CASE REPORT

Case reports to suggest an algorithm for management of total fertilisation failure prior to use of donor gametes

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Abstract

Purpose Total fertilisation failure (TFF), even with intracytoplasmic sperm injection (ICSI), occurs in approximately 3 % of cycles, can be recurrent and the exact cause is difficult to elucidate. Differentiation between oocyte and sperm-related cause of TFF is possible using mouse oocyte-activation techniques, but is not an option within most clinical settings. Therefore, the management of these couples is clinically driven, and the endpoint, if recurrent, is often the use of donor gametes. However, with the invariable lack of a definitive cause of TFF, any decision between the use of donor sperm or oocytes remains an emotive one. We present two case reports demonstrating the importance of appropriate investigation, activation techniques (mechanical and chemical) and clinical management options to develop a clinical algorithm prior to the use of donor gametes.

Methods This study is composed of two case reports of assisted reproduction investigation and treatment within an assisted conception unit for couples with recurrent total fertilisation failure.

Results Using appropriate investigation (endocrine, urological and embryological) and treatments (ICSI, IMSI, oocyte-activation techniques), a fertilisation rate of 48 % was achieved in two cycles in couples following a total of nine previous cycles (and 200 previously collected eggs) with TFF.

Capsule Case reports to develop an algorithm to highlight the importance of oocyte-activation techniques and investigation prior to the use of donor gametes.

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Conclusions Oocyte activation requires the triggering of intracellular calcium oscillations by the release of a sperm-specific factor (phospholipase C zeta (PLC ζ)) into the oocyte cytoplasm. Although, PLC ζ deficiencies have been demonstrated as putative causes of failed activation, impaired oocyte responsiveness may also be a factor. The use of donor gametes is often recommended and is often the required endpoint of treatment. However, these reports outline a clinical algorithm that potentially offers success without donation, and also offers a systematic approach to help decide whether donor oocytes or sperm should be recommended.

Keywords Donor gametes · Fertilisation failure · ICSI · Oocyte activation

Introduction

Human fertilisation involves a complex interplay of oocyte and spermatozoal processes. Intracytoplasmic sperm injection (ICSI), bypassing the steps of cumulus cell penetration, zona and oolemma binding allowed acceptable fertilisation and live birth rates for male factor couples [1]. However, total fertilisation failure (TFF), even with ICSI, occurs in approximately 3 % of cycles [2], can be recurrent and the exact cause is difficult to elucidate.

The initial increase seen in intracellular calcium concentration that occurs on sperm-oocyte fusion in natural conception or IVF occurs artificially with ICSI with the immediate influx of the calcium-containing media on sperm injection. Subsequent oocyte activation requires the triggering of intracellular calcium oscillations by the release of a sperm-specific factor (phospholipase C zeta (PLC ζ)) [3] into the oocyte cytoplasm. Although abnormal PLC ζ deficiencies or mutations have been demonstrated as putative causes of failed activation [4],

impaired oocyte responsiveness to PLC ζ and impaired oocyte-related generation and maintenance of calcium oscillations [5] as a likely consequence of asynchrony between nuclear and cytoplasmic maturation [6] may also be a factor.

Although the use of mouse oocyte-activation tests has been suggested to distinguish sperm- from oocyte-related cause of TFF [7], within strictly regulated UK clinical practice [8], this test is prohibited without a specific research licence.

The diagnosis of cause of TFF therefore remains clinically driven with the recommendation of donor gametes often the endpoint of management. However, in the absence of certainty as to whether the deficiency in activation is sperm- or egg-related, further guidance towards donor oocytes or sperm becomes difficult.

Assisted oocyte-activation (AOA) techniques, whether mechanical [9] or chemical, have been suggested as potential tools to overcome TFF but with variable success, lack of consistency in techniques and lack of long-term safety data for their use.

We therefore present the first two published case reports of patients with multiple TFF culminating in a successful outcome using calcium ionophore chemical AOA in the UK, outlining the diagnostic tools and activation techniques at our disposal to demonstrate their potential efficacy and suggest a clinical algorithm for the management of couples presenting with TFF.

Materials and methods

This study is composed of two case reports of patients within an assisted reproduction clinic. As case reports, this paper did not require institutional ethical approval and were reported with the patient's informed consent.

Results

Case 1

Patient 1 presented at 37 years with a 4-year history of couple subfertility having had a previous termination of pregnancy in 2002 with a different partner. She was hypothyroid with mild endometriosis, regular cycles and excellent age-related ovarian reserve (AMH 19.1 pmol/l). Male history was unremarkable. Their working diagnosis was mild male factor subfertility by virtue of variable sperm concentration (11.7–17 million/ml), variable progressive motility (18–40 %) and normal morphology.

Table 1 outlines their two ICSI cycles at another institution where a total of 27 oocytes were collected, of which 24 were mature and injected with ICSI

techniques with both cycles ending with TFF. In the latter cycle, note was made of significant refractile bodies in most of the oocytes and significant vacuoles in three of the oocytes.

Initial discussion focused on potential further investigation and treatment options to help elucidate whether the TFF was sperm- or oocyte-related to aid further management. Both partners were demonstrated to have normal peripheral karyotypes and the sperm was demonstrated to have normal levels of DNA damage (8.0 %, HALO technique) and aneuploidy (2.7 %, both tests Reprogenetics, Oxford).

Oocyte-activation techniques (both mechanical and chemical using calcium ionophore), not routinely performed within our clinic, were thereafter tentatively discussed as well as the potential use of donor sperm in a split cycle where patient's oocytes could be divided and fertilised between partner and donor sperm to help elucidate aetiology of TFF.

After multidisciplinary discussion, a decision was made to proceed with a milder stimulation regime with altered gonadotrophin choice. Nineteen eggs were collected, 16 mature and injected with intracytoplasmic morphologically selected partner sperm (IMSI) using our standard unit IMSI protocol (6000 times magnification, Research Instruments Limited, UK). All eggs were injected with grade I sperm [10], although the IMSI procedure was deemed of little additional value as the vast majority of morphologically normal sperm using standard selection techniques were deemed grade I when assessed using the IMSI microscope. Mechanical assisted oocyte-activation (AOA) techniques [11] were used on eight of the 16 oocytes and our standard unit injection technique on eight of the oocytes. Mechanical AOA was used predominantly due to difficulties sourcing validated chemical activators, lack of consensus of protocol for their laboratory preparation and regulatory barriers to their use. Time-lapse imaging (EmbryoScope, Fertilitech, UK) was also used to ensure optimal monitoring of potential pronuclear formation. Table 1 confirms TFF after a total of 40 injected oocytes with no oocyte activation seen.

A final attempt was undertaken and on this occasion using donor sperm to attempt fertilisation with half the oocyte cohort. Following the failure of mechanical AOA techniques, a decision was made to proceed with the use of calcium ionophore chemical activation. This decision was aided by the identification of a commercially available ready-to-use solution (GM508 Cult-Active, Gynemed, Germany) and the Human Fertilisation and Embryology Authority (HFEA) clarifying a position on its use. The use of calcium ionophore had been licensed for use, the authority stating '*given the theoretical risks of using Calcium Ionophore, centres using it are expected to do so only in selected patients, such as those with*

Table 1 Clinical treatment outcomes for two patients with TFF

		Treatment	Oocytes collected	Oocytes Injected	No of 2PN embryos	Outcome
Patient 1	Cycle 1	ICSI	10	9	0	TFF
	Cycle 2	ICSI	17	15	0	TFF
	Cycle 3 (Lister)	IMSI	20	16	0	TFF
		Embryoscope Mechanical AOA				
Patient 1	Cycle 4 (Lister)	IMSI	20	8 (partner)	3	Embryo transfer on day 3 Ongoing pregnancy
		Embryoscope Ca ionophore Donor spilt		8 (donor)	7	No ET / freeze
Patient 2	Cycle 1	ICSI	13	6	0	TFF
	Cycle 2	ICSI	30	—*	0	TFF
	Cycle 3	ICSI	16	12	1	Day 2 embryo transferred
	Cycle 4	ICSI	21	17	1	Day 2 embryo transferred
	Cycle 5	ICSI	21	—*	0	TFF
	Cycle 6	ICSI	18	9	0	TFF
	Cycle 7	ICSI	20	9	0	TFF
	Cycle 8 (Lister)	IMSI	14	9	0	TFF
	Cycle 8 (Lister)	Embryoscope				
	Cycle 8 (Lister)	IMSI	21	11	3	Embryo transfer on day 5 Ongoing pregnancy
		Embryoscope Ca ionophore				

*Indicates exact data unavailable

PLCz deficiency. Centres are expected to document their rationale for using Calcium Ionophore for individual cases. As with all treatments and processes, centres should ensure that patients are fully informed about the efficacy and potential risks and that validation is carried out.'

An in-house protocol was developed for its use and for patient consent, and the available solution was confirmed to have the Conformité Européenne marking, and hence is compliant with EU legislation for use. This was confirmed in discussion with the HFEA.

The product was used as per manufacturer's instructions with equilibration for 4 h in a vial not firmly closed at 5–7 % CO₂ and 37 °C prior to use. Following the sperm injection step, the oocytes were incubated for 15 min in the pre-equilibrated Ca²⁺—Ionophore GM508 Cult-Active drop, before removal and two washes performed in HEPES media (Origio, Denmark). The oocytes were subsequently placed in our standard unit cleavage culture media.

Table 1 outlines the outcome with successful fertilisation in oocytes injected with both partner (3 from 8) and donor (8 from 8) sperm. In view of the emotional impact of previous cycles, the couple were extremely hesitant to culture all embryos to blastocyst (our standard protocol where more than two top-quality embryos are available). Two top-quality partner sperm embryos (both 8-cell, grade 1) were transferred on day 3 and none of the remainder (from either sperm source) were suitable for freezing on day 5.

A positive pregnancy test was demonstrated 2 weeks following oocyte collection, and the couple is now in the third trimester of an uncomplicated singleton pregnancy.

Case 2

Patient 2 presented at 27 years with regular ovulatory cycles, no significant medical history and a 7-year history of primary subfertility. Their working diagnosis at initial fertility assessment elsewhere was unexplained infertility with normal sperm parameters, an excellent ovarian reserve (AMH 42.6 pmol/l) and normal tubal patency at laparoscopy.

Table 1 outlines the treatment prior to our input. Following three failed cycles of intrauterine insemination, seven cycles of ICSI yielded a total of 139 oocytes with five cycles ending in TFF. In two cycles, a single oocyte achieved successful fertilisation and a cleavage-stage embryo transferred on day 2 (details of quality unavailable).

In view of a history of previous varicocele, the male partner underwent formal urological work up which demonstrated a normal scrotal ultrasound scan, peripheral karyotype and sperm DNA fragmentation (11.0 %). An enterococcus infection was demonstrated that was treated prior to commencement of a treatment cycle. Stimulation was commenced performing IMSI, assessing development in the embryoscope, and in view of variable previous oocyte maturity rates, oocyte triggering was performed with lead follicles at 20–22 m

(rather than the norm of 18–20 mm in our clinic). Although IMSI was a useful tool with grade II sperm identified and excluded before injection of all oocytes with grade I sperm, TFF was again demonstrated.

In view of recent treatment success with patient 1, a similar final cycle was planned but with the addition of the calcium ionophore protocol described above. Twenty-one oocytes were collected, of which 11 were injected with grade I IMSI sperm and successful fertilisation demonstrated in three. All three embryos were of good quality on day 3 of culture so they were developed to day 5 when two cavitating morulas of average quality were transferred.

A positive pregnancy test was demonstrated 2 weeks following oocyte collection, and the couple is now in the second trimester of an uncomplicated twin pregnancy.

Conclusion

These cases should act to stimulate debate on the options for recurrent TFF and encourage clinics to consider AOA options prior to recommending donor gametes. A fertilisation rate of 48 % was achieved in the two cycles using calcium ionophore following a total of 9 cycles with TFF and two fertilised oocytes from 200 collected eggs.

Direct diagnostic assessment of couples with TFF is limited by the restricted clinical application of mouse oocyte-activation tests and tests of PLC ζ levels. Although some studies using immunofluorescence techniques have suggested quantitative defects in PLC ζ levels and qualitative defects in PLC ζ distribution in sperm from men with TFF, there is significant variability within individuals and overlap between men with normal fertilisation and TFF. A pragmatic and systematic clinical approach to the investigation and management of these couples is therefore essential. The aim of such an approach is to identify couples where AOA techniques may be worthy of consideration before the use of donor gametes becomes the only option.

Although AOA techniques have been described, their use has historically been limited by the lack of long-term data and calcium ionophore has only recently obtained a licence for use in selected patients. Concerns have been raised that the sudden influx of calcium ions that does not mirror physiological changes may have a potential effect on gene expression and embryo development in animal models [12]. Limited long-term data at birth and 3 year follow-up has been reported in children born with chemical AOA with no effect on birth weight, gestational age or later cognitive function [13]. Despite this reassuring data and the availability and licensing of calcium ionophore, its use should remain limited to carefully selected couples.

Figure 1 suggests an algorithm for clinical use. Following the exclusion of obvious factors that would dictate gamete

donation as first-line treatment, focus should initially be on investigation. Although genetic markers of sperm have not necessarily been related to TFF, both increased sperm DNA fragmentation [14] and sperm aneuploidy levels [15] have been demonstrated to impact negatively on fertilisation rates. Furthermore, a correlation between increased sperm DNA fragmentation and loss PLC ζ activity has also been suggested [16]. In the presence of impaired sperm parameters, a formal urological review is recommended to exclude any reversible factors impacting on seminal quality and therefore fertilising capacity. Where no further negative prognostic factors are identified, management options should be discussed with the patients, counselling them on the level of evidence for their use and the level of long-term safety data available.

IMSI has been demonstrated to increase pregnancy outcomes for those with previous ICSI failure [17] and has been demonstrated to have a potential role in identifying increased levels of PLC ζ in sperm relative to samples that did not use higher magnification techniques for sperm selection [18]. The selection of such sperm and the potential visualisation of sperm with otherwise missed acrosomal buds within a globozoospermic population make IMSI a potential valuable tool in this patient group.

Although the potential benefit of routine use on pregnancy outcome remains an area of considerable debate [19], in the context of TFF, any role of time-lapse embryo imaging is yet to be reported. However, the increased use of this technology has demonstrated that the signs of fertilisation may appear early and syngamy occurs before the conventional fertilisation check that occurs at a single time point at between 18 and 20 h post insemination. Time-lapse may therefore allow full visualisation of the oocytes and any possibility of early reactivity or polar body extrusion in the hours after injection. Furthermore, with the unknown potential of calcium ionophore to increase the calcium oscillations during the fertilisation process, it was surmised that this might alter the speed of the cell cycle and disrupt the presentation of the signs of fertilisation. In these two particular cases, signs of fertilisation developed at the expected timescale, but if available, time-lapse technology remains a tool that may be of benefit in future cases.

In the majority of those with recurrent TFF where a final cycle is planned, it remains uncertain whether the activation failure is an oocyte or sperm issue. Should this cycle again fail, with or without AOA techniques, the subsequent options are realistically limited to the use of donor gametes. Offering the option of a cycle split using donor sperm and partner sperm (to each attempt to fertilise half of the oocyte cohort) may yield important diagnostic information as well as offering a chance of success. Such cycles require sensible consideration and planning and as such should only occur within a carefully

Fig. 1 Suggested clinical algorithm for management of couples with TFF

designed framework. As such, within our unit, there is a mandatory four-step process that requires completion before a cycle can proceed. Initially, there must be a clinical and embryology consensus as to the merit of such a split, after which two counselling appointments follow. The first, a standard gamete donation session that includes a discussion of the regulatory and anonymity issues [8], and a second to ensure that the couple are particularly aware of the potential difficult decisions they may be faced with at each stage of treatment. Thereafter, a final multidisciplinary team meeting confirms that a split cycle is appropriate.

Both chemical and mechanical AOA techniques have been successfully reported but with little consistency. Mechanical techniques have varied from initially piercing the oolemma using a micro needle to increase calcium influx before ICSI [20] to repeated dislocation of the central ooplasm to the periphery to increase calcium influx or increase intracellular calcium release [9]. More recently, a modified ICSI technique (mICSI) has been reported where aspiration of highly

polarised mitochondria from pericortical areas to the centre of the oocyte attempts to increase energy levels to the area of sperm injection [11]. The lack of consistent technique, safety data on such techniques, minimal reported success and the benefit of the chemical agents we have demonstrated would lead us to recommend chemical AOA techniques as first line. The licensing for use of calcium ionophore in the UK and the availability of a validated ready-to-use solution further supports this as the agent of choice. Where there is long-term safety data following AOA, it is following the use of calcium ionophore [13].

In conclusion, we have demonstrated the difficulty in clinical management of couples with recurrent TFF. Our cases are the first reported ongoing pregnancies in the UK using now licensed AOA techniques, and with such a history of multiple TFF. They allow us to present a clinical algorithm for management that utilises appropriate investigation, embryological assessment and techniques and clinical judgement. It offers a pathway to potential pregnancy for these couples, or at worse,

an appropriate pathway to aid their decision making with regard to the need for donor gametes, and if so, whether sperm or oocyte donation would offer their optimal chance of success.

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