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# Obstetric and neo-natal outcomes of ICSI cycles using pentoxifylline to identify viable spermatozoa in patients with immotile spermatozoa

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**KEY MESSAGE**

Safety concerns have been raised regarding the use of pentoxifylline to identify viable spermatozoa for intracytoplasmic sperm injection. In the current study this treatment does not appear to increase adverse obstetric and neo-natal outcomes; however, the cohort was small.

A B S T R A C T

Pentoxifylline (PF) represents an effective tool in stimulating motility and identifying viable spermatozoa in intracytoplasmic sperm injection (ICSI) patients presenting exclusively with immotile spermatozoa. However, its use is not universally accepted for its possible detrimental effects on oocytes, embryos or newborns. To evaluate whether PF use may affect obstetrical/neo-natal outcomes, 102 patients achieving a clinical pregnancy after a PF-ICSI in four IVF units in Spain and Italy were followed up after delivery. Neo-natal malformations were classified according to the World Health Organization *International Classification of Diseases* (ICD-10, range Q00-Q99). Malformation rate was compared with data published by other groups regarding children conceived by conventional IVF or ICSI reporting a 5.3% and 4.4% frequency of ICD-10 codes, respectively. Of 134 clinical pregnancies, 122 babies (82 singletons and 40 twins) were registered. Among singletons, the rates of low birthweight (≤2500 g) and preterm birth (<37 weeks) were 6.1% and

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12%, respectively. Regarding malformation rate per live births, 4/122 (3.3%, 95% confidence interval: 0.9–8.2%) babies with ICD-10 malformations were recorded. This is the first report on neo-natal outcomes deriving from PF-ICSI. Although based on a limited cohort, results do not suggest an increase of adverse outcomes, including malformation rates, following this procedure.



## Introduction

The development of intracytoplasmic sperm injection (ICSI) has been a breakthrough in the treatment of infertility. This technique also allows the access to IVF procedures to patients with a severe male factor such as those presenting exclusively with immotile spermatozoa, a condition known as 'absolute asthenozoospermia' (Ortega et al., 2011; Rubino et al., 2016). It represents a rare condition due either to sperm cell death (necrozoospermia) or to defects of the sperm motor apparatus related to specific genetic disorders such as the primary ciliary dyskinesia or the dysplasia of fibrous sheath (Dávila Garza and Patrizio, 2013; Nagy, 2000). Moreover, since sperm motility is enhanced during epididymal maturation, gametes retrieved by testicular sperm extraction (TESE) are often characterized by the absence of sperm motility, especially after a freezing/thawing procedure. For these conditions, ICSI represents a demanding technical procedure since immotile viable spermatozoa cannot be easily distinguished from immotile non-viable spermatozoa (Rubino et al., 2016). Three main strategies have been developed in order to improve ICSI results when it is not possible to obtain any motile spermatozoa or to identify living immotile sperm spermatozoa from the ejaculate/TESE samples: (i) the use of the hypo-osmotic swelling test; (ii) the sperm tail flexibility test; and (iii) the in-situ use of pharmacological agents such as caffeine (Garbers et al., 1971) or xanthine derivates such as theophylline (Loughlin and Agarwal, 1992) and pentoxifylline (PF) (Griveau et al., 2006; Kovacic et al., 2006; Sharma and Agarwal, 1997). More specifically, the use of PF is widely used in the daily laboratory routine as a selection method to identify viable spermatozoa. It can be easily added to the sperm sample before the ICSI procedure in order to drive the flagellar movement of live sperm cells through the inhibition of 3',5'-nucleotidase phosphodiesterase and the resulting increased concentration of intracellular cyclic nucleotides (Nassar et al., 1999; Yovich, 1993).

The effectiveness of PF in stimulating motility and improving ICSI outcomes has been previously described (de Mendoza et al., 2000; Rubino et al., 2016). However, its use is not universally accepted for its purported harmful effects on oocytes (artificial activation and morphological changes), embryos (developmental retardation or arrest) or newborns (teratogenic effects) (Fisher and Gunaga, 1975; Scott and Smith, 1995; Tournaye et al., 1993). Indeed, three decades ago, methylxanthine had been reported to cause malformations in animal models such as mouse, xenopus and chicken (Bruyere et al., 1983; Dawson and Bantle, 1987; Nakatsuka et al., 1983; York et al., 1986). Of note, all of these negative effects were derived from the direct exposure of oocytes or embryos to experimentally high concentrations of xanthine derivates. Conversely, when PF is used for sperm selection during ICSI, its concentration in the culture drop is negligible and oocyte/embryo exposure is virtually absent. The French Agence de la biomédecine included in 2013 the selection of live spermatozoa before ICSI using inhibitors of phosphodiesterase into the official list of allowed techniques used for the amelioration of assisted reproductive technology procedures (Agence de BioMédecine, 2013). However, surprisingly in this regard, published data about its safety in humans are very scant. Thus, this study was designed to evaluate whether the use of PF for the stimulation of sperm motility affects neo-natal outcomes including congenital malformations in children born after ICSI with the use of PF (PF-ICSI).

## Materials and methods

### Study design

A retrospective observational cohort multi-centric study was conducted. From 2005 onwards, clinical pregnancies ensued after PF-ICSI because of immotile spermatozoa were identified in the data sets of four IVF units: Unidad Reproducción – Complejo Hospitalario Universitario Granada–Spain; Clínica MasVida y CEIFER Biobanco–Sevilla–Spain; Infertility Unit- Fondazione IRCCS Ca' Granda- Ospedale Maggiore Policlinico–Milan- Italy; Centro Scienze Natalità–IRCCS San Raffaele Hospital–Milan- Italy. Exclusion criteria were oocyte donation cycles and maternal age >45 years.

### Treatment

Infertility treatments, including hormonal stimulation, oocyte retrieval, oocyte vitrification, embryo culture, vitrification and transfer methods were performed in a standardized manner as described in details elsewhere (Busnelli et al., 2014; Corti et al., 2013; Intra et al., 2016; López-Regalado et al., 2014; Restelli et al., 2014; Sarais et al., 2016). In couples presenting exclusively with immotile spermatozoa in the ejaculate or frozen samples, ICSI was performed after exposing spermatozoa to PF. The PF (Trental, Sanofi, Origgio, Italy or Hemovàs, Ferrer, Barcelona, Spain) solution was prepared in a concentration 3 or 5 mmol in HEPES buffered medium and stored at –20°C. After centrifugation of the sperm sample (10 min, 600*g*), a volume of 1 – 2 µl of washed spermatozoa was added to 5 µl-drops of PF solution in an ICSI Petri dish, overlaid with pre-warmed mineral oil and cultured for 10 to 20 min at 37°C. On an inverted microscope, motile spermatozoa were subsequently selected and aspirated with an ICSI micropipette, washed in a drop of polyvinylpyrolidone and microinjected into fresh or thawed oocytes. Resulting embryos were either transferred between day 2 and day 6 of culture or vitrified, according to the characteristics of the cycle and of the patient.

### Outcomes

The final analysis was performed considering the clinical pregnancies achieved after the embryo transfer of fresh or vitrified embryos deriving from fresh or vitrified oocytes fertilized with PF-ICSI. Serum human chorionic gonadotrophin (HCG) was used to determine a pregnancy 2 weeks after embryo transfer; this level was subsequently

tested serially to monitor the rise in titres. A clinical pregnancy was defined as the presence of a gestational sac with fetal heart activity on ultrasound examination 5 weeks after embryo transfer. Live birth was defined as the delivery of at least one viable newborn after 24 weeks' gestation. Miscarriage was defined as the loss of a clinical pregnancy before 20 weeks' gestation. Stillbirth was defined as the fetal death at or after 20 completed weeks of gestational age.

According to the clinical routine, all pregnant women were actively followed-up and data on pregnancy outcomes were systematically obtained. A telephonic follow up of pregnancies was performed after a period of 1 to 3 months after the estimated date of birth and specific questions/answers on neo-natal malformations were registered. In case of positive feedback for adverse obstetrical events or neo-natal malformations, clinical charts were thereafter analysed in order to record obstetric and neo-natal outcomes.

Neo-natal malformations were classified according to the International Classification of Diseases and health related problems ICD-10, 10th revision (ICD-Code, 2007) including congenital malformations, deformations and chromosomal abnormalities in the coding range Q00-Q99. The neo-natal malformation rate was calculated as the number of affected live births divided by the total number of live births (EUROCAT Guide 1.4 and Reference Documents, 2013).

### *Data analysis*

The rate of malformations of children born after PF-ICSI was compared with historical data previously published regarding children conceived by conventional IVF who underwent a prospective clinical follow-up study, but not a systematic screening programme (at least two ultrasounds), at birth reporting a frequency of ICD-10 codes = 5.3% (139/2608) (Bonduelle et al., 2002). As a secondary analysis, malformation rate was compared with historical data previously published regarding children conceived by ICSI (Bonduelle et al., 2002) with a frequency of ICD-10 codes = 4.4% (114/2612) (Bonduelle et al., 2002).

This incidence was used to calculate the expected confidence interval for the cohort of PF-ICSI children. A sample size of 100 newborns was estimated to be large enough to demonstrate a two-fold higher frequency of neo-natal malformations (proportion of ICD-10 codes) in the PF-ICSI group compared with those detected in the previously published dataset considered for comparison (Bonduelle et al., 2002) (type I and II errors at 0.05 and 0.20 respectively).

The total malformation rate was obtained from the following formula: (affected live births + affected stillbirths + induced abortion for malformation) divided by (total number of live births + stillbirths) (EUROCAT Guide 1.4 and Reference Documents, 2013).

Continuous data are presented as absolute, mean with standard deviations (SD). Categorical variables are presented as absolute, percentage frequency with 95% confidence interval (95% CI). Data were analysed using the software SPSS 18.0 (Chicago, IL., USA) and compared using Fisher's exact test or Wilcoxon's non-parametric test, as appropriate.

### *Ethical approval*

The Ethical Committee Comitato Etico Milano Area B approved the study (ref. 639–2015, 29/09/2015). All women were routinely requested to provide an informed consent for their data to be used for research purposes.

## Results

The biochemical pregnancy rate was 12% (95% CI: 7 – 19%), however this was not included in the main analysis. Out of 139 clinical pregnancies 106 (76%) births of at least one baby were registered. One hundred and twenty-seven babies (85 singletons and 42 twins) were born and the neo-natal follow up was available for 82 singletons and 40 twins. Final data analysis included 134 clinical pregnancies with known obstetrical/neo-natal outcome for a total of 122 newborns from 102 deliveries, 28 miscarriages, two stillbirths and two induced abortions (**Figure 1**). The main characteristics of the studied cycles and the obstetrical outcomes are shown in **Tables 1** and **2**, respectively. Clinical pregnancies were mostly derived from fresh cycles (84%). Neo-natal outcomes are reported in **Table 3**. Considering singletons and twins as one group, the male:female ratio at birth was 0.85, with an

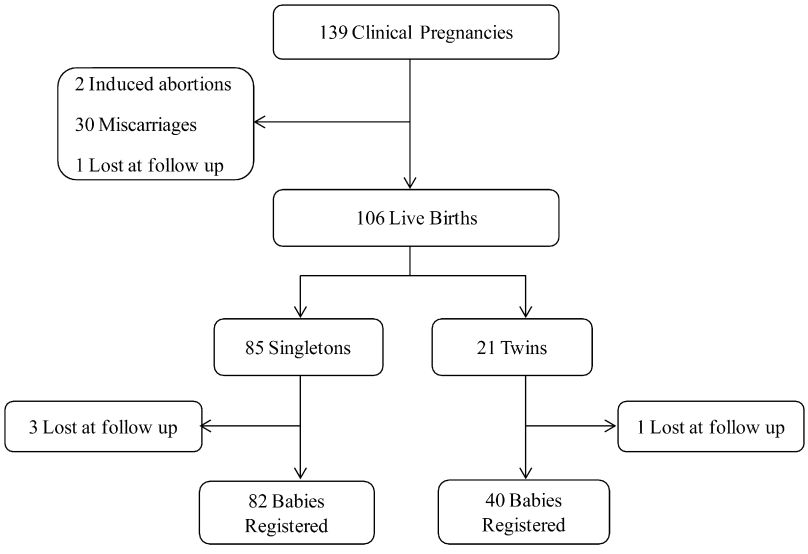


Figure 1 – Flow chart of clinical pregnancies included in this study.

**Table 1 – Clinical characteristics of the studied cycles.**

| Characteristics | Number (%) or mean ± standard deviation |
|---|---|
| No. of patients | 120 |
| No. of clinical pregnancies | 134 |
| Age at conception (years) | 34.1 ± 4.4 |
| Use of folic acid supplement | 81 (60) |
| Type of treatment | |
| Fresh cycle | 112 (84) |
| Vitrified oocytes | 3 (2) |
| Vitrified embryos | 19 (14) |
| Sperm origin | |
| Testicular | 113 (84) |
| Epididymal | 9 (7) |
| Ejaculated | 12 (9) |
| No. of embryos transferred | 1.9 ± 0.5 |
| 1 | 29 (22) |
| 2 | 94 (70) |
| 3 | 11 (8) |
| Day of embryo transfer | |
| Day 2–3 | 110 (82) |
| Day 4 | 13 (10) |
| Day 5–6 | 11 (8) |

**Table 3 – Obstetric/neo-natal outcomes of the studied deliveries (*n* = 102).**

| Characteristics | Singletons, *n* = 82 deliveries, 82 newborns | Twins, *n* = 20 deliveries, 40 newborns |
|---|---|---|
| Gestational weeks $^{(+days)}$ at delivery[a] | $39^{+2} \pm 2^{+5}$ [b] | $36^{+6} \pm 2^{+2}$ [b] |
| Preterm delivery (<37 weeks): *n* (%) | 10 (12)[b] | 9 (45)[b] |
| Birth weight (grams) | 3242 ± 577[b] | 2434 ± 481[b] |
| Birth weight <1500 grams: *n* (%) | 2 (2) | 2 (5) |
| Birth weight 1500–2500 grams: *n* (%) | 3 (4)[b] | 18 (45)[b] |
| Birth weight >4500 grams (%) | 0% | 0% |
| Neo-natal death *n* (%) | 1 (1) | 0 (0) |
| ICD-10 malformations (newborns) *n* (%) | 1 (1) | 3 (8) |
| Congenital intestinal anomaly (Q43.9) *n* (%) | 0 (0) | 1 (3) |
| Positional plagiocephaly (Q67.3) *n* (%) | 0 (0) | 1 (3) |
| Hip dysplasia (Q65.89) *n* (%) | 0 (0) | 1 (3) |
| Congenital macular changes (Q14.1) *n* (%) | 1 (1) | 0 (0) |

[a] Calculated from the day of embryo transfer + 14 days + age of the embryo.
[b] $P \leq 0.002$ within rows.

incidence of male sex equal to 46% (95% CI: 37 – 55%). Mean gestational age at the delivery was $38^{+6} \pm 2^{+6}$ weeks, with differences between singleton pregnancies ($39^{+2} \pm 2^{+5}$ weeks) and twins ($36^{+6} \pm 2^{+2}$ weeks). Mean birth weight at delivery was 2984 ± 669 g, with significant differences ($P < 0.001$) between singletons (3242 ± 577 g) and twins (2434 ± 481 g).

Regarding malformation rate per live births, 4/122 (3.3%, 95% CI 0.9 – 8.2%) babies with ICD-10 malformations were recorded, namely one Q67 and one Q65 affecting the musculoskeletal system, one Q14 affecting the eye and one Q43 affecting the intestine. The sperm origin in relation to the clinical pregnancy is indicated in **Table 1**. Considering deliveries (*n* = 102), sperm origin was testicular in 89 cases, epididymal in six and deriving from ejaculated spermatozoa in seven cases. The four babies with ICD-10 malformations were born in PF-ICSI cycles with testicular spermatozoa. Of note, three out of four malformations were recorded in babies born from twin pregnancies. Moreover, one woman had a preterm premature rupture of membranes at 24 weeks of gestation and the baby died one week after delivery without reported malformations.

**Table 2 – Clinical characteristics of the studied clinical pregnancies (*n* = 134).**

| Characteristics | Number (%) |
|---|---|
| Order of clinical pregnancies | |
| Singleton | 106 (79.1) |
| Twin | 28 (20.9) |
| Adverse pregnancy outcome | |
| Miscarriage rate | 28 (20.9) |
| Miscarriage <12 weeks | 26 (19.4) |
| Miscarriage 12 – 19 weeks | 2 (1.5) |
| Stillbirth ≥20 weeks | 2 (1.5) |
| Therapeutic abortion | 2 (1.5) |
| Livebirths | 102 (76.1) |
| Order of deliveries | |
| Singleton | 82 (80.4) |
| Twin | 20 (19.6) |
| Gestational hypertension | 4 (3.0) |
| Gestational diabetes | 11 (8.2) |
| Placenta previa | 1 (0.7) |
| Premature rupture of membranes | 11 (8.2) |

Based on data previously published on the expected prevalence rate of ICD-10 malformations among children born with conventional IVF (Bonduelle et al., 2002), the risk of having an affected offspring among PF-ICSI patients would be 5.3%, with a 95% CI ranging from 1.8% to 10.4%. The observed 3.3% of ICD-10 malformation codes is included in this CI, indicating a non-increased risk for PF-ICSI babies compared with IVF babies. The comparison between neo-natal outcomes in PF-ICSI babies compared with the previously published data is reported in **Table 4**. The analysis was also repeated using ICSI babies based on previously published data (Bonduelle et al., 2002). The expected prevalence of ICD-10 malformations in ICSI newborns according to Bonduelle et al. (2002) was 4.4%; this value corresponds to a 95% CI ranging from 1.3 to 9.3% in the study group, which includes the 3.3% rate of ICD-10 codes observed herein.

Considering singletons and multiples separately, the observed malformation rates were 1.2% (95% CI: 0.0 – 6.7%) in singletons and 7.5% in twins (95% CI: 1.6 – 20.4%), respectively.

The total malformation rate according to ICD-10 classification was 4 (affected live births) + 1 (affected stillbirth) + 2 (induced abortions) divided by 122 (live births) + 2 (stillbirths) = 7/124 = 5.6% (95% CI: 2.3 – 11.3%). The affected stillbirth had a trisomy 18; the two induced abortions were affected by trisomy 18 and 16, respectively. All of the malformations were observed in fresh cycles with the exception of the Q67.3, which derived from a vitrified embryo.

Since none of the neo-natal malformations was seen in couples using ejaculated spermatozoa and one affected stillbirth was registered in one couple using ejaculated spermatozoa, the subgroup of children born after PF-ICSI with testicular or epididymal spermatozoa was also analysed and a 3.5% (4/114) rate of ICD-10 codes per live birth and a 5.2% (6/116) rate of total malformation rate was observed.

**Table 4 – Obstetric/neo-natal outcomes of the study group compared with previously published data.**

| Characteristics | Expected incidence according to Bonduelle et al., 2002 (%) | Expected 95% CI in the study group | Observed incidence (95% CI) in the study group |
|---|---|---|---|
| Stillbirths | 1.3 | 0.2–5.7 | 1.6 (0.3–5.2) |
| Perinatal death | 2.2[a] | 0.2–6.8 | 0.08 (0.02–4.4) |
| Prematurity in newborns[a] | 31.2[b] | 23.1–40.2 | 23.0 (15.8–21.4) |
| ICD-10 malformations in newborns | 5.3 | 1.8–10.4 | 3.3 (0.9–8.2) |

95% CI = 95% confidence interval.
[a] < 37 weeks of gestation.
[b] Singletons + twins.

## Discussion

This is the first study addressing the neo-natal outcomes of babies born after ICSI using spermatozoa treated with PF. The drug acts via the cyclic AMP pathway to induce downstream sperm tail protein phosphorylation stimulating motility and therefore it is widely used when motility is not yet induced such as for testicular spermatozoa. Obviously, it is not useful for the treatment of sperm structural dysfunctions. Some concerns have been raised about its embryotoxic potential (Rubino et al., 2016). The most important consideration in all IVF procedures is indeed patients' safety, both for the women undergoing the procedures and for any potential resulting offspring.

Reassuringly, gestational age at delivery in single pregnancies and in pregnancies of twins after PF-ICSI were similar to those observed in two cohorts of assisted reproductive technology pregnancies from Italy and Spain (Levi Setti et al., 2016; Ricciarelli et al., 2013), the comparison with which could include variability due to geographical heterogeneity. The rate of biochemical pregnancies was in line with that reported for the previously published dataset to which we have referred (Bonduelle et al., 2002). Similarly, early and late abortion rates, birth weight and male to female ratio both were in line with those reported in the international literature (Farquhar et al., 2015; Qin et al., 2016; Tarín et al., 2014).

A 0.85 male:female ratio has been observed in this study; although during PF-ICSI a selection bias of X- or Y- chromosome bearing spermatozoa cannot be excluded, a significant effect of PF-ICSI on the male:female ratio in the newborns was not recorded. However, due to the sample size, the cohort was not suitable to highlight possible subtle modifications of the sex ratio with the use of PF. Interestingly, other studies have found significantly lower sex ratios (fewer males) being produced under ICSI than under other treatment methods (Fedder et al., 2013; Maalouf et al., 2014).

This study found an incidence of 12% for prematurity in singleton pregnancies, very similar to the rate reported for other cohorts of assisted reproductive technology babies (Levi Setti et al., 2016; Ricciarelli et al., 2013). Among multiple pregnancies, 45% had premature delivery, which is consistent with the 42 – 68% rate reported by other European countries (Ricciarelli et al., 2013).

The percentage of stillbirth observed was similar to that reported for assisted reproductive technology pregnancy (Bonduelle et al., 2002; Koudstaal et al., 2000; Ricciarelli et al., 2013; Westergaard et al., 1999). It seems to be slightly, but not significantly, higher than the observed in natural conceptions (Fedder et al., 2013). An increased risk of early neo-natal and infant deaths in assisted reproductive technology treatment might be partially explained by the increased incidence of preterm birth among assisted reproductive technology children since gestational age is a crucial risk factor for perinatal death (Henningsen et al., 2014).

The risk of congenital anomalies after assisted reproductive technology treatment is still a debated and controversial issue. Several cohort studies showed a higher risk of congenital defects in assisted conception children (Chen et al., 2014; El-Chaar et al., 2009; Tararbit et al., 2011) while others suggested a slight or no increased risk. Overall, two large meta-analyses showed an increased risk for congenital malformations in children following IVF and ICSI compared with those naturally conceived (Hansen et al., 2005; Rimm et al., 2004). This finding was confirmed in a recent meta-analysis of 46 studies assessing the effect of IVF and ICSI on birth defects compared with naturally conceived children (Wen et al., 2012). In any case, in a meta-analysis taking into account the contribution of subfertility as a risk factor for major congenital malformations, the risk diminished substantially (Rimm et al., 2011). Although preliminary, the percentage of congenital malformations observed in newborns of this study was 3.3%, which is similar to the prevalence of congenital anomalies recorded in Europe (2.6%) based on population-based registers (EUROCAT Guide 1.4 and Reference Documents, 2013) (Dolk et al., 2010). This estimate is also not increased compared with the rate of congenital malformation found by Allen and Douglas (2006) in Canada (4.2%) and that reported in the South Australia Registry (5.8%) (Davies et al., 2012) in the normal conception population. It is also not higher than that found in assisted reproductive technology pregnancies (3.8%) (Rimm et al., 2011). The incidence of congenital malformations of the musculoskeletal system and central nervous system has also been reported previously as the most frequent in assisted reproductive technology pregnancies (Wen et al., 2012).

In this study, the frequency of congenital malformations in multiple pregnancies was higher than in singleton pregnancies although the difference did not reach significance. The literature assessing possible differences in malformations between multiple and singleton pregnancies is also controversial. Findings reported can be different according to the congenital malformations considered, if only major or not, and the number of cases observed. However, a recent meta-analysis showed a higher risk of congenital malformations (RR = 1.26, 95% CI 1.09–1.46) in dichorionic twins born after assisted reproductive technology treatment (Qin et al., 2016).

Several syndromes involving epigenetic alterations have been associated with assisted reproductive technology, especially where ICSI was used to achieve fertilization (Cox et al., 2002; DeBaun et al., 2003; Gicquel et al., 2003; Moll et al., 2003). These include Beckwith Wiedemann syndrome, Angelman syndrome and retinoblastomas. No epigenetic disorders were reported in the present series of PF-ICSI births.

There are some weaknesses in this study. First of all, the lack of an adequate control group is a major drawback for this report. The results have been compared with historical data previously published regarding a large cohort of assisted reproductive technology children born in Belgium between 1983 and 1999 (Bonduelle et al., 2002) but that cohort cannot strictly be considered an ideal comparison group for the present study. In fact children considered in the study by Bonduelle *et al.* (Bonduelle et al., 2002) underwent an *ad hoc* physical examination during a prospective clinical follow-up study while data from this study were initially obtained from parents, not directly from medical sheets; for this reason, in this study's cohort congenital malformation rate can be underestimated and there is a risk of reporting/coding errors. Thus, the real birth defect rate may be higher than data imply. Secondly, since most of the babies from this study derived from PF-ICSI cycles with testicular spermatozoa, the ideal comparison group would be ICSI cycles performed without motility enhancers. Unfortunately, such a control group could not be supplied or found in the literature. Therefore, this study firstly compared PF-ICSI babies with those conceived with conventional IVF; we believe that this previously published cohort represents the most suitable for our purpose given the virtual absence of cycles with the use of PF. In fact the use of PF in a standard IVF is not beneficial (Dimitriadou et al., 1995; Tournaye et al., 1994). On the other hand, comparison has also been provided with babies born after ICSI. This comparison has the advantage of the same insemination technique but could not completely exclude the confounding effect of the use of motility enhancers also in the comparison group. Of note, sperm origin and severe male infertility have been indicated as possible confounders when studying congenital malformations in newborns with particular regard to cryptorchidism and hypospadias (Asklund et al., 2007; Fedder et al., 2013; Skakkebæk et al., 2001); however, in this study, no malformations linked to the male reproductive dysfunctions were observed.

Thirdly, only neo-natal information was collected and the study lacked a long follow-up for most children. However, two-thirds of major malformations are detected within the first seven days of life, so we can assume that results from this study in congenital malformations are not far from the actual situation. Finally, the sample size is not enough to estimate the possible effect of confounding factors such as age of parents, sperm origin or use of vitrified oocytes/embryos. Strengths of the study include its novelty, the multicentric/international nature and with a sample size, as calculated, able to detect a statistically significant increase in neo-natal malformation rate in the PF-ICSI group of at least two-fold higher compared with that observed by Bonduelle and co-workers (Bonduelle et al., 2002) in a series of children conceived by conventional IVF or ICSI. Furthermore, the cycles included in this analysis are quite heterogeneous (type of treatment, embryo stage at transfer) and this heterogeneity may influence the results. However, since no increased risk for malformations was seen, a specific analysis of confounding factors may be avoided.

In conclusion, this is the first report on neo-natal outcomes deriving from PF-ICSI. The percentage of congenital malformations observed in newborns was 3.3%, which is not increased compared with the rate of congenital malformations observed in IVF babies and in the general European population reported by EUROCAT. Multiplicity seems to be the most important factor behind the increased incidence of newborn malformations and complications such as preterm birth. Findings derived from this study need however to be considered with caution. The number of observations considered in this analysis is very limited for a contribution reporting on neo-natal outcomes. Larger studies and a nationwide registry including all IVF units are needed to confirm these data.

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