Original Article

Selection of fertilization strategies for different sperm parameters in vitro fertilization

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Background: Fertilization is a prerequisite for successful human reproduction. The choice of clinical fertilization strategy is crucial and directly affects clinical outcomes. This study analyzes the most appropriate assisted reproductive technology (ART) strategy based on sperm parameters.

Methods: Semen samples were divided into six groups based on semen progressive motility (PR) and semen density (SD): HMLD (high motility-low density) (PR ≥32% and sperm density <15×10^6/mL, n=60), HMID (high motility-intermediate density) (PR ≥32% and 15×10^6/mL ≤ SD <30×10^6/mL, n=106), HMHD (high motility-high density) (PR ≥32% and SD ≥30×10^6/mL, n=1,009), LMLD (low motility-low density) (PR <32% and SD <15×10^6/mL, n=99), LMID (low motility-intermediate density) (PR <32% and 15×10^6/mL ≤ SD <30×10^6/mL, n=77), and LMHD (low motility-high density) (PR <32% and SD ≥30×10^6/mL, n=164). We analyzed hyaluronic acid binding (HAB) assay and acrosin activity, along with fertilization, embryonic development, and pregnancy outcomes, to demonstrate the correlation of sperm parameters with fertilization function.

Results: In the PR <32% groups, the rate of intracytoplasmic sperm injection (ICSI) treatment decreased with increasing sperm concentration. Specifically, approximately 10% of in vitro fertilization (IVF) treatment cycles required a rescue ICSI when sperm PR was <32% accompanied by SD ≥15×10^6/mL and PR ≥32% accompanied by SD <30×10^6/mL, which was significantly higher than HMHD group, P<0.001. Sperm acrosin activity and HAB ability were significantly higher in the groups with good sperm parameters, P<0.05.

Conclusions: The findings of this study suggest, fertilization ability of sperm is closely related to sperm motility and density. In clinical practice, IVF strategies should be refined based on male sperm parameters.

Keywords: Fertilization strategies; in vitro fertilization (IVF); sperm density; sperm motility

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Introduction

Semen parameters (i.e., sperm density, motility, morphology) significantly correlate with fertilization rates *in vitro*. Fertilization is the fusion of gametes, and a critical step in the initiation of embryonic life, and is the culmination of a multitude of intricately regulated cellular processes. In successful natural fertilization, spermatozoa swim to reach the oocyte in the upper fallopian tube, and then complete the acrosome reaction (AR) at the oolemma to activate the egg, all of which involve receptor-ligand

interactions, ion channel regulation, membrane fusion, and proteolysis (1-4).

Asthenozoospermia alone or accompanied by other abnormal sperm parameters, is a common cause of male infertility and low fertilization rate, and there are several *in vitro* strategies to overcome the latter (5,6). For example, *in vitro* fertilization and embryo transfer (IVF-ET) or intracytoplasmic sperm injection (ICSI) can overcome infertility in male patients with asthenozoospermia, oligospermia, or severe teratospermia (7). However, because ICSI is invasive, the long-term impact on current or future pregnancies needs to be further explored and clarified. For IVF failure through low fertilization rate, the alternative is a rescue ICSI (RICSI) in the current cycle. RICSI was first described 20 years ago in relation to late-RICSI (ICSI performed at 20 h of insemination) to overcome chromosomal abnormalities (oocyte aneuploidy) in a 1-day-old unfertilized oocyte (8) or because of the aging process *in vitro* (9). Early-RICSI (ICSI performed at 6 h of insemination) is superior to micromanipulation performed 1 day after ovum pick-up, with pregnancy rates ranging from 43% to 51% (10-13). In contrast to studies of late-RICSI, early-RICSI contributes to improved pregnancy rates. Moreover, it also guarantees timely fertilization of oocytes compared with traditional IVF, while reducing the necessity for ICSI as the first therapeutic choice, and thus limits the additional risks, costs, and laboratory workload that are typical of ICSI (14).

Standard semen analysis is widely used as a fundamental test of male fertility, but the results do not provide precise diagnostic or prognostic information for human fertility *in vivo* or *in vitro* (15). The correlation between sperm characteristics and the selection of fertilization strategy, particularly with regard to the strategy for low sperm motility with normal sperm density, still needs further clarification. In recent years, many studies have focused improving the ability of sperm to fertilize *in vitro* (16-18). In assisted reproductive technology (ART), the purpose of sperm cell preparation is to select qualified sperm with the highest fertilization potential. Moreover, the semen standards of the WHO for evaluating male fertility are gradually decreasing, which makes people ignore the sperm characteristics in the man's semen. In clinical practice, there are still some patients who fail to fertilize due to missing the best fertilization timing, especially semen with moderate characteristics. There are limitations to directly judging sperm fertilization ability according to the existing WHO standards, especially since there are different fertilization methods in clinical practice, sperm quality should be more refined to judge sperm function. Although many studies have been conducted to identify election techniques, many questions and disagreements remain. In the present study, we compared the fertilization rates with sperm parameters, explored the value of sperm parameters as the basis of the fertilization strategy, and estimated the best time for oocyte fertilization, thereby improving egg utilization and embryo quality. We present the following article in accordance with the STROBE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4308/rc).

Methods

Patients and experimental design

A total of 1,545 patients enrolled from January 2017 to September 2020 were divided into six groups according to sperm density and motility. We excluded couples within fertility due to the female factor, such as advanced age women (≥38 years old), polycystic ovary, ovarian cancer, ovarian insufficiency. Cases of severe teratozoospermia (<1% of spermatozoa with strictly normal morphology), azoospermia, sperm with high rates of DNA fragmentation (>25% fragmentation), and no oocytes able to be retrieved were excluded, leaving a study population of 1,515, which was divided into two groups based on sperm motility: PR ≥ 32% (n=1,175) and PR <32% Group (n=340). These two groups were further divided into three subgroups according to sperm density, which was shown as *Figure 1*. Fertilization method, fertilization rate, embryonic development and clinical outcome among different groups were the main evaluation indicators. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Biomedical Research Ethics Review Committee of Fujian Medical University [(2021)Fuyi Ethics Review No. (376)] and informed consent was taken from all the patients.

Stimulation protocols, follow-up, and oocyte retrieval were performed as previously described (13). After IVF insemination, the rate of fertilization was recorded. The non-fertilized oocytes were then washed free of sperm and RICSI was performed. Motile sperm from the original sample were used in all cases, and evidence of fertilization was evaluated after 10 h. Normally fertilized and cleaved oocytes were re-incubated and transferred 3 days after retrieval.

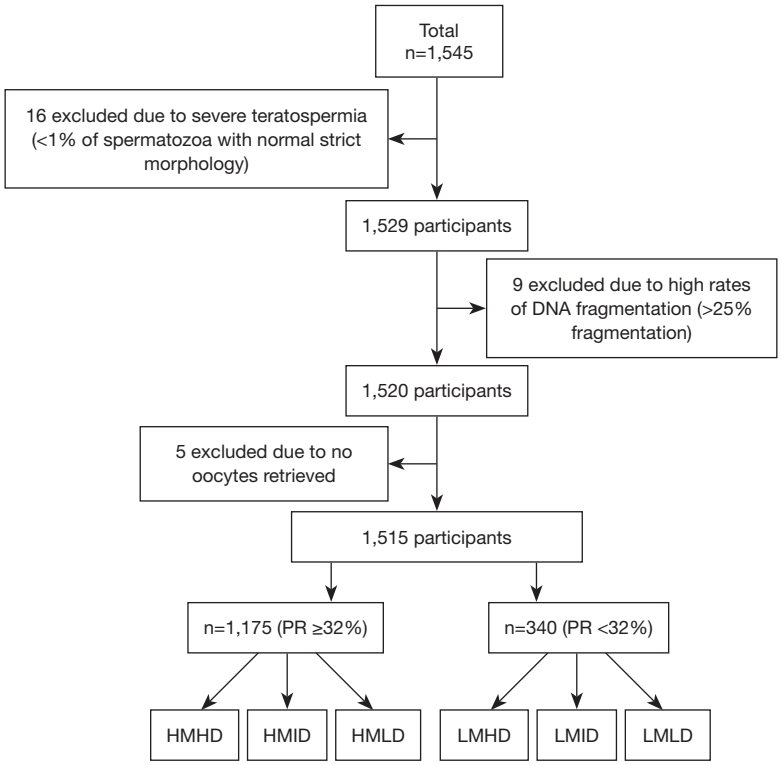

Figure 1 Flow chart of patients and experimental design. PR, progressive motility; LMLD, low motility-low density; LMID, low motility-intermediate density; LMHD, low motility-high density; HMLD, high motility-low density; HMID, high motility-intermediate density; HMHD, high motility-high density.

Semen analysis

Semen samples were obtained after 3–4 days of sexual abstinence, and samples were allowed to liquefy for at least 20 min at 37 ℃ before analysis. Sperm density and motility were determined according to the recommendations of the World Health Organization, and sperm morphology was assessed using strict criteria (19). Sperm retrieved by ejaculation were prepared by the swim-up technique, and sperm suspensions were kept at 37 ℃ in an incubator (5% O_2, 5% CO_2, and 90% N_2) in IVF insemination medium (Medicult 10310500; Imperial Laboratories, Andover, UK) until the time of fertilization.

Detection of sperm DNA fragmentation

Sperm DNA fragmentation was detected by the sperm chromatin dispersion (SCD) test. Briefly, a 60-μL sample of semen from each group was mixed with 1% low-melting-point agarose and maintained at 37 ℃. Approximately 30 μL of this mixture was layered onto a slide pre-coated with 0.65% of normal-melting-point agarose, over which we carefully placed a cover slip, and the gel was allowed to solidify. The slides were then immersed in freshly prepared acid denaturation solution (0.08 M HCl) for 7 min at room temperature in the dark. Proteins were removed by incubating the slides in lysing solution (0.4 M Tris, 20 mM DTT, 1% SDS, and 50 mM EDTA; pH 7.5) for 28 min, followed by incubation in lysing solution 2 (0.4 M Tris and 2 M NaCl; pH 7.5) for 15 min at room temperature. Slides were washed in Tris buffer (0.4 M Tris, pH 7.5) for 2 min, serially dehydrated in a graded series of ethanol, and air dried. Cells were stained with Switzerland pigment (0.2%) and scored under an optical microscope (Olympus-CX31, Japan). We scored a minimum of 500 spermatozoa per slide. Sperm DNA fragmentation was indicated by no halo or a unilateral halo of less than one-third of the minimal diameter of the sperm head, whereas intact sperm DNA was indicated by a large halo. Samples were counted separately under an oil immersion objective at 1,000× magnification. The percentage of sperm with intact DNA was determined

Table 1 Patients' characteristics and their semen parameters

Parameter	Mean ± SD/%
Age (years)	30.4±4.35
BMI (kg/m^2)	21.5±1.49
Tobacco smoking	36.80%
Alcohol consumption	41.75%
Duration of infertility (years)	3.50±2.5
Sexual abstinence (days)	4.00±3.5
Semen volume (mL)	2.50±0.54
Normal sperm morphology (%)	8.14±4.38
DFI (%)	8.91±4.65

T-tests and χ^2 tests analyzed differences in parameters. There was no significantly statistical difference. BMI, body mass index; DFI, DNA fragmentation index; SD, standard deviation.

from the number of spermatozoa having a large halo relative to the total number counted.

IVF protocol

Ovulation induction was achieved with Metrodin HP (Serono, Welwyn Garden City, UK) following a long agonist protocol of pituitary desensitization with buserelin acetate (Suprefact; Hoechst, Hounslow, UK) or Synarel (Syntex Pharmaceuticals Ltd; Maidenhead, UK). Human chorionic gonadotrophin (Organon, Oss, The Netherlands) was administered when there were at least four follicles with a mean diameter of 17 mm, 36 h prior to egg retrieval. Mature, metaphase II oocytes obtained by vaginal ultrasound-guided aspiration were cultured in universal IVF medium (Medicult 10310500; Imperial Laboratories, Andover, UK) at 37 ℃ in 5% CO_2. IVF insemination was performed using 100,000 motile sperm per dish, and each dish contained 3–5 oocytes. The oocytes were observed 16–18 h later for the presence of two pronuclei, cytoplasmic contraction, and extrusion of the second polar body.

Acrosin activity

The activity of sperm acrosin, the major protease involved in the acrosomal reaction, was determined with a commercially available kit (BERD Life Science, Shenzhen, China), which was a modification of previously published methods (20,21). The liquefied semen sample was rinsed with the washing buffer provided in the kit. A pre-calculated sample volume containing 7×10^6 spermatozoa was then added to a polytetrafluoroethylene (PTFE) membrane fixed in a tube and centrifuged. After the washing buffer was removed, 200 μL of reaction buffer containing N-α-benzoyl-DL-arginine-para-nitroanilide HCl (BAPNA) substrate was added to the sperm cells on the PTFE membrane, and the mixture was incubated at 24 ℃ for 1 h. Activated acrosin hydrolyzes BAPNA into a chromophoric product, 4-nitroaniline, the absorbance of which can be detected at 405 nm on a SpectraMax M2 spectrophotometer (Molecular Devices LLC, Sunnyvale, CA, USA). In each experiment, sperm test samples were processed along with control samples that contained the same solution but no spermatozoa. Results are presented as μIU/10^6 sperm.

Hyaluronic acid binding (HAB) assay

A drop of 10 μL of the selected sperm suspension was loaded on a HAB ability assay slide from MidAtlantic Diagnostics (Marlton, USA), according to the manufacturer's instructions (22). Around 200 unbound and bound motile spermatozoa were counted. The HAB percentage was calculated by dividing the motile bound spermatozoa by the total motile (bound and not bound) spermatozoa, multiplied by 100. All HAB analyses were performed by the same operator to reduce variability. The intra- and inter-assay coefficients of variation for all assessments were <12%.

Statistical analysis

Descriptive results are presented as the mean ± SD or number (%). Statistical analysis of the data was performed using Student's *t*-test, analysis of variance, or Chi-squared test. Two-sided P value less than 0.05 was defined as representing a significant difference. Database management and statistical analysis were performed using the Statistical Package for Social Sciences, version 20.0 (IBM Corp., USA). We used Pearson's correlation co-efficient to analyze the correlation between the fertilization rate and the semen parameters.

Results

IVF-ET

A total 1,515 IVF-ET cycles were included in this study. The characteristics of the study population are summarized in *Table 1*. Male participants were 30.4±4.35 years old,

Table 2 Baseline information for different sperm-density classes

Groups	Total No. cycles	No. cycles per woman	Average infertility duration (years)	Women diagnosed with primary infertility (n)	Rate of conventional IVF treatment cycles (%)	Rate of ICSI treatment cycles (%)	Rate of rescue-ICSI treatment cycles (%)
HMLD	60	1.22 (73/60)	5.1±2.5	70% (42/60)	50% (30/60)	40% (24/60)	10% (6/60)
HMID	106	1.25 (133/106)	5.4±2.3	62.26% (66/106)	76.42% (81/106)	14.15% (15/106)	9.43% (10/106)
HMHD	1,009	1.01 (1,023/1,009)	2.6±2.0	40.83% (412/1,009)	85.83% (866/1,009)	9.02% (91/1,009)	5.15% (52/1,009)
LMLD	99	1.16 (115/99)	6.5±2.8	81.81% (81/99)	10.1% (10/99)	88.89% (88/99)	1.01% (1/99)
LMID	77	1.16 (89/77)	5.8±2.1	72.73% (56/77)	40.26% (31/77)	45.45% (35/77)	14.29% (11/77)
LMHD	164	1.12 (183/164)	3.8±1.9	46.34% (76/164)	61.59% (101/164)	24.39% (40/164)	14.02% (23/164)

Values are shown as the mean ± standard. IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; HMLD, high motility-low density; HMID, high motility-intermediate density; HMHD, high motility-high density; LMLD, low motility-low density; LMID, low motility-intermediate density; LMHD, low motility-high density.

with a mean body mass index (BMI) of 21.5 kg/m^2, and an average sexual abstinence period of 4.0 days. Approximately 36.8% of the subjects were currently tobacco smokers, and approximately 41.75% were currently alcohol drinkers.

Comparison of fertilization strategies with respect to different sperm characteristics

We divided the 1,515 cycles into six groups according to semen motility and sperm density, and predicted the fertilizing ability of sperm by retrospective analysis of the different groups' fertilization strategies. For groups with PR ≥32%, couples with the SD <30×10^6/mL had a significantly longer duration of infertility than couples with SD ≥30×10^6/mL (P<0.01). As shown in *Table 2*, the proportion of women diagnosed with primary infertility decreased as the semen sperm concentration increased (70.0%, 62.3%, and 40.8%). If the sperm PR was <32% and SD <15×10^6/mL, 81.8% (81/99) of women diagnosed with primary infertility and 88.9% (88/99) of IVF cycles necessitated ICSI. With improvement in sperm motility and density, sperm fertilizing ability increased. For semen with PR ≥32%, the rates for conventional IVF treatment cycles increased in groups HMLD, HMID, and HMHD (50%, 76.4%, and 85.8%, respectively), and consequently the rates for ICSI treatment cycles (40.0%, 14.1%, and 9.0%, respectively), and the rates for RICSI cycles (10.0%, 9.4%, and 5.1%) were reduced. Similarly in groups LMLD, LMID, and LMHD, for semen with a PR <32%, the rates for conventional IVF treatment cycles were 10.1%, 40.26%, and 61.59%, while the rates for ICSI treatment cycles were 88.89%, 45.45%, and 24.39%, and the rates for RICSI treatment cycles were 1.01%, 14.29%, and 14.02%, respectively. The result suggests approximately 10% of IVF treatment cycles required a rescue ICSI when sperm PR was <32% accompanied by SD ≥15×10^6/mL and PR ≥32% accompanied by SD <30×10^6/mL, which was significantly higher than HMHD group, P<0.001.

Compared with a PR ≥32%, the female partners exhibited a higher probability of being diagnosed with primary infertility relative to men with semen PR <32%. The rate of conventional IVF treatment cycles for semen containing sperm with a PR ≥32% was significantly higher than for those with a PR <32% [83.14% (977/1,175) *vs.* 41.28% (142/340), P<0.001 respectively]; conversely, the rate for ICSI treatment cycles was significantly lower than a PR <32% [11.06% (130/1,175) *vs.* 47.94% (163/340), P<0.001, respectively]. In particular, the probability of RICSI was 6.8% (103/1,515) of the total cycles, lower than in groups HMLD, HMID, LMID, and LMHD (10%, 9.43%, 14.29%, and 14.02%, respectively).

Fertilization, embryonic development, and pregnancy

The numbers of oocytes fertilized, embryos developed, and clinical pregnancies in all groups are shown in *Table 3*. There was no statistically significant difference in implantation rates, clinical pregnancy rates or birth rates among the six groups of patients, irrespective of the semen parameters. As a result, there were no statistically significant differences in fertilization, embryonic development, or pregnancy. Our data suggest that sperm concentration and motility in semen are closely related to sperm fertilization ability, but not to subsequent embryonic development and pregnancy and live birth rates.

Table 3 Clinical and laboratory data from IVF cycles in groups with different sperm characteristics

Groups	Fertilization rate	2PN rate	High-quality embryo rate	Blastocyst formation rate	Clinical pregnancy rate per transfer cycle (n)	Implantation rate per transfer cycle (n)	Live birth rate
HMLD	81.43%	70%	38.81%	66.03%	56% (14/25)	45.71% (16/35)	64.29% (9/14)
HMID	79.01%	65.17%	39.26%	69.56%	52.5% (21/40)	43.33% (26/60)	66.67% (14/21)
HMHD	80.82%	67.68%	36.78%	71.12%	54.08% (179/331)	47.65% (193/405)	65.92% (118/179)
LMLD	79.50%	70.65%	35.48%	70.25%	50% (19/38)	43.75% (21/48)	47.37% (9/19)
LMID	78%	69.14%	37.93%	67.52%	51.52% (17/33)	44.9% (22/49)	47.06% (8/17)
LMHD	79.44%	69.90%	37.40%	72.62%	47.37% (27/57)	43.48% (30/69)	62.96% (17/27)

IVF, in vitro fertilization; 2PN, bipronuclear; HMLD, high motility-low density; HMID, high motility-intermediate density; HMHD, high motility-high density; LMLD, low motility-low density; LMID, low motility-intermediate density; LMHD, low motility-high density.

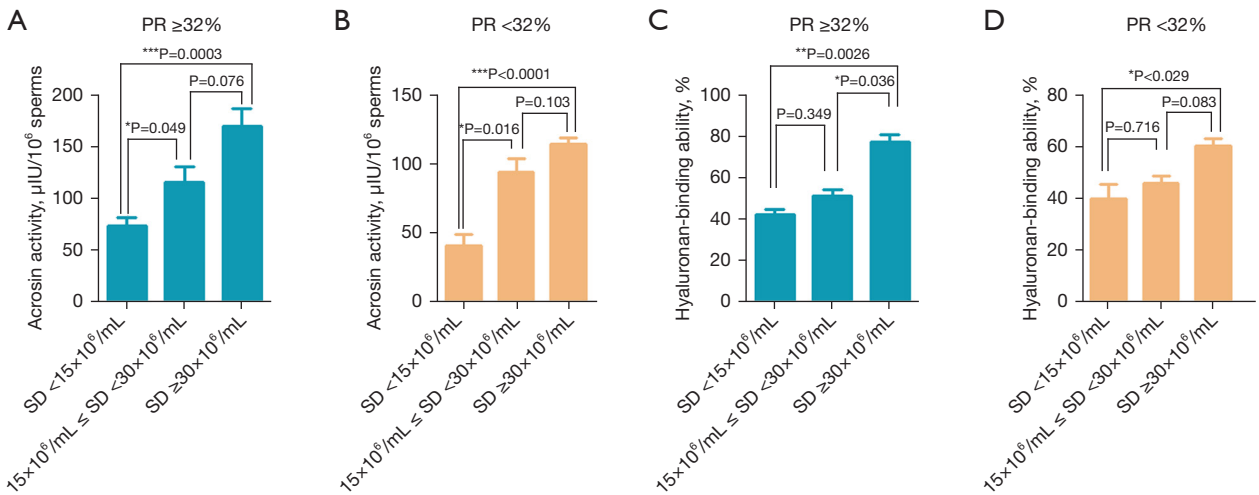

Figure 2 Acrosin activity and hyaluronic acid binding ability of sperm with different sperm concentration and motility. For PR ≥32% and PR <32%, acrosin activity increased as sperm density increased (A,B); Hyaluronidase binding ability increased as the concentration and motility of sperm increased (C,D); *P<0.05 considered statistically significant; **, P<0.01; ***, P<0.001. PR, progressive motility; SD, semen density.

Function analysis for different sperm characteristics

As shown in *Figure 2A,2B*, the acrosin activity of sperm increased with sperm motility (HMLD *vs.* HMID, P=0.049; Group HMID *vs.* HMHD, P=0.076; HMLD *vs.* HMHD, P=0.0003) and density (LMLD *vs.* LMID, P=0.016; LMID *vs.* LMLD, P=0.103; LMID *vs.* LMHD, P=0.0001). HAB ability of sperm has been suggested as predictive test of fertility *in vitro* (23). Hence, we evaluated whether sperm with good characteristics has stronger HAB ability. We found that sperm with good characteristics has higher HAB scores (*Figure 2C,2D*). The HAB ability of sperm also increased with sperm motility (HMLD *vs.* HMID, P=0.349; Group HMID *vs.* HMHD, P=0.036; HMLD *vs.* HMHD, P=0.0026) and density (LMLD *vs.* LMID, P=0.716; LMID *vs.* LMLD, P=0.083; LMID *vs.* LMHD, P=0.029).

Discussion

This study retrospectively analyzed the fertilization strategies adopted for assisted reproduction based on different semen parameters, and examined functional experiments related to sperm fertilization, such as acrosin activity and HAB ability, to analyze differences in sperm fertilization ability. This study aims to guide the clinic to pay attention to sperm concentration and motility, and be alert of possibly doing RICSI in case of intermediate semen parameters in order not to miss the optimal fertilization

timing. Common external insemination strategies include conventional IVF, ICSI, and RICSI. ICSI may be a solution to IVF failure due to unsuccessful sperm penetration, wherein the oocyte membrane is mechanically pierced, bypassing the biological barriers. RICSI is another approach in which unfertilized oocytes can be microinjected post-insemination with the aim of achieving fertilization and thereby preventing cycle cancellation (12). Early-RICSI is performed for fertilization failure or low fertilization rate at 6 h post-insemination, whereas late-RICSI is used to rescue oocytes unfertilized by ICSI at 20-h post-insemination, although attempts to rescue oocytes unfertilized by ICSI at 20-h post-insemination generally have poor results (24). In contrast, better fertilization, implantation, and pregnancy rates are obtained with early-RICSI (10). RICSI has no detrimental effects on clinical outcomes in human IVF or in patients at high risk of fertilization failure, and has acceptable pregnancy outcomes (12).

Determining the sperm parameters that predict fertilization potential is important in guiding insemination strategies. The standard semen analysis obtained by most clinicians for evaluating fertilization usually consists of sperm concentration, percent motility, quality of motility, and sperm morphology (25), and few investigators have described a correlation between sperm parameters and the fertilizing capacity of sperm in human IVF (12). In this study, we divided semen into six groups according to sperm motility and density, and compared the differences in sperm fertilizing ability of the groups through a retrospective analysis. For sperm with PR ≥32% and SD ≥30×10^6/mL, 85.83% of cycles were selected for conventional IVF whereas for sperm PR ≤32% and SD <15×10^6/mL, 88.89% of cycles underwent ICSI. The proportion of RICSI increased with semen PR ≥32% and SD <30×10^6/mL versus PR ≤32% and SD >15×10^6/mL, which indicates that cumulus cells should be removed at 6 h of coincubation with gametes in human IVF to allow early-RICSI for unfertilized oocytes.

The AR is another process in fertilization that is essential for enabling sperm to pass through the zona pellucida. A spermatozoon penetrates the zona pellucida with the help of mechanical force and is assisted by acrosin bound to the inner-acrosomal membrane (26). Previous study has examined the determinants of sperm fertilizing capacity according to AR capability and sperm motility (27). Indeed, it was reported that knockout of acrosin made mutant male hamsters completely infertile because their spermatozoa were unable to penetrate the zona (26). We also analyzed the differences in the AR in the six groups, and found that acrosin activity was elevated when sperm motility and density increased. For sperm quality outside these two conditions, conventional IVF or ICSI fertilization strategies can be selected according to sperm acrosin activity, and early-RICSI can also be used to improve oocyte utilization.

Having a choice of various insemination strategies is very common in routine IVF. Prior to insemination we can thus choose the most appropriate insemination method according to the sperm parameters presented in order to obtain the best egg utilization, instead of making adjustments in the subsequent cycle. Herein, we conclude from our retrospective research that sperm motility and density are significantly related to sperm fertilizing ability; and that acrosin activity and HAB were also important indicators of sperm fertilizing ability, especially for sperm showing medium motility and density parameters (PR ≥32%, SD <30×10^6/mL; and PR ≤32%, SD >15×10^6/mL). For acrosin activity <60 μIU/10^6 sperm, using the ICSI insemination strategy was ranked high, while early-RICSI insemination strategy was selected for sperm exhibiting acrosin activity of approximately 100 μIU/10^6 spermatozoa. In addition, the conventional IVF fertilization strategy was selected for semen containing sperm with medium motility and density, accompanied by acrosin activity > μIU/10^6 sperm.

The presence of peripheral granulosa cells improves oocyte developmental competence and embryonic quality (28,29). Therefore, in the process of artificial reproduction, care must be taken when removing the granulosa cells. Embryologists also need to consider sperm and oocyte factors in a comprehensive manner in order to obtain the best embryos *in vitro*. From this study, we gained additional valuable information with respect to the sperm characteristics that are most important for the selection of IVF strategy to increase the utilization of fertilized eggs and enhance pregnancy outcome.

However, the study still had some limitations, such as being unable to distinguish the effects of other parameters besides motility and density on sperm fertilization, such as sperm morphology and sperm DNA fragmentation. We also could not rule out some genetic abnormalities that may cause IVF disorders.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4308/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4308/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4308/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Biomedical Research Ethics Review Committee of Fujian Medical University [(2021)Fuyi Ethics Review No. (376)] and informed consent was taken from all the patients.

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