The association between polypronucleate zygote formation with certain motion characteristics of sperm and IVF outcome

Tsung-Hsien Lee · Chung-Hsien Liu ·
Chun-Chia Huang · Hsui-Hui Chen · Shee-Uan Chen ·
Maw-Sheng Lee

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Abstract

Purpose To determine the efficiency of sperm motion characteristics as predictors for normal (2PN) and polypronucleate (PPN) zygotes in IVF.

Methods A retrospective cohort analysis for a total of 230 couples undergoing IVF treatment in a single infertility center.

Result(s) Subsequent to semen analysis and hemizona assay, unexpected fertilization failure would appear to have

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Capsule The combination of CASA and hemi-zona assay is adequate for directing IVF or ICSI treatment; further, sperm motion characteristics correlate with the polypronucleate zygote formation.

T.-H. Lee · S.-U. Chen
Department of Obstetrics and Gynecology, College of Medicine,
National Taiwan University,
Taipei, Taiwan

T.-H. Lee · C.-H. Liu · M.-S. Lee
Department of Obstetrics and Gynecology,
Chung Shan Medical University Hospital,
Taichung, Taiwan

C.-C. Huang · H.-H. Chen · M.-S. Lee (✉)
The Infertility Clinic Division, Lee Women's Hospital,
No. 263, Pei-Tun Road,
Taichung 406, Taiwan, Republic of China
e-mail: msleephd@giga.net.tw

C.-C. Huang
Department of Biotechnology,
Central Taiwan University of Science and Technology,
Taichung, Taiwan

M.-S. Lee
Department of Medicine, China Medical University,
Taichung, Taiwan

occurred only extremely rarely (1/236, 0.4%). The rate of PPN, however, did arise and appeared to be related to certain sperm motion characteristics, such as lateral head displacement and concentration of progressive motile sperm. Interestingly, the patients featuring a high PPN rate (>20%) was associated with a greater pregnancy rate than those featuring a low PPN rate (<20%).

Conclusion The sperm motion characteristics examined herein could be utilized to predict the rate of PPN in IVF. In order to enhance the rate of 2PN and maintain the relative high rate of clinical pregnancy, an efficient method needs further investigation and development.

Keywords In vitro fertilization ·
Computer assisted semen analysis · Normal fertilization ·
Polypronucleate zygotes

Introduction

Semen analysis is an essential part of clinical investigation for a couple's infertility evaluation. The World Health Organization (WHO) has outlined guidelines for the clinical interpretation the results of basic semen analyses, with an emphasis being placed upon various sperm characteristics such as concentration, motility, and morphology [1]. The current criteria for sperm morphology within the WHO guideline are similar to the strict criteria recommended by Kruger in 1986 [2]. The results of the examination of such basic semen characteristics are the main criteria used to determine the potential for effective fertilization and to direct the specific treatment modality for infertility by means of either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).

Unexpected fertilization failure do occur for some IVF cycles, however, especially the first one, directed by the results of conventional semen analysis [3]. Therefore, an appropriate semen diagnostic scheme has been suggested to need to include basic semen analysis and functional sperm testing [4]. Several functional sperm tests, including computer-assisted semen analysis (CASA) [5, 6], hemizona assay (HZA) [7, 8], induced-acrosome reaction testing [9, 10], and sperm penetration assay (SPA) [11, 12] have been developed, over time, in order to further define the fertilization capacity of sperm. If clinicians are able to identify infertile couples who would likely be at a rather high risk for fertilization failure, then ICSI or hemi-ICSI treatment may be chosen in order to accomplish adequate fertilization and to provide a sufficient large number of embryos to achieve successful pregnancy.

In the current study we have sought to assess the fertilization rate in a retrospective cohort of IVF cycles based upon traditional semen analysis criteria and HZA. Then we attempted to identify certain semen motion parameters which were predictive of fertilization rate as assessed by the Copenhagen Rigshospitalet Image house sperm Motility Analysis System (CRISMAS) [13] in an attempt to increase the relative accuracy for differentially predicting normal and abnormal in-vitro fertilization rates. The impact of PPN rate on clinical pregnancy was also explored in this study.

Materials and methods

Patient selection

Subsequent to clinical evaluation for the factors of infertility, the infertile couples featuring a relative high fertilization capability were assigned to undergo IVF treatment with conventional insemination. For the infertile couples destined to undergo ICSI treatment for all or a split procedure (half ICSI and half IVF) for the mature oocytes collected, they were excluded from this study.

The traditional semen analysis procedure in the Lee Women's hospital was routinely performed by the assistance of Copenhagen Rigshospitalet Image house Sperm Motility Analysis System (CRISMAS; ImageHouse A/S, Copenhagen, Denmark) and manual methods for sperm morphology analysis according to Kruger's criteria [2]. The protocol of this study was basically analyzed according to medical records without any further intervention; therefore, the approval of institutional ethical review board was not obtained.

From March 2005 to February 2006 inclusively, 264 IVF cycles were conducted in the Lee Women's Hospital, Taichung, Taiwan. When two or more IVF cycles were performed repeatedly for one couple during the study

period, only the first cycle was included for this study. As a result, a total of 230 IVF procedures were included for analysis.

The criteria for conventional insemination for infertility management included: (1) Basic semen analysis revealed results of normal (sperm concentration exceeded $20 \times 10^6/\text{ml}$, percentage of motile sperm was $> 40\%$, and the figure for normal sperm morphology was 14% or greater) for at least two of three separate repeat tests at the occasion of initial sperm evaluation, sperm storage, and the day of oocyte retrieval; (2) The age of the female partner was less than 38 years; (3) The couples underwent their first cycle of IVF treatment or had a fertilization rate $> 65\%$ for prior IVF cycles; (4) More than four MII matured oocytes were available for insemination; and (5) Normal results were revealed by hemi-zona assay (hemizona index; HZI $> 36\%$). For subfertile couples who did not meet the above described criteria, ICSI or hemi-ICSI treatment would be performed.

Hemizona assays

Fresh unfertilized oocytes from our assisted reproduction program were utilized as the source of zona pellucida. Subsequent to basic semen analysis, the sperm concentration was diluted to the level of $10 \sim 20 \times 10^6/\text{ml}$ and a total of 20,000 sperm were put into a droplet of medium. The pair of hemizona were co-incubated, at 37°C in an atmosphere of $5\% \text{CO}_2$ in air for 4 h, either with spermatozoa from the patients (test) or from a fertile man (control). The number of spermatozoa tightly bound to the zona was counted and the results of HZA were expressed as HZA index: the ratio of number of spermatozoa bound in test droplet vs. the number of spermatozoa bound in the control droplet. A HZA index level greater than 36% was considered to be featured effective fertilization potential [8].

Controlled ovarian stimulation

The female partner of infertile couples participating in this study followed a stimulation protocol that began with daily subcutaneous injections of leuprolide acetate (LA Lupron, Takeda Pharmaceuticals, Germany), 0.1 mg day 21 of their pre-stimulation cycle (long protocol) or on day 2 of their stimulation cycle (short protocol), and was continued until human chorionic gonadotrophin (hCG) was administered. Gonadotropins administration (Gonal-F; Serono, Bari, Italy), 225 IU/day s.c. , was performed in order to stimulate follicular development. The resultant ovarian response was monitored by transvaginal ultrasound and serum estradiol levels. When two or more follicles reached a maximum diameter of 18 mm and the serum estradiol levels exceeded 600 pg/ml , $10,000 \text{ IU hCG}$ (Profasi; Serono) was admin-

istered. Transvaginal oocyte retrieval was performed 32–34 h subsequent to hCG injection.

Determination of semen motion characteristics

Fresh semen samples were collected from the male partners of infertile couples on the day of oocyte retrieval for their female partners undergoing standard IVF treatment at Lee Women's Hospital, Taichung, Taiwan. The semen samples were kept at 37°C for liquefaction for an average of one hour (range from 0.5 to 1.5 h) prior to analyses for motion characteristics.

Sperm motion characteristics were analyzed by the CRISMAS, the results of which have been shown to be more reliable and reproducible than previous version of CASA and the results reported by experienced technicians would be at a median difference of < 5% [13]. The software version used for this purpose was CRISMAS Clinical 4.6 (ImageHouse A/S). The setting parameters for analysis included: 80 Hz for image-acquisition rate, two seconds for track-sampling time, at least 200 sperms sampled, and at least one microscopic field sampled microscopically with 10×20 magnification. The chamber utilized for sperm analysis was 0.01 square mm in surface area and 0.01 mm in depth (Sefi-medical instruments, Haifa, Israel).

CRISMAS was used to determine various sperm parameters, including concentration, motility, average path velocity (VAP), straight-line velocity (VSL), straightness of sperm motion (STR = VAP/VSL), and the amplitude of lateral head displacement (ALH). Other semen parameters measured included the concentration of progressive motile sperm featuring a VAP > 25 µm/s and a STR > 80% (Conc. P), and ratio of progressive motile sperm (Conc. P/ sperm concentration). The definition of progressive motile sperm is similar to the grade 'a' sperm motility determined according to WHO guideline [1].

Insemination process and evaluation of zygotes and embryos

The motile spermatozoa were selected by gradient separation technique with the application of 60% PureSperm (Nidacon International AB, Molndal, Sweden). Neat semen (1 mL) was layered on top of the gradient and centrifuged at 1,160 g for 5 min, followed by resuspension in fresh media (2–10 mL) and a further concentration step by centrifugation at 1,160 g for 5 min. The final sperm preparation was suspended in a suitable volume of modified HTF medium supplemented with 2.5% human serum albumin to a concentration of 10×10^6 /mL before insemination of the oocyte. The retrieved oocytes were inseminated between 4 and 6 h later with an average of 100,000 motile sperm in groups of three to five in 1 mL

modified HTF medium supplemented with 2.5% human serum albumin.

Mature oocytes were identified by the presence of the first polar body after removal of the corona cells in the IVF cycles at the fertilization check. The normal and abnormal fertilization rate was the quotient of number of 2PN and PPN zygotes, respectively, over the number of matured oocytes inseminated. At the fertilization check, which was performed 16–18 h after insemination, the unfertilized oocytes, 2PN and PPN zygotes were identified and counted. Diploid zygotes (2PN) were cultured individually into 15 µL microdroplets of G2.1/G2.2 (Scandinavian IVF Science/Vitrolife, Gothenburg, Sweden) medium overlaid with 2.5 mL oil in Falcon 3002 culture dishes (Becton Dickinson Labware, Franklin Lakes, NJ).

The embryos were classified according to the criteria proposed by Steer at 1992 [14], namely: score 4, equally-sized blastomeres, no fragmentation; score 3, equally- or unequally-sized blastomeres, < 20% overall fragmentation; score 2, equally- or unequally-sized blastomeres, 20–50% fragmentation and score 1, equally- or unequally-sized blastomeres, > 50% fragmentation. The embryos with score 4 or score 3, together with six to eight blastomeres on day 3 were considered as good quality embryos.

Statistical analysis

Multivariate linear regression were utilized for estimation of the predictive power of certain sperm parameters for the prediction of normal fertilization (2PN) and PPN zygote formation rate, such sperm parameters including concentration, certain motility parameters, and morphology. The comparison of the means between two groups was performed by Student t test or Mann–Whitney test as the condition determined. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 14.0; SPSS Inc., Chicago, IL, USA). A confidence level of $P < 0.05$ was considered to be the limit of statistical significance for comparison purposes.

Results

From March 2005 to February 2006, medical record for semen analysis results were collected from the male partners of 230 women undergoing standard IVF treatment at Lee Women's Hospital. A summary of the basic and advanced sperm characteristics of these semen samples is presented in Table 1. Only one couple suffered from a zero fertilization rate ($1/230 = 0.4\%$), an incidence which was relatively slight compared to the reported rate of 5–10% for complete failure of fertilization for standard IVF techniques [15]. In this study, the fertilization rate was more than 20%

Table 1 Patient characteristics, semen characteristics, and in vitro fertilization outcomes ($n=230$) (progressive motile sperm = sperm featuring average path velocity $> 25 \mu\text{m/s}$ and straightness of sperm motion $> 80\%$)

Variable	Mean (range)	Standard deviation
Age of female partner (years)	32.92 (22–38)	4.76
Duration of infertility (years)	4.00 (0.5–9)	3.01
Cycle day of oocyte retrieval (days)	14.35 (13–24)	1.45
Number of oocytes collected	13.98 (2–43)	8.30
Number of mature oocytes	12.17 (2–35)	7.42
Total volume of semen (ml)	3.17 (2.0–9.0)	1.44
Sperm concentration ($10^6/\text{ml}$)	87.0 (20–303)	55.1
Motility (% of active sperm)	77.1 (40–99)	16.2
Average path velocity ($\mu\text{m/s}$)	32.51 (18–57)	6.63
Straight line velocity ($\mu\text{m/s}$)	18.81 (10–33)	4.11
Straightness of sperm motion (%)	56.87 (31–81)	7.13
Amplitude of lateral head displacement (μm)	1.03 (0.7–1.7)	0.20
Normal morphology (%)	30.15 (14–38)	7.91
Concentration of progressive motile sperm ($10^6/\text{ml}$)	9.31 (0.00–52.81)	8.96
Hemizona assay index (%)	57.6 (36–100)	8.4
Number of polypronucleate zygote	2.25 (0–14)	2.61
Number of fertilized oocytes (2PN)	8.13 (0–29)	5.22
Normal fertilization rate (%)	68.34 (0–100)	21.41
Abnormal fertilization rate (%)	17.24 (0–80)	16.35

for all the couples who underwent conventional insemination except one failure of fertilization. Furthermore, 191 couples (83.0%) and 139 couples (60.4%) featured a fertilization rate more than 50 and 65%, respectively.

Utilizing a linear regression model, we determined those semen characteristics which were most predictive of the normal fertilization rate for IVF cycles. The ALH and Conc. P were identified as being significant factors for predicting a normal fertilization rate (Table 2). Specifically, the ALH level decreased the normal fertilization rate by 27.9% per increase of $1 \mu\text{m}$ ($P<0.05$; Table 2). On the other hand, the Conc. P increased the normal fertilization rate by 0.69% per increase of $10^6/\text{ml}$ ($P<0.05$; Table 2).

With regard to abnormal fertilization (PPN zygote), 85 couples (37.0%) featured a PPN formation rate more than 20% in this study. If the fertilization status was evaluated based on individual oocyte, a total of 534 PPN zygotes were found amongst 2,478 inseminated mature oocytes. The rate (518/2788, 18.6%) was much higher compared to

the reported rate of 5–8% for PPN zygote formation for IVF treatment [16].

The characteristics of sperm motility leading to an abnormal fertilization rate differed from the characteristics associated with normal fertilization. As shown in Table 2, the number of mature oocytes, concentration of sperm, and ALH level increased the rate of PPN by, respectively, 0.29% per increase of one oocyte, by 0.05% per increase of $10^6/\text{ml}$ and by 20.8% per increase of $1 \mu\text{m}$ ($P<0.05$; Table 2), while the Conc. P decreased the PPN zygote rate by 0.40% per increase of $10^6/\text{ml}$ ($P<0.05$; Table 2).

We were also interested in the effect of high PPN rate on the IVF outcome; therefore, the patients were further divided into two groups according to a level of 20% PPN rate (Tables 3 and 4). As shown in Table 2, the associated sperm motion characteristics with the rate of PPN included ALH and number of mature oocytes (both $P<0.05$). However, the association of Conc. P with rate of PPN was not evident in Table 3. The association of VAP and

Table 2 The correlation between the variable parameter and normal fertilization rate (2PN) and polypronucleate zygote (PPN) by multivariate linear regression ($n=230$)

Variable	Normal fertilization (2PN)		Polypronucleate zygote (PPN)	
	B value (95% CI ^a)	P value	B value (95% CI ^a)	P value
Amplitude of lateral head displacement (μm)	−27.9 (−48.1, −7.7)	0.007	20.8 (10.1, 31.5)	0.000
Concentration of progressive motile sperm ($10^6/\text{ml}$)	0.69 (0.11, 1.27)	0.020	−0.40 (−0.71, −0.10)	0.010
Number of mature oocytes	—	—	0.29 (0.02, 0.57)	0.038

(progressive motile sperm = sperm featuring average path velocity $> 25 \mu\text{m/s}$ and straightness of sperm motion $> 80\%$)

Model for normal fertilization rate: Rate of 2PN = $97.3 + (-27.9) \times \text{ALH} + (0.69) \times \text{Conc. P}$.

Model for polypronucleate zygote: Rate of PPN = $-8.4 + (20.8) \times \text{ALH} + (-0.4) \times \text{Conc. P} + 0.29(\text{M II oocyte})$

^a CI = Confidence Interval

Table 3 The sperm motion characteristics and hemizona index for the semen samples together with number of oocytes divided into two groups by a rate of polypronucleate zygote (20%) and *p* value was calculated by Mann–Whitney test

Polypronucleate zygote rate (%)	≤20% (<i>n</i> =145)	> 20% (<i>n</i> =85)	<i>P</i> value
Normal fertilization rate (%)	74.7±1.8	57.5±1.7	0.000
Total volume of semen (ml)	3.2±0.1	3.2±0.2	0.831
Sperm concentration (10 ⁶ /ml)	88.0±4.3	85.2±6.6	0.324
Motility (% of active sperm)	76.7±1.4	77.8±1.7	0.667
Concentration of progressive motile sperm (10 ⁶ /ml)	10.1±0.8	8.0±0.9	0.120
Normal morphology (%)	30.0±0.6	30.4±0.8	0.789
Amplitude of lateral head displacement (μm)	0.99±0.01	1.09±0.02	0.000
Average path velocity (μm/s)	31.5±0.5	34.2±0.7	0.002
Straight line velocity (μm/s)	18.4±0.3	19.4±0.5	0.124
Straightness of sperm motion (%)	57.4±0.6	55.9±0.8	0.103
Hemizona assay index	56.4±1.1	59.2±0.4	0.033
Number of collected oocytes	13.2±0.7	15.3±0.9	0.050
Number of mature oocytes	11.3±0.6	13.5±0.8	0.012
Rate of immature oocytes (%)	14.6±1.4	11.2±1.4	0.237

HZI with rate of PPN appeared in Table 3 (both $P<0.05$). Nonetheless, the IVF cycles featuring high PPN rates ($>20\%$) reached a greater pregnancy rate than did the cycles featuring low PPN rates ($\leq 20\%$; $P<0.05$). The age of female partner, number of 2PN zygotes, number of good quality embryos, and number of transferred embryos were not significantly different between those two groups ($P>0.05$).

Discussion

Our results indicated that the combination of basic semen analysis, including sperm concentration, motility, and morphology, with bioassay of sperm functional testing (HZA in this study) is useful to direct clinical treatment for IVF or ICSI, since only one fertilization failure from 230 (0.4%) IVF cycles arose for this study. In a meta-analysis, a high predictive power for normal fertilization has been demonstrated for certain bioassay of sperm functional characteristics, such as HZA and induced-acrosome reaction testing, but not so for sperm penetration assaying [17]. The suggested diagnostic scheme for fertilizing potential offers a reliable index for decision making in clinical management of infertility, although a high rate of PPN zygote formation was noted for some IVF cycles in our study.

Some of the sperm motile characteristics as revealed by CRISMAS, such as ALH and the Conc. P, correlated well with fertilization rate in a simple linear regression model (Table 2). From previous work, however, most of the values for these motility traits have not shown to follow a normal distribution and some authors have suggested that median and range are considered to be statistically more meaningful than mean values [18]. Therefore, we also performed an analysis based upon stratification according to the rate of PPN (20%) in addition to the linear regression model.

The fertilization process for mammals could be divided into several sequential parts, namely, the sperm-zona binding, induced acrosome-reaction, zona penetration, and oolemma fusion [3]. The capability for zona binding of sperm obviously could be identified by hemi-zona assay testing. Meanwhile, most semen variables, such as an intact acrosome, are correlated with the results of sperm-zona binding tests, which indicate that HZA reflect multiple sperm function [3]. Furthermore, sperm that is able to bind zona pellucida feature normal DNA chromatin [19]. Table 3 revealed that a high rate of PPN ($>20\%$) is associated with a high HZA index, although the difference of HZA index is not so robust to be a predictor for the rate of PPN. In our study, a normal result from HZA and basic semen analysis did guarantee the occurrence of fertilization (a low rate of fertilization failure), but at the same time, a higher rate of PPN zygotes formation than a previous report [16] arose.

Table 4 The patients were further divided into two groups by a rate of polypronucleate zygote (20%) and IVF outcome related factors were compared by means of Mann–Whitney test

Polypronucleate zygote rate (%)	≤20% (<i>n</i> =145)	> 20% (<i>n</i> =85)	<i>P</i> value
Age of female partner (years)	32.8±0.4	33.2±0.5	0.600
Number of mature oocytes	11.3±0.6	13.5±0.8	0.012
Number of 2PN embryos	8.3±0.5	7.9±0.8	0.856
Number of embryo transferred	3.8±0.2	3.6±0.2	0.869
Number of good embryos on day 3	1.4±0.2	1.6±0.2	0.756
Rate of clinical pregnancy (%)	47.9±4.2	62.4±5.3	0.035

Capacitation of sperm, by definition, is characterized by the acquired ability of sperm to undergo the acrosome reaction to bind to the zona pellucida and to acquire hyperactivated motility either in vivo or in vitro. By defining sperm motion characteristics, CASA is able to identify hyperactivated sperm and then to be utilized for prediction of fertilization rate [20]. Furthermore, the number of capacitated sperm at the site of fertilization has been reported to be related to PPN zygotes formation for mammals [21]. However, CASA was utilized to define the motion characteristics of sperm in seminal plasma and was not able to identify the hyperactivated motility sperm in the setting of this study due to shallow depth of chamber (only 10 μm). Nevertheless, sperm featuring a rapid progressive motile pattern in seminal plasma was able to predict the rate of 2PN and PPN in our study. The relationship between rapid progressive motile pattern in seminal plasma and hyperactivated motility during sperm capacitation warrants further investigation.

The formation of PPN embryos in mammals is associated with factors related to oocytes, sperm concentration, zona pellucida [22], and insemination medium [21]. Several reports have shown that aged or immature oocytes were associated with a high proportion of polyspermy following IVF for most mammals [23–25]. In the linear regression model for PPN zygote rate in our study, the number of mature oocytes (MII) was one of the factors that were clearly associated with this rate. This result may be explained by the possibility that some aged oocytes or abnormal zona pellucida might exist within a large cohort of matured oocytes retrieved from a female patient in IVF cycles. Further, in 2003, Shibahara et al. reported that the numbers of oocytes collected during IVF cycles that obtained some PPN zygotes were higher than those collected during IVF cycles featuring only 2PN zygotes [26].

Those progressive motile sperm as defined in this study by a VAP of $> 25 \mu\text{m/s}$ and a STR $> 80\%$, which are categorized as the rapid progressive motile pattern or WHO grade 'a' motility of sperm [1], characterized with high fertilization potential. Further, ALH levels and progressive forward motile pattern may be considered different pattern of sperm motion trajectory. In the linear regression model of this study, the Conc. P and ALH level were negatively and positively correlated with the rate of PPN zygotes, respectively (Table 2). In addition, higher mean levels of ALH and VAP were found in patients featuring higher levels of PPN rate (Table 3). These results in Table 3 were quite similar to those reported in 2003 by Shibahara et al., namely, VCL (curvilinear velocity), VAP, and ALH have been associated with PPN zygotes formation in human IVF [26].

A rate of production of PPN zygotes greater than 20% would appear to significantly decrease the rate of 2PN zygote (Table 4) available for transfer in order to achieve

successful pregnancy for IVF cycles. In 2006, Jun et al. reported that ICSI would likely benefit those patients who featured a high rate ($>20\%$) of triploidy embryos in prior IVF cycles [27]. However, the implantation rate and pregnancy rate were lower in ICSI cycles than was the cases in IVF cycles even though the number of 2PN zygotes was elevated subsequent to ICSI treatment [27]. It seems that the embryos result from IVF featured higher implantation potential than those embryos from ICSI for those patients with a high ($>20\%$) rate of PPN, although the embryo quality was similar between IVF and ICSI cycles.

In this study, the pregnancy rate of IVF cycles with a high rate of PPN was higher than those with a low rate of PPN. The high rate of PPN was associated with rapid progressive sperm motile characteristics. In our previous study, sperm velocity parameters have been negatively correlated with sperm DNA fragmentation rate [28]. The result of our present study (Table 3 and 4) would suggest that the rate of PPN was closely related with quality and quantity of rapid progressive sperm in seminal plasma and might be viewed as a sign of high fertilization potential. Furthermore, the 2PN zygote within the embryo cohort from that with a high rate of PPN might exhibit higher implantation potential and then increase the subsequent pregnancy rate. Alternative explanation would be that the aging or abnormal matured oocytes were de-selected by PPN zygotes formation and the residual healthy oocytes would result in 2PN zygotes. The implantation potential of these embryos was not able to be identified by morphology criteria alone in this study and that research reported by Jun et al. in 2006 [27].

In effect, we could identify those patients who featured a high rate of PPN zygote formation prior to conventional insemination, and thus, perhaps undertake ICSI for all of, or half of, the cohort of mature oocytes available. However, this modality of treatment might result in higher number of 2PN zygote but could not increase the implantation potential of embryo according to the results reported by Jun et al. in 2006 [27]. The alternative way to decrease the rate of PPN zygotes might be by means of decreasing the number or time of progressive sperm contact with individual oocyte during insemination. For example, if a semen sample features exaggerated ALH level ($> 1 \mu\text{m}$), the insemination may only require a reduced number of rapid progressive sperm for insemination (from 100,000 sperm per oocyte to 50,000 sperm per oocyte). However, the effectiveness of this treatment modality warrants further investigation.

In conclusion, the combination of semen analysis and sperm-zona binding-assay testing provides an efficient treatment strategy for clinical management of infertility, although a high rate of PPN zygotes formation would occur for patients selected based on these criteria. Motion characteristics of rapid progressive sperm in seminal plasma

for those patients could be utilized in order to predict the rate of PPN zygotes. However, the necessity and efficiency to decrease the rate of PPN by ICSI or decreasing sperm concentration at fertilization sites in terms of improving pregnancy rate needs further elucidation.

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