

Fertilization failure is not associated with sperm motility following human *in vitro* fertilization

Abstract

In reproductive medicine, total and partial fertilization failure (TFF/PFF) following conventional *in vitro* fertilization (cIVF) has frustrated patients. To address this issue of the role of sperm motility in TFF/PFF, we have conducted a systematic analysis of the sperm parameters that affect TFF/PFF following human cIVF. We show here that the sperm motility becomes more vigorous in TFF/PFF patients, and sperm hyper activation induced by progesterone is unlikely to induce TFF/PFF. However, we found that the lower number of oocytes retrieved and the reduction in sperm binding to the zona pellucida observed among TFF/PFF patients might induce fertilization failure. We strongly believe that sperm motility is not sufficient to induce TFF/PFF, whereas the interaction between sperm and oocyte might help enhance fertilization and prevent fertilization failure during human IVF.

Keywords: fertilization failure, sperm motility, hyperactivation, sperm zona binding

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Abbreviations: TFF, total fertilization failure; PFF, partial fertilization failure; cIVF, conventional *in vitro* fertilization; CASA, computer-assisted sperm analysis; ZP, zona pellucida; IVF, *in vitro* fertilization

Introduction

In reproductive medicine, total and partial fertilization failure (TFF/PFF) following conventional *in vitro* fertilization (cIVF) has frustrated patients, adding emotional pain and financial burden to painful treatment. The incidence of TFF/PFF following cIVF, in the presence of sperm with progressive motility, has been reported to be 4-16%.¹ To address this issue of the role of sperm motility in TFF/PFF, we have conducted a systematic analysis of the sperm parameters that affect TFF/PFF following human cIVF. These include semen parameters, sperm motility, the sperm's ability to bind to the zona pellucida (ZP), fertilization parameters, and the progesterone concentration in the insemination media used.

Materials and methods

This retrospective study was approved by our Institutional Ethical Board on 21th, August 2014. This study evaluated 613 cycles from patients who had been treated at the Department of Infertility, Angel Bell Hospital between May 2010 and December 2013, and at the Royal Bell Clinic between April and December 2013. Sperm were obtained from the male partner following masturbation, and were processed using the discontinuous gradient and swim-up technique. Three hours after oocyte retrieval, oocytes were fertilized using cIVF. Five hours after insemination, denuded oocytes without a second polar body were considered to be unfertilized.² After 18-20 hours insemination, fertilization was determined by checking for the presence of two pronuclei and two polar body. Sperm analysis was performed using computer-assisted sperm analysis (CASA), according to published guidelines.^{3,4} For statistical analyses, differences were evaluated using the t-test, and considered significant at $P < 0.05$.

Results

The CASA parameters for patients with and without TFF/PFF

are listed in Table 1. Among patients with TFF/PFF, most CASA parameters had increased by the time the sperm were suspended following swim-up ($P < 0.05$), with the exception of linearity and amplitude of lateral head displacement. As well, we observed a significant increase in all CASA parameters for patients without TFF/PFF ($P < 0.05$). The linearity of sperm motility was steady at approximately 0.4 among TFF/PFF patients. As well, the amplitude of lateral head displacement exhibited a constant trend at approximately 12.0 Hz. Sperm hyperactivation following swim-up was detected in 68.4% and 78.9% of patients with and without TFF/PFF, respectively. There was no significant difference in the average progesterone concentration for the insemination media used to treat patients with and without TFF/PFF (119 and 100 ng/mL). A significant difference was detected in the number of oocytes retrieved for patients with and without TFF/PFF (5.6 vs. 8.7 oocytes; $P < 0.01$). The prevalence of two pronuclei among TFF/PFF patients was significantly lower than that among patients without TFF/PFF (1.9% vs. 73.6%; $P < 0.0001$). For TFF/PFF patients, the number of sperm bound to the ZP was substantially lower than that for patients without TFF/PFF.

Discussion

Regarding sperm motility, the significant increase in TFF/PFF patients' sperm parameters with the exception of linearity and amplitude of lateral head displacement after swim-up indicates increase in vigor, whereas the non-TFF/PFF patients' parameters also increased significantly. Moreover, we found that sperm hyperactivation in most patients was induced by progesterone, and the progesterone concentrations were similar for both groups of patients. Progesterone is present throughout the female genital tract, reaching a peak concentration in the cumulus matrix that surrounds the oocyte. Progesterone is also known to stimulate several sperm functions, including hyperactivation⁵ and acrosome reaction.⁶ Hyperactivated motility might assist the sperm in penetrating the ZP, which is a critical process for fertilization.⁷ Thus, we conclude that sperm motility becomes more vigorous in TFF/PFF patients, and sperm hyperactivation induced by progesterone is unlikely to induce TFF/PFF.

Table 1 Semen and sperm parameters for patients with total and partial fertilization failure (TFF and PFF)

	Patients		P value
	Without TFF/PFF (n = 580)#	With TFF/PFF (n = 33)#	
Maternal age	35.7±0.2	36.6±0.9	NS
Paternal age	36.6±0.2	37.2±1.1	NS
No. of oocyte retrieved	8.7±0.3	5.6±0.9	P<0.01
Total fertilization (%)	91.2±0.7	6.8±2.5	P<0.0001
Two pronuclei (%)	73.6±1.1	1.9±1.0	P<0.0001
Semen volume (ml)	3.0±0.1	3.0±0.3	NS
Sperm concentration (×106/ml) in semen	67.1±2.4*	52.6±6.5*	NS
Sperm concentration (×106/ml) after swim-up	11.0±0.4	8.3±6.5	NS
Motility (%) in semen	45.5±0.1*	40.4±0.3*	NS
Motility (%) after swim-up	98.7±0.2	93.9±2.7	NS
Straight-line velocity (µm/s) in semen	20.2±0.4*	22.1±2.4*	NS
Straight-line velocity (µm/s) after swim-up	40.5±0.8	42.1±3.0	NS
Curvilinear velocity (µm/s) in semen	47.5±1.0*	50.0±4.6*	NS
Curvilinear velocity (µm/s) after swim-up	119.8±1.6	114.7±6.0	NS
Linearity in semen	0.4±0.01*	0.4±0.03	NS
Linearity after swim-up	0.36±0.01	0.4±0.03	NS
Beat cross frequency (µm) in semen	1.2±0.03*	1.2±0.1*	NS
Beat cross frequency (µm) after swim-up	2.7±0.04	2.6±0.1	NS
Amplitude of lateral head displacement (Hz) in semen	12.0±0.1*	12.0±0.4	NS
Amplitude of lateral head displacement (Hz) after swim-up	13.0±0.2	12.4±0.6	NS
Hyperactivation (%) in semen※	0.50%*	0%*	NS
Hyperactivation (%) after swim-up※	78.90%	68.40%	NS
No. of sperm binding zona pellucida (No. of sperm/oocyte)	3.5±0.4 (n=55)	1.5±0.4 (n=34)	P<0.05
Progesterone concentration in insemination media	100.0±16.0 (n=28)	118.5±13.6 (n=4)	NS

*n=number of patients. The number of patients assessed by CASA: 220 patients without TFF/PFF and 19 patients with TFF/PFF.

*Significant differences between semen before swim-up and sperm suspension after swim-up (t-test P<0.05).

※The number of patients with induced sperm hyperactivation divided by the total number of patients. Induction of hyperactivation in patients was assessed using standard criteria to identify hyperactivation, ≥100µm/s average curvilinear velocity, ≤0.5 average linearity, ≥8Hz average amplitude of lateral head displacement.

Interestingly, we found the reduction in sperm binding to the ZP, oocyte retrieval, and fertilization observed among patients with TFF/PFF. Recently, it has been reported that the O-glycan of ZP3 can act as a sperm head receptor.^{8,9} As well, the interaction between the Izumo protein on the sperm membrane¹⁰ and the Juno receptor on the oolemma¹¹ are required for successful fertilization. Therefore, we suggest that the interactions between sperm and oocyte should be considered to promote fertilization and prevent TFF or PFF.

We strongly believe that sperm motility is not sufficient to induce TFF/PFF, whereas the interaction between sperm and oocyte might help enhance fertilization and prevent fertilization failure during IVF.

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None.

Conflicts of interest

Author has no any conflict of interest to declare.

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