percent
Delivery	1867	77.3	439	76.0
Abortion <12 weeks	418	17.3	103	17.8
Abortion >12 weeks	34	1.4	8	1.4
Ectopic pregnancy	65	2.7	15	2.6
Therapeutic abortion	23	1.0	4	0.7
Lost to follow-up	7	0.3	13	2.2

between singleton pregnancies (38.7 ± 2.1 weeks) and twins (35.1 ± 2.2 weeks). Mean birth weight at delivery was 2740.3 ± 530.9 g, with significant differences between singletons (3126.2 ± 672 g), twins (2201 ± 709 g) (Table 3).

Although a significant difference was found between ART and spontaneous pregnancies in the twin rate (21.3 vs 2.3 %) and triplets rate (2.3 % vs 0 %), no statistically significant differences were found between the two groups in gestational age at delivery, birth weight, and male to female ratio both in single and in twins pregnancies (p = ns).

In ART deliveries, major congenital anomalies were observed in 90 (3.8 %) babies, while minor congenital anomalies were found in 11 (0.5 %) babies (Table 4). The most frequent major congenital defects involved cardiovascular (25, 1.1 %), genitourinary (21, 0.9 %), and gastrointestinal (9, 0.4 %) systems; other anomalies were two congenital deafness and one right-hand cutaneous mastocytoma. The distribution of congenital defects is reported in Table 4. Forty nine of all major anomalies were detected in singleton pregnancies representing an incidence of 3.4 % (49/1426), 36 were reported in twins for an incidence of 4.5 % (36/796), and 5 in triplets for an incidence of 3.9 % (5/129).

Minor anomalies were detected in 11 newborns (0.5 %) with 4 involving the genitourinary system, 1 the cardiovascular system, and 1 the gastrointestinal system. Other minor anomalies were two inguinal hernias, one auricular malformation, one polythelia, and one lacrimal duct stenosis (Table 4).

In the control group, major congenital anomalies were observed in 15 (3.3 %) babies, while minor congenital anomalies were found in 4 babies (0.9 %). Of all the major anomalies, 4 (25 %) involved the cardiovascular system, 3 (18.8 %) the nervous system, 2 (12.5 %) the genitourinary, and 2 (12.5 %) the gastrointestinal system, 2 (12.5 %) were chromosomal defects, 1 (6.3 %) was a muscle and bone anomaly and 1 (6.3 %) was a cleft palate abnormality. Minor anomalies (0.9 %) involved the genitourinary system (n = 2), 1 cardiovascular and 1 gastrointestinal system (Table 3). Major anomalies were detected in 13 singleton pregnancies and 2 in twins (Table 4).

There were no statistically significant differences in the rates of anomalies between babies born after fresh IVF/ICSI transfer and our controls of infertile patients conceiving spontaneously without any treatment. No significant differences was found in the incidence of major anomalies between single and multiple pregnancies in ART vs spontaneous conceptions (p = 0.071).

The rates of major congenital anomalies in the general population reported by EUROCAT (data uploaded by all full member registries during the period 1996–2009 were (221, 849/10,824,229) 2.05 versus 3.75 % (105/2800) in our total infertile population (p = <0.0002) and for Italy in the same period 1.46 % (28,258/1,930,337) [22].

Discussion

The risk of congenital anomalies after ART is still a debated and controversial issue. In this comprehensive obstetrical and neonatal follow-up, the rates of congenital anomalies after ART were not significantly different than those observed in spontaneous conceptions from our infertile population. However, when these rates were compared to those reported by the EUROCAT (which records major but not minor congenital anomalies in the general population), they were significantly higher than in the general population.

The EUROCAT recorded a total prevalence of major congenital anomalies of 2.05 % for the years 1996–2009. The prevalence of chromosomal anomalies was 0.15 %, while congenital heart defects were the most common in the

Table 3 Obstetrical outcome of ART and spontaneous pregnancies in infertile patients

	ART Single	Spontaneous	ART Twins	Spontaneous	ART Triplets[b]
Babies delivered	1426	429	796	20	129
Gestational age[a]	38.6 ± 2.3	38.7 ± 2.1	35.5 ± 2.6	35.1 ± 2.2	32.6 ± 2.2
Birth weight[a]	3.078 ± 609	3.126 ± 672	2228 ± 534	2.201 ± 709	1639.1 ± 420.6
Males	690 (48.4 %)	203 (47.3 %)	392 (49.3 %)	9 (45.0 %)	55 (42.6 %)
Females	736 (51.6 %)	226 (52.7 %)	404 (50.7 %)	11 (55.0 %)	74 (57.4 %)

[a] Mean ± SD

[b] No triplets in spontaneous pregnancies

Table 4 Types and frequency of congenital defects in newborns from ART and spontaneous pregnancies

	ART	Percent	Spontaneous	Percent
Newborns	2351		449	
Major congenital anomalies[a]	*90*	3.8	*15*	3.3
Nervous system	5	0.2	1	0.2
Cardiovascular system	25	1.1	3	0.7
Gastrointestinal system	9	0.4	2	0.4
Genitourinary system	21	0.9	3	0.7
Muscle and/or bone anomalies	6	0.3	1	0.2
Chromosomal defects	7	0.3	2	0.4
Cleft lip/palate abnormalities	1	0.0	0	0.0
Metabolic anomalies	2	0.1	0	0.0
Other genetic defects	3	0.1	2	0.4
Malformation syndromes	7	0.3	0	0.0
Other	3	0.1	1	0.2
Minor congenital anomalies	*11*	0.5	*4*	0.9
Nervous system	0	0.0	1	0.2
Cardiovascular system	1	0.0	1	0.2
Gastrointestinal system	1	0.0	1	0.2
Genitourinary system	4	0.2	1	0.2
Other	5	0.2	0	0.0

The complex malformation syndromes in ART pregnancies include the following:

Congenital heart disease (pulmonary valve stenosis) + cystic fibrosis

Pierre-Raden syndrome + cleft palate

Malformations of the kidneys, bowel, and bones

Left cryptorchidism, feet varus-supine

Hydrocephalus + hip dysplasia

Down syndrome + cardiac malformation (single chamber)

Cardiac malformation (interventricular defect) + hypospadia

Chromosome anomalies

1 Robertsonian translocation

1 Down syndrome 45XY,der (21,22) q10, q10

4 Down syndrome

3 Klinefelter syndrome (47XXY)

Metabolic anomalies

2 Phenylketonuria

Genetic anomalies

1 Multiple bone exostosis

1 Epidermolysis bullosa

1 Cystic fibrosis

[a] In ART deliveries of all the major anomalies, 49 were detected in singleton pregnancies (54.4 %), 36 (40 %) in twins, and 5 (5.6 %) in triplets. In spontaneous conception of infertile patients of all the major anomalies, 13 were detected in singleton pregnancies (86.7 % and 2 (13.3 %) in twins ($p = 0.071$))

non-chromosomal subgroup, at 0.70 %, followed by limb defects (0.39 %) and anomalies of the urinary (0.27 %) and nervous system (0.12 %) [22].

In our group of ART children, the prevalence of major anomalies was higher (3.8 vs 2.05 % in the EUROCAT), but the distribution of the different anomalies was quite similar: the most frequent anomalies were heart defects both in the major and minor anomaly categories (1 % in our newborns vs 0.7 % in the EUROCAT), followed by chromosomal anomalies (0.2 % in our patients vs 0.15 %) and anomalies of the urinary system (0.8 vs 0.2 %).

Reassuringly, our data showed that in pregnancies after ART, both the early and late abortion rates as well as the incidence of therapeutic abortions and ectopic pregnancies

were comparable with that observed in our controls of infertile patients after spontaneous conceptions (SC).

In our ART patients, the principal complication leading to a reduced gestational age at the delivery and low birth weight was related to the high rate of multiple pregnancies.

However, based on meta-analyses, singleton births after assisted conception (AC) have a doubled risk of preterm birth [16, 23]. According to another study, low birth weight and premature delivery were not statistically significant after adjustment for age, area of residence, BMI, smoking, and twin births [24].

Moreover, in the USA, clinical data births after ART showed that infertile women using treatment for the first time or nulliparous women were more likely to have low birth weight babies when compared with population-based birth certificate data [12, 25].

Multiple pregnancies are the most powerful predictive factor for adverse maternal, obstetrical, and perinatal outcomes, so these complications may be prevented using single embryo transfer.

According to a recent article, analyzing data reported to the US National Assisted Reproductive Technology Surveillance System during 2011, for patients younger than 35 years of age undergoing IVF with a favorable prognosis, single embryo transfer was associated with the highest chance for a good perinatal outcome [26].

Another recent paper has analyzed results of ART after the Turkish government implemented the policy of limiting the number of transferred embryos to one for women under the age of 35 and to two for women older than 35 years. The results demonstrated that single embryo transfer had a pregnancy rate comparable to double-embryo transfer with significantly reduced multiple pregnancy rates [27].

Single embryo transfer may be considered a feasible policy to improve not only clinical outcomes but also cost-effectiveness of ART procedures, without affecting pregnancy rate in selected cases and should be strongly encouraged in the couples with good reproductive prognosis.

Concerning the risk of congenital anomalies in babies born from infertile couples enrolled in ART program, there is increasing evidence that infertility in itself is an independent risk factor for obstetrical complications and adverse perinatal outcomes, even without the addition of ART. The results obtained in this study confirm the concept proposed by many researchers that infertility, with or without ART, is a condition leading to increased risk of birth defects [28–31]. Several studies have indicated that ART is associated with a significantly higher risk of congenital defects in newborns when compared to spontaneous conception [32]. Moreover, a recent meta-analysis published by Wen et al. reviewed 46 studies consisting 124,468 ART babies (ICSI/FIVET) with a pooled risk estimation of 1.37 (95 % CI 1.26–1.48) [33]. Only one Chinese multicenter study provided different results showing a lower total rate of birth defects of 1.23 %, comparable to that of the Chinese general population (1.35 %) [34]. Rimm et al. [30] in their meta-analysis reviewed 19 studies on major congenital anomalies and reported an overall odds ratio (OR) of 1.29 (95 % CI 1.01–1.67) for babies conceived with ART compared to spontaneously conceived ones. However, when taking infertility into account, the new adjusted OR became 1.01 (95 % CI 0.82–1.23). Our study on a large number of pregnancies (2414) and deliveries (1867) all from a single center, with a nearly complete follow up and inclusive of complications at birth and at any stage of gestational age, confirms that infertility in itself is a risk factor for increased incidence of congenital anomalies [28, 31, 35–37].

Women undergoing ART are often older and therefore at a plausible increased risk of producing abnormal gametes responsible for poorer obstetrical and perinatal outcomes. Likewise, it is also plausible to associate poorer outcomes to the assisted reproduction procedures themselves in addition to the patients' characteristics. As it stands, however, the effect of assisted reproduction procedures on the health of children born using ART is not fully understood and our data is suggestive that infertility in itself is a risk factor for congenital anomalies given the equal rate of anomalies recorded in infertile patients conceiving spontaneously [27, 38].

Acknowledgments We thank the medical and embryological staff of the Department of Gynecology, Division of Gynecology and Reproductive medicine, Humanitas Fertility Center Humanitas, who contributed to the results of this study. All the authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Financial support and disclosure This investigation has been partially supported by a finalized grant from Lombardy County, Italy (DGR 7255, July 3, 2008).

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