



The influence of processed total non-forward and non-motile sperm count on the outcome of artificial insemination with the husband's semen

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Background: Artificial insemination with the husband's semen (AIH) is an economical and noninvasive method of infertility treatment. However, AIH's pregnancy rate is much lower than in vitro fertilization (IVF) as its multiple and complex uncertainty factors. Semen quality has been one of the main factors which affect the pregnancy outcome of AIH.

Methods: The relevant parameters of 1,142 AIH cycles were retrospectively studied, including the general parameters and the semen quality parameters among clinical pregnancy, biochemical pregnancy, non-pregnancy group, age, infertility duration, infertility type, body mass index (BMI), cycle count, morphology in previously semen examination, and semen quality parameters on the day of AIH.

Results: The statistically significant difference was only found on processed total non-forward and non-motile sperm count (N-TFMSC). The mean processed N-TFMSC in the biochemical pregnancy group was 6.37 ± 4.27 million, significantly higher than the other two groups (*vs.* 4.40 ± 3.15 million or *vs.* 4.48 ± 3.60 million, $P < 0.05$). The study was then divided into two groups according to processed N-TFMSC, Group 1 ≤ 5.0 million, and Group 2 > 5.0 million. A statistical increase in biochemical pregnancy rate was observed when the processed N-TFMSC was > 5.0 million (2.72% *vs.* 0.90%).

Conclusions: Processed N-TFMSC may be one of the independent factors on AIH's outcome; it should be given equal attention the same as processed total forward motile sperm count (TFMSC).

Keywords: Artificial insemination with husband's semen (AIH); total non-forward and non-motile sperm count (N-TFMSC); biochemical pregnancy

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Introduction

Artificial insemination with the husband's semen (AIH) is an economical and noninvasive method of infertility treatment; the optimized semen is delivered directly into the female uterine to achieve a pregnancy (1,2). However, AIH's pregnancy rate is much lower than *in vitro* fertilization (IVF) as its multiple and complex uncertainty factors (3,4). AIH, as an assisted reproductive technology which is closer

to the natural pregnancy process, is the first choice for the treatment of infertility such as semen abnormality, sexual dysfunction and female cervical factors. The pregnancy rate of AIH reported in the literature fluctuated from 8% to 22%, which was affected by the age of the woman, infertility factors, ovulation promotion program, semen quality and other factors. Semen quality is one of the main factors which affect the pregnancy rate of AIH. More

research concerns the processed total forward motile sperm count (TFMSC) as the critical parameter of semen quality (5,6) because only forward motile sperm can reach to the oviduct and occur fertilization. Simultaneously, the non-forward and non-motile sperm is also delivered directly into the female uterine cavity, together with the forward motile sperm. Although they do not compete for fertilization, do they have any other adverse effects on AIH?

We retrospectively analyzed 1,142 AIH cycles performed in our Reproductive Medical Center between January 2016 and December 2019. Series parameters are compared among clinical pregnancy, biochemical pregnancy, and non-pregnancy groups, including age, infertility duration, infertility type, body mass index (BMI), cycle count, morphology in previously semen examination, and semen quality parameters on the day of AIH. Here, TFMSC, total non-forward and non-motile sperm count (N-TFMSC) are defined as the relevant semen quality parameters.

We present the following article in accordance with the MDAR reporting checklist (available at <http://dx.doi.org/10.21037/tau-20-1385>).

Methods

Study design

The relevant parameters of 1,142 AIH cycles performed in our Reproductive Medical Center between January 2016 and December 2019 were retrospectively analyzed. The study was approved by The Affiliated Hospital (Group) of Putian University (NO.[2015]229). All infertile patients who conformed to the AIH indications were enrolled in the study and signed the informed consent. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The general parameters (including age, infertility duration, infertility type, BMI, cycle count, morphology in previously semen examination) and the semen quality parameters (pre-processed TFMSC, pre-processed N-TFMSC, processed TFMSC, processed N-TFMSC) were compared among clinical pregnancy, biochemical pregnancy, and non-pregnancy group. The semen quality parameters (motility and density) were tested by a sperm quality analyzer (WLJY-9000, China), the semen morphology was analyzed with the modified Papanicolaou staining method. The study was then divided into two groups according to the processed N-TFMSC to explore the influence on the outcome of AIH, Group 1

≤ 5.0 million, and Group 2 > 5.0 million of the processed N-TFMSC.

Ovulation monitoring

Females with regular menstrual cycles could adopt AIH in natural cycles. Follicular development was monitored by transvaginal ultrasound from day 10 of the cycle or onward according to the menstrual cycle length. When the dominant follicle diameter reached 14 mm, the urine luteinizing hormone (LH) peak was monitored. AIH was performed about 24 h after the occurrence of the LH peak. The stimulated cycle was controlled primarily by letrozole (Jiangsu, Hengrui Pharmaceuticals, Lianyungang, China) or gonadotropin (HMG, Lizhu Pharmaceuticals, Zhuhai, China; FSH, Lizhu Pharmaceuticals, Zhuhai, China). Transvaginal ultrasound monitored follicular development (GE, Voluson E6, USA) and the urine LH. An intramuscular injection of 10,000 IU HCG (Maanshan, Fengyuan Pharmaceuticals, Maanshan, China) was administered when the dominant follicle diameter reached 18 mm, or the LH peak appeared. AIH was performed about 24–48 h after the intramuscular injection.

Semen collection and processing

Semen collection and processing is done according to the guidelines of the World Health Organization (WHO) procedures. Masturbation was used to collect semen after abstinence for 2–7 days and placed in a CO₂ incubator to liquefaction. After the semen was processed through the density gradient centrifugation or the upstream method, 0.5 mL of semen suspension was prepared and used for the artificial insemination within an hour. Routine analysis of semen was performed before or after processing. Here, we defined the TFMSC as the multiplication of density, activity ratio of (a+b) grade and volume of the sperm, and defined the N-TFMSC as the multiplication of density ratio (c+d) grade and the volume of the sperm.

Artificial insemination

Patients were placed in a lithotomy position. After vaginal and cervical secretions were cleaned, 0.5 mL of semen suspension was slowly injected into the uterine cavity through a disposable intrauterine insemination tube (COOK, USA). The patients were instructed to keep in the dorsal decubitus for 30 min after the operation.

Table 1 Profile of patients in the clinical pregnancy, biochemical pregnancy, and non-pregnancy group

General parameters	Clinical pregnancy	Biochemical pregnancy	Non-pregnancy
Cycles (n)	143	17	982
Female age (year)	29.95±3.53	30.41±3.36	30.38±3.89
Male age (year)	31.53±4.14	32.24±3.27	31.91±4.60
Infertility duration (year)	2.76±1.84	3.00±2.15	2.87±1.95
Primary infertility (%)	61.54 (88/143)	64.71 (11/17)	62.42 (613/982)
Stimulated AIH cycle (%)	46.15 (66/143)	29.41 (5/17)	51.12 (502/982)
Female BMI	21.97±3.59	23.45±3.57	21.90±3.36
Cycle count	1.90±1.03	1.94±0.83	1.85±0.89
Morphology (%)	4.40±2.56	4.72±1.82	4.27±2.12

Values are mean ± SD; One-way ANOVA or non-parametric test was used in this analysis. AIH, artificial insemination with the husband's semen; BMI, body mass index.

Diagnosis of pregnancy

Blood β -hCG was determined in patients 14–16 days after AIH. B-ultrasound was performed in patients with positive β -hCG 35 days after AIH. Gestational sac and primitive heart tube pulsation are regarded as clinical pregnancy. Patients with positive β -hCG while no gestational sac are regarded as biochemical pregnancy.

Statistical analysis

Statistical analysis was performed with SPSS 19.0 software. The measurement data in multiple groups were compared with randomized analysis of variance, and the comparison among groups was performed with LSD test (One-way ANOVA). If the measurement data disobey the Gauss distribution, a non-parametric test is used to analyze. A comparison between the two groups was constructed with an independent sample *T*-test. The enumeration data in multiple groups were displayed as crosstabs and were compared with the χ^2 test. Statistical significance was established at $P < 0.05$.

Results

The general data on the outcome of AIH

The study involved 1,142 AIH cycles; the total clinical pregnancy rate was 12.52% (143/1,142), the total biochemical pregnancy rate was 1.49% (17/1,142). The general data were divided into three groups according to

the outcome of AIH. As shown in *Table 1*, there was no statistically significant difference in the general parameters among the three groups, including female age, male age, infertility duration, primary infertility, insemination cycle, female BMI, cycle count, and the morphology in previously semen examination (all $P > 0.05$).

Semen quality parameters on the outcome of AIH

Here, TFMSC and N-TFMSC before or after processing were determined to be the semen quality parameters. As shown in *Table 2*, the mean processed N-TFMSC in the biochemical pregnancy group was 6.37 ± 4.27 million, significantly higher than the other two groups (*vs.* 4.40 ± 3.15 million or *vs.* 4.48 ± 3.60 million, $P < 0.05$). There were no statistically significant differences in pre-processed TFMSC, pre-processed N-TFMSC, and processed TFMSC.

The influence of processed N-TFMSC on the outcome of AIH

The study was divided into two groups according to processed N-TFMSC, Group 1: ≤ 5.0 million (786 AIH cycles), and Group 2: > 5.0 million (356 AIH cycles). The significant difference we observed between them tend to be more male parameters, including male age (31.68 ± 4.46 *vs.* 32.27 ± 4.66), morphology in previously semen examination (4.08 ± 2.01 *vs.* 4.74 ± 2.43), and all the semen quality parameters on the day of AIH (in *Table 3*). The enumeration

Table 2 Semen quality parameters of patients on the day of AIH in the clinical pregnancy, biochemical pregnancy, and non-pregnancy

Semen quality parameters	Clinical pregnancy	Biochemical pregnancy	Non-pregnancy
Pre-processed TFMSC (million)	92.21±70.21	96.67±62.91	84.71±60.26
Pre-processed N-TFMSC (million)	135.53±82.91	150.99±97.64	127.16±83.53
Processed TFMSC (million)	36.25±22.74	40.28±23.93	33.85±21.68
Processed N-TFMSC (million)	4.40±3.15	6.37±4.27*	4.48±3.60

Values are mean ± SD; One-way ANOVA was used in this analysis. *P<0.05, compared with clinical pregnancy or non-pregnancy. AIH, artificial insemination with the husband's semen; TFMSC, total forward motile sperm count; N-TFMSC, total non-forward and non-motile sperm count.

Table 3 The parameters of patients in processed N-TFMSC ≤5.0 million and >5.0 million

N-TFMSC (million)	≤5.0	>5.0
Cycles (n)	786	356
Female age (year)	30.25±3.83	30.49±3.85
Male age (year)	31.68±4.46	32.27±4.66*
infertility duration (year)	2.83±1.86	2.93±2.09
Primary infertility (%)	61.83 (486/786)	63.48 (226/356)
Ovulation induction cycle (%)	46.69 (367/786)	56.74 (202/786)**
Female BMI	21.76±3.32	22.33±3.51**
Cycle count	1.84±0.91	1.88±0.91
Morphology (%)	4.08±2.01	4.74±2.43**
Pre-processed TFMSC (million)	67.19±47.99	126.98±68.19**
Pre-processed N-TFMSC (million)	104.63±64.16	181.40±96.63**
Processed TFMSC (million)	26.85±16.68	50.59±23.04**
Processed N-TFMSC (million)	2.70±1.15	8.47±3.84**

Values are mean ± SD; an independent sample T-test was used in this analysis. *P<0.05 or **P<0.01, compared with N-TFMSC ≤5.0 million groups. N-TFMSC, total non-forward and non-motile sperm count; BMI, body mass index; TFMSC, total forward motile sperm count.

data in multiple groups were displayed as crosstabs and were compared with the χ^2 test. The significance value of Pearson χ^2 was 0.045 (<0.05); the contingency coefficient was only 0.073 (in Table 4). That was presumed to be a weak correlation between the processed N-TFMSC and the outcome of AIH.

Discussion

Semen processing is the product of aided reproductive technology (ART). Unlike natural fertilization, semen suspension is optimized before AIH, removing the non-sperm components and abnormal sperms (7,8). It has

generally been presumed the pregnancy rate is associated with female age, the infertility duration, infertility type, the insemination cycle, the dominant follicles, and semen quality (9,10). The processed semen suspension was equipped with hyperactivated motility (HAM) and fertilization to look forward to an improved AIH pregnancy rate. Many reports showed that the processed total motile sperm count (PTMS) could represent semen's quality more than other semen parameters (3,5). Badawy *et al.* (11) suggested that PTMS should not be less than 5 million. With the increase of PTMS, the pregnancy rate of AIH is also increased. When it reached 10 million, a satisfactory pregnancy rate can be obtained (12). Here, we care more

Table 4 The influence of processed N-TFMSC on the outcome of AIH

The processed N-TFMSC (million)	Clinical pregnancy	Biochemical pregnancy	Non-pregnancy
≤5.0			
Count	100	7	679
The percent of the group	12.72%	0.90%	86.39%
>5.0			
Count	43	10	303
The percent of the group	12.08%	2.72%	85.11%

The χ^2 test analyzes the crosstabs among the multiple groups; 0% of the table's expected count is less than 5; the minimum expected count is 5.30. N-TFMSC, total non-forward and non-motile sperm count; AIH, artificial insemination with the husband's semen.

about the forward motile sperm for their HAM and fertilization ability. Therefore, TFMSC and N-TFMSC are used to be the semen quality parameters on the day of AIH. Even a perfectly processed semen suspension cannot completely remove the non-forward and non-motile sperm. We hypothesized that there might be some effects on AIH, as the non-forward and non-motile sperm is also delivered directly into the female uterine cavity.

In the study, we divided the pregnancy outcome into three parts rather than two parts: the clinical pregnancy, biochemical pregnancy, and non-pregnancy. It is interesting to note that the statistically significant difference is only found on processed N-TFMSC. The mean processed N-TFMSC in the biochemical pregnancy group was significantly higher than the other two groups. It reminded us that we should pay more attention to process N-TFMSC. Then the study was divided into two groups according to processed N-TFMSC. The significant difference we observed between them tend to be more male parameters, including male age, morphology in previously semen examination, and all the semen quality parameters on the day of AIH. The older the male, accompanied by more processed N-TFMSC. Whereas the highest of normal morphology does not mean lower processed N-TFMSC, it may be due to the previously semen examination's morphology. Also, the highest of processed N-TFMSC was often accompanied by higher processed TFMSC.

A statistical increase in biochemical pregnancy rate was observed when the processed N-TFMSC was >5.0 million. Biochemical pregnancy due primarily to the embryo implantation process has begun but has not been completed; its mechanism is still unclear. The zygote begins to bed on day 6–7 after fertilization; it experiences position, adhesion, and implantation. Any problem with the step may lead to

the final failure of implantation. Embryo qualities affect the pregnancy outcome through its ability to implant and its development potential (13,14). Semen is the participant in ensuring the quality of the embryo. Sometimes we observed that more processed N-TFMSC happened to the same patient in different AIH cycles regardless of any processing measure. Does that mean the semen of the patient itself has an unknown defect? Their sperm can escape apoptosis and cannot be easily eliminated by conventional semen processing. And the remaining defective sperms may release reactive oxygen species (ROS) or toxins, which may be harmful to the forward motile sperm. As we know, an excess of ROS causes oxidative stress and therefore causes DNA damage in the nucleus and mitochondria (15). However, with the higher processed TFMSC in group 2 (processed N-TFMSC >5.0 million), excessive motility semen is injected into the female reproductive tract, which may increase the chances of polyspermic fertilization (16).

The maternal environment is another crucial factor in a biochemical pregnancy. Endometrium receptivity, endocrine factors, infection, immune factors, psychosocial factors, etc. (17). We suspected that non-forward and non-motile sperm remained in the uterine cavity and adhered to the endometrium, which may change the endometrium morphology and the expression of its related factors. It may affect the adhesion space between pinopodes and embryos. Factors involved in endometrial receptivity, including the expression of integrin, leukemia, inhibitory factor, interleukin-1, etc., may also change as the stress of the non-forward and non-motile sperms.

Implantation failure is presumed to occur due to impairment of embryo developmental potential and/or impairment of endometrial receptivity and the embryo-uterine dialogue (18,19). Although non-forward and non-

motile sperms do not compete for fertilization, they do deliver directly into the uterine cavity together with the forward motile sperm and result in adverse effects on implantation. As reported above, the biochemical pregnancy rate was 2.72% when processed N-TFMSC was >5.0 million ($P < 0.05$). The contingency coefficient was 0.073; there was a weak correlation between the N-TFMSC and AIH outcome. This was a retrospective study with a limited sample size, especially since the number of biochemical pregnancies was only 17, which may lead to bias; further study is needed to confirm our conclusion.

In summary, the processed N-TFMSC may be one of the independent factors on AIH's outcome; it should be given equal attention the same as the processed TFMSC. We should control the processed N-TFMSC at least ≤ 5 million; sometimes, the upstream method is necessary to lessen the processed N-TFMSC after the density gradient centrifugation of semen processing.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at <http://dx.doi.org/10.21037/tau-20-1385>

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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 approved by The Affiliated Hospital (Group) of Putian University (NO.[2015]229). All patients in the study signed informed consent. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

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