Prediction of Human Sperm Fertilizing Ability by the Hyperactivated Motility Patterns

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Abstract: Human sperm cannot fertilize oocytes immediately upon ejaculation, but must acquire the ability to bind and penetrate the zona pellucida. Hyperactivation, which is linked to the process of capacitation, is a vigorous pattern of sperm motility marked by wide-amplitude, high-velocity, whiplash movements of the flagellum. This study was performed to investigate the correlation between hyperactivated (HA) motility patterns assessed by computer-aided sperm analysis (CASA) and the fertilization rate (FR) in vitro. Swim-up sperm were collected in 135 IVF cycles with at least 3 oocytes collected. Because no cases satisfied the HA motility pattern of "Star-spin", patients were divided into 3 groups: Sperm with curvilinear velocity (VCL) ≥ 100 μM/sec, linearity (LIN) < 60% and amplitude of lateral head displacement (ALH) ≥ 5 μM were "All HA". Sperm with straight-line velocity (VSL) ≥ 40 μM/sec, LIN ≥ 60% and ALH < 5 μM were "Non-HA". Others were defined as "Transition phase". The FRs in 81 "All HA" cycles, 33 "Non-HA" cycles, and 21 "Transition phase" cycles were 79.5 ± 26.6%, 65.4 ± 32.5%, and 80.8 ± 27.3% respectively. There was a significant difference between "All HA" and "Non-HA" cycles in the FRs (P=0.018). In 27 (20.0%) of 135 IVF cycles, the FRs were ≤ 50% ("poor" group). Eleven (13.6%) of 81 "All HA" cycles, 12 (36.3%) of 33 "Non-HA" cycles, and 4 of 21 "Transition phase" cycles belonged to the "poor" group. There was a significant difference between "All HA" and "Non-HA" cycles (P=0.006) in these incidences. The better FRs were obtained in patients with "All HA" cycles, and lower FRs were obtained in those with "Non-HA" cycles. These findings suggest that the assessment of HA motility patterns by means of CASA could be one of the predictors of human sperm fertilizing ability.

Key words: Hyperactivation, Sperm motility, Computer-aided sperm analysis, Fertilization

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Introduction

Mammalian sperm cannot fertilize oocytes without undergoing capacitation, which is defined as a process of providing mature sperm with the reaction pathways necessary to undergo the exocytotic acrosome reaction in response to an inducing signal from the zona pellucida [1]. Capacitation physiologically begins in the uterus with the removal of some sperm surface components during passage through cervical mucus due to the high shear forces to which the sperm are exposed [2]. *In vitro* capacitation can also be induced with appropriate culture media supplemented with blood serum or follicular fluid [3] so that, the ability to capacitate sperm *in vitro* is an integral step in successful *in vitro* fertilization (IVF).

The final phases of capacitation are generally characterized by the development of sperm hyperactivation and acrosome reaction. Hyperactivation is a vigorous pattern of sperm motility marked by wide-amplitude, high-velocity, whiplash movements of the flagellum. The potential physiological role of sperm hyperactivation in fertilization was first suggested by the fact that the high amplitude flagellar beats and vigorous movement patterns were observed in situ through the walls of the oviductal ampulla of golden hamsters [4]. It was suggested that in human

sperm they also show signs of a hyperactivated (HA) motility pattern after swim-up into synthetic culture medium [5]. It was also suggested by Hoshi *et al.* [6] who used high-speed videomicrography that hyperactivation occurs before the acrosome reaction takes place.

Computer-aided sperm analysis (CASA) has significantly advanced the field of male fertility testing. In the present study, the CASA system was used to investigate the sperm motility characteristics in semen samples from infertile patients treated by IVF-embryo transfer (ET). The development of CASA systems, which can identify and track human sperm has revolutionized research on the movement of human sperm [7–9]. CASA has also been used for the kinematic analysis of capacitating sperm populations to identify the proportion exhibiting HA motility. Some investigators have shown that samples exhibiting good fertilization rates (FRs) *in vitro* express substantial HA motility during capacitation, whereas those showing poor FRs *in vitro* demonstrate less HA motility [10–14]. But, other researchers arrived at the opposite conclusion [15, 16]. The aim of this study is to investigate whether the HA motility patterns could predict the fertilizing ability of human sperm.

Materials and Methods

Semen analysis with the CASA system

Fresh ejaculated semen samples were obtained by masturbation. In the present study, altogether 135 conventional IVF cycles, in which at least 3 oocytes were collected, were retrospectively analyzed. The mean age of 98 female subjects was 33.5 years. The medical indications for conventional IVF-ET treatment included a tubal factor in 46 cases, a male factor in 8 cases, a female immunological factor in 2 cases, and unexplained in 42 cases.

The swim-up procedure of sperm followed our protocol as previously described [17]. In brief, semen samples were mixed with 7 mL of Sydney IVF Sperm Buffer (COOK IVF, Australia) containing human serum albumin (HSA) and centrifuged at 569-× g (1800 rpm) for 5 minutes. The pellet was resuspended in 0.5 mL of the same medium. Swim-up from semen was performed with a combined migration-sedimentation method [18] in a BIO-LABO tube (Jyuji Field Co., Tokyo, Japan). Spermatozoa migrate from semen contained in a ring-shaped well that is completely overlaid with a layer of culture medium. The central hole of the ring constitutes the collection well into which motile spermatozoa settle within 1 to 2 hours at 37°C in air. The upper two-thirds of the supernatant was collected and mixed with Sydney IVF Sperm Medium (COOK IVF, Australia), followed by centrifugation at 569-× g for 5 minutes. The pellet was resuspended in 0.5 mL of the same medium and the sperm characteristics were immediately assessed with a CASA system (Hamilton Thorne Research, Beverly MA, USA) as previously described [19, 20]. A 5 μL aliquot of the semen sample was placed in a Makler chamber. The sperm concentration, sperm motility and sperm motion variables including amplitude of lateral head displacement (ALH), beat cross frequency (BCF), curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN=VSL/VCL) and straightness (STR=VSL/VAP) were assessed. At least 200 sperm were counted by CASA. The CASA settings followed the manufacturer's instructions. Sperm morphology was assessed by the strict criteria following the method as previously reported [17].

The criteria for sperm hyperactivation followed that by Mortimer & Mortimer [21]. The HA motility patterns were evaluated by the average values of the CASA estimates that were analyzed for all the spermatozoa contained in the semen sample. After swim-up, spermatozoa with VSL ≥ 40 μM/sec, LIN ≥ 60% and ALH < 5 μM were classified as non-HA ("Non-HA"). Tracks with VCL ≥ 100 μM/sec, LIN < 60% and ALH ≥ 5 μM showed characteristics of hyperactivation and were defined as all HA ("All HA"). Because no cases in the present study satisfied the motility pattern of "Star-spin [VCL ≥ 100 μM/sec; VSL < 30 μM/sec; LIN < 60%; STR < 60; ALH ≥ 5 μM]", others were classified as "Transition phase [VCL ≥ 100 μM/sec; VSL ≥ 30 μM/sec; LIN < 60%; STR ≥ 60; ALH ≥ 5 μM]".

IVF protocol

The patients were stimulated with a combination of a gonadotropin releasing hormone (GnRH) agonist started in the luteal phase (suppression protocol) followed by gonadotropins as we reported previously [22, 23]. On the second or third day after oocyte retrieval, the morphological assessment of embryos was performed under an inverted microscope and a maximum of three embryos of good quality were transferred. Clinical pregnancy was diagnosed when the gestational sac was detected by transvaginal ultrasonography.

Statistical analysis

Statistical analysis of the data was performed by

Table 1. Relationship between the sperm motility pattern and fertilization rate (FR)

Motility pattern[a]	n	FR (%)[b]
Non-hyperactivated	33	65.4 ± 32.5[c]
Transition phase	21	80.8 ± 27.3
All hyperactivated	81	79.5 ± 26.6[c]
Total	135	76.3 ± 28.7

[a]Mortimer, S.T. and Mortimer, D. [21]. [b]Values are the mean ± SD. [C]P=0.018.

Table 2. Prediction of poor fertilization rate (FR≤50%) by using the sperm motility patterns assessed by CASA

Motility pattern[a]	n	No. of patients with poor FR	Incidence (%)
Non-hyperactivated	33	12	36.3[b]
Transition phase	21	4	19.0
All hyperactivated	81	11	13.6[b]
Total	135	27	20.0

[a]Mortimer, S.T. and Mortimer, D. [21]. [b]P=0.006.

Student's t-test with Stat view 4.5 (Abacus Concepts, Berkeley, CA) for Macintosh, and P<0.05 was defined as representing a significant difference.

Results

Semen characteristics of infertile men treated with IVF-ET

The semen characteristics of a total of 135 fresh semen samples from 98 infertile men were assessed. The mean ± SD for semen volume, sperm concentration, sperm motility, progressive motility, total motile count, and normal sperm morphology were 4.1 ± 1.5 mL, 183.0 ± 110.9 × 10^6/ mL, 58.3 ± 18.5%, 37.2 ± 18.5%, 442.0 ± 323.0 × 10^6 and 23.1 ± 9.2, respectively, (data not shown).

Results of IVF-ET

The average number of oocytes collected was 11.1 ± 6.6 (mean ± SD) in the 135 IVF-ET treatment cycles. The overall FR per cycle was 76.3 ± 28.7%. No embryo was available in 6 cycles. ET was intentionally canceled to avoid developing severe ovarian hyperstimulation syndrome (OHSS) in 7 cycles. The established clinical pregnancies included 29 single, 5 twins and 3 triplets out of 122 fresh ET, giving a pregnancy rate per ET of 30.3%. The implantation rate per embryo transferred was 11.7% (48/412).

Relationship between sperm motility pattern and fertilization rate (FR)

As shown in Table 1, the FRs in 81 "All HA" cycles, 33 "Non-HA" cycles, and 21 "Transition phase" cycles were 79.5 ± 26.6%, 65.4 ± 32.5%, and 80.8 ± 27.3%, respectively. There was a significant difference in the FRs between "All HA" and "Non-HA" cycles (P=0.018). In 27 (20.0%) of 135 IVF treatment cycles, the FRs were ≤ 50% ("poor" group). Eleven (13.6%) of 81 "All HA" cycles, 12 (36.3%) of 33 "Non-HA" cycles, and 4 (19.0%) of 21 "Transition phase" cycles belonged to the "poor" group. There was a significant difference between the incidences for "All HA" and "Non-HA" cycles (P=0.006) (Table 2).

Discussion

Male infertile patients have been assessed on the basis of a semen profile including sperm concentration, sperm motility, and sperm morphology incorporating descriptive criteria by WHO [24]. Such descriptive criteria are adequate to identify the most severe cases of male factor, but prospective studies have shown that the conventional semen profile is incapable of discriminating between fertile and infertile men especially in idiopathic infertility [25–27]. Therefore, *in vitro* tests have been developed to assess the functional capacity of human sperm to predict fertility. While various attributes of sperm function have been studied in some detail, many of the assays involved are technically complex. Sperm motility is commonly believed to be one of the most important characteristics related to fertility [27, 28]. Recent studies have indicated that sperm motility data obtained by CASA may also be predictive of fertility [16, 29, 30]. We also demonstrated that some of the CASA estimates provide reliable estimation of the fertilizing ability of human sperm [19].

HA motility of sperm has been perceived to have a potential physiological role in fertilization in various species [3, 31]. Robertson *et al.* [32] reported the first detailed description of centroid-based kinematic definition of human sperm hyperactivation by using a CellSoft CASA instrument. Afterwards, Mortimer & Mortimer [21] established criteria that are applicable to any automated system to determine human sperm hyperactivation. In the present study, we used the criteria of Mortimer & Mortimer [21] and found that the better FRs were obtained in patients with "All HA" cycles, whereas lower FRs were obtained in those with

"Non-HA" cycles (Table 1). Moreover, a significantly higher incidence of "poor" FR could be predicted in patients belonging to "Non-HA" cycles (Table 2). These results support the conclusions of some previous studies [10–14].

In conclusion, samples undergoing HA motility during capacitation are expected to exhibit good FRs *in vitro*, but those demonstrating "Non-HA" are supposed to show poor FRs *in vitro*. Such information would be useful when counseling couples before they make the decision to proceed with IVF-ET. Furthermore, the information could aid the laboratory in planning its strategy at the time of insemination.

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