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ARTICLE

Neonatal and neurodevelopmental outcome of children aged 3–10 years born following assisted oocyte activation

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Frauke Vanden Meerschaut was born in Belgium in 1984 and concluded her medical studies at the University of Ghent, Faculty of Health Sciences and Medicine in 2009. Since then, she has been part of the research team of the Centre for Reproductive Medicine at the Ghent University Hospital. She is interested in different aspects of reproductive medicine. She recently started working as an obstetrician gynaecology trainee, while finalizing her PhD on assisted oocyte activation.

Abstract Assisted oocyte activation (AOA) using a calcium ionophore has been used for more than a decade following intracytoplasmic sperm injection (ICSI) fertilization failure. However, since AOA does not mimic precisely the physiological fertilization process, concerns exist about its use in human assisted reproduction. This study assessed the neonatal and neurodevelopmental outcome of children aged ≥3 years who had been born following AOA in our centre. Twenty-one children participated in the study (81% response rate; mean age 63.6 ± 21.07 months). Neonatal data were collected via questionnaires. Neurodevelopmental outcome was tested using the Reynell Developmental Language Scales or Clinical Evaluation of Language Fundamentals, Wechsler Preschool and Primary Scale of Intelligence or Wechsler Intelligence Scale for Children, and the Movement Assessment Battery for Children III. Behaviour was scored by the Social Communication Questionnaire, the Child Behaviour Checklist and the Teachers Report Form. For all tests and questionnaires, the mean outcomes lay within the expected ranges. These are first data on the developmental outcome of AOA children. The high response rate and the robustness of the tests support the data, which are reassuring although still considered preliminary. Therefore, AOA should still be performed only in selected couples.

KEYWORDS: assisted oocyte activation, child follow up, failed fertilization, ICSI, ionomycine

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Introduction

Intracytoplasmic sperm injection (ICSI) is typically used in cases of severe semen dysfunction or following failure of conventional IVF. In general, fertilization rates of 70—75% are obtained (Palermo et al., 2009). In contrast, failed fertilization still occurs in 1—3% of ICSI cycles, even when a sufficient number of mature oocytes and motile spermatozoa are available (Esfandiari et al., 2005; Flaherty et al., 1995). The principal reason for fertilization failure following ICSI is defective oocyte activation (Flaherty et al., 1998; Liu et al., 1995; Rawe et al., 2000). The primary cellular event leading to mammalian oocyte activation is the initiation of intracellular calcium oscillations in the oocyte's cytosol. Although doubts have risen recently, substantial evidence suggests that the spermatozoon supplies an activating protein, phospholipase C zeta, into the oocyte's cytosol which triggers calcium oscillations via an inositol-3-phosphate-mediated pathway (Aarabi et al., 2012; Saunders et al., 2002). The oocyte responds to this by the calcium-dependent activation of several down-stream pathways, which are necessary for successful oocyte activation, pre- and post-implantation embryo development (Ducibella et al., 2002; Kashir et al., 2010; Parrington et al., 2007). Therefore, both the spermatozoon and the oocyte are considered to be responsible for successful oocyte activation and embryo development. In this study centre, the mouse oocyte activation test (MOAT), a heterologous ICSI model, is performed to diagnose the activation capacity of a patient's spermatozoa (Rybouchkin et al., 1996).

Several reports show that most, but not all, of the couples suffering from ICSI failure benefit from the application of ICSI combined with assisted oocyte activation (AOA) (Heindryckx et al., 2005, 2008; Montag et al., 2012; Vanden Meerschaut et al., 2012). Different activating agents have been used successfully in human assisted reproduction, e.g. calcium ionophore, electrical pulses and strontium chloride (Baltaci et al., 2010; Kyono et al., 2008; Rybouchkin et al., 1997; Yanagida et al., 2006). However, until adequate scientific evidence is provided regarding its safety and efficacy, ICSI combined with AOA cannot yet be considered an established treatment. The current study centre has been performing ICSI combined with AOA during more than a decade, using the injection of calcium chloride ($CaCl_2$) along with the spermatozoon into the oocyte, followed by a 2-fold calcium ionophore exposure of the injected oocyte (Rybouchkin et al., 1997) and has built up a large experience in the treatment of this rare but challenging group of patients. Rewarding results have been obtained regarding fertilization and pregnancy rates (Heindryckx et al., 2008). Nevertheless, the artificially induced calcium rises do not mimic precisely the physiologically sperm-induced calcium oscillations and little is known yet about the possible adverse effects of ionophores on post-implantation embryo development. Therefore, concerns exist about the use of a calcium ionophore as oocyte-activating agent in human assisted reproduction. However, the influence of ionomycin exposure during the zygote stage on pre- and post-implantation development has been evaluated in the mouse (Heytens et al., 2008). Zygotes treated with ionomycin showed normal pre- and post-implantation development, and normally developing and fertile pups were born originating from ionomycin-treated zygotes. Nevertheless, since these results were obtained from highly fertile animals, results should not be extrapolated without caution to a subfertile human population (Harper et al., 2012). Even though the exposure to the calcium ionophore for the purpose of AOA is short and limited to the zygote stage, it is appropriate to examine possible adverse effects of ionomycin in children born following AOA.

Since the main aim of a IVF-clinic is to ensure the birth of healthy babies, the goal of this study was to report on the neonatal outcome of children born following AOA and on their cognitive, language and motor development and behaviour from the age of 3 years onwards and to compare their outcomes with age-matched norms.

Materials and methods

Participants

All children born following AOA at University Hospital Ghent from October 2001 until September 2008 were eligible for this study. Inclusion criteria for the neurodevelopmental and behavioural assessment were: (i) age $\geq$3 years; (ii) the ability to visit the outpatient clinic; and (iii) Dutch as the mother tongue. Written informed consent was given by the parents and the study was approved by the Ethical Committee of the Ghent University Hospital (EC number: 2011/188; Belgian registry number: B670201110978, approved 19 May 2011).

Assisted oocyte activation

ICSI combined with AOA was performed as previously described (Heindryckx et al., 2008). Briefly, a spermatozoon was injected into the oocyte together with a small amount of 0.1 mol/l $CaCl_2$ (corresponding to the diameter of the oocyte). Thereafter, the oocytes were incubated for 30 min at 37°C in a 6% CO_2 air atmosphere. Next, the oocytes were exposed to a calcium ionophore (10 μmol/l ionomycin, I9657; Sigma-Aldrich, Bornem, Belgium) for 10 min. Following ionophore exposure, the oocytes were washed with Cook Cleavage medium (Cook Ireland, Limerick, Ireland) and were incubated again. After another 30 min, the calcium ionophore treatment was repeated for 10 min. Finally, the oocytes were washed and incubated overnight under 5% O_2.

Neonatal data

A questionnaire concerning obstetric and neonatal parameters was filled in by the parents shortly after the birth of their child. This questionnaire was retrieved from the patients' file at the moment of this study and informed consent was given by the parents to use this questionnaire for data analysis. Additionally, some parents were consulted on the day of the neurodevelopmental assessment if data were missing or unclear.

Follow up by assessment

All children aged $\geq$3 years were invited to the study centre for neurodevelopmental follow up using a set of three