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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