Title: A randomized trial comparing conventional semen parameters, sperm DNA fragmentation levels, and satisfaction levels between semen collection at home and at the clinic

Running head: Site of semen collection and semen parameters

Key words: satisfaction level, semen analysis, site of semen collection, sperm DNA fragmentation

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Abstract

The aim of the randomized trial was to compare conventional semen parameters, sperm DNA fragmentation levels, and satisfaction levels between semen samples collected at home and at the clinic. We recruited 110 men with a history of infertility for at least one year from the outpatient andrology clinic. Each man collected two semen samples, one at home and one at the clinic. Men were randomly assigned into the home first (n = 55) or clinic first (n = 55) groups. The primary outcome was sperm concentration. There was no significant difference in sperm concentration, sperm DNA fragmentation levels, or other conventional semen parameters between home first and clinic first samples ($p > 0.05$), while satisfaction levels were significantly higher for home first samples ($p < 0.01$). Consistent results were obtained when comparing home-collected and clinic-collected samples within individuals. Men can be offered the option to collect semen samples at home for examination or assisted reproduction without compromising semen quality, especially for those with difficulty in producing semen samples at the clinic.

1 INTRODUCTION

Infertility affects approximately 15% of couples, and male factor contributes to 20-70% of infertile cases. The percentage of infertile men ranges from 2.5-12.0%, amounting to at least 30 million patients globally (Agarwal, Mulgund, Hamada, & Chyatte, 2015).

During investigation and treatment, men are usually requested to produce semen samples in a private room near the andrology laboratory at the clinic. However, some men find it difficult to collect samples in an unfamiliar environment and prefer to collect samples at home. A retrospective study showed that the sperm concentration and rapid progressive motility were higher in samples collected at home than at the clinic (Elzanaty & Malm, 2008). Evidence from randomized controlled trials is lacking.

Sperm DNA integrity has received increased attention due to its impact on the success of assisted reproduction treatment. Systematic reviews have concluded that a high DNA fragmentation index is associated with decreased live birth rates in conventional in vitro fertilization (IVF) cycles (Osman, Alsomait, Seshadri, El-Toukhy, & Khalaf, 2015), and increased miscarriage rates in intracytoplasmic sperm injection (ICSI) cycles (Deng et al., 2019; Zhao, Zhang, Wang, & Li, 2014). Moreover, spermatozoa with damaged DNA have fertilizing potential, and their unrepaired damage sites are associated with disease phenotypes in offspring (Aitken, 2018).

The major contributor to sperm DNA damage is oxidative stress from reactive

oxygen species (ROS). Excessive ROS are counteracted by a variety of enzymatic (e.g. superoxide dismutase) and nonenzymatic (e.g. vitamins C and E) antioxidants in the seminal plasma (Agarwal, Virk, Ong, & du Plessis, 2014). The prostasomes, which are secreted by the prostate into the seminal plasma, have the capacity to inhibit superoxide anion production by neutrophils (Saez, Motta, Boucher, & Grizard, 1998). Notably, when sexual erotic materials were provided for semen sample collection via masturbation, patients' satisfaction levels were higher (van Roijen et al., 1996) and their samples contained increased prostate secretion and spermatozoa with improved motility and oocyte fusion ability (Yamamoto, Sofikitis, Mio, & Miyagawa, 2000). It is not known whether the site of semen collection can also influence patient satisfaction level, leading to a change in the secretion of sex accessory glands and the level of sperm DNA fragmentation.

The aim of this randomized trial was to compare conventional semen parameters, sperm DNA fragmentation levels, and satisfaction levels between samples collected at home and at the clinic for infertile men. The hypothesis of the study was that samples collected at home would show improvements in conventional semen parameters, sperm DNA fragmentation levels, and patient satisfaction levels.

2 MATERIALS AND METHODS

2.1 Study population

The study was approved by the Institutional Review Board of the University of Hong Kong-Shenzhen Hospital (HKU-SZH) (Approval Number: Ethics [2015]19) and was

registered at the Chinese Clinical Trial Registry (http://www.chictr.org.cn) (Registration Number: ChiCTR-IOR-17014224). Men between 18 and 55 years of age with a history of infertility for at least one year were recruited from the andrology clinic of HKU-SZH. The exclusion criteria were as follows: (1) presence of dysuria, urinary urgency and increased frequency of urination; (2) erectile or ejaculatory dysfunction; (3) inability to follow instructions due to impaired cognition, and (4) being recruited into other research projects.Patients received full counselling and signed an informed consent before participating in the study.

2.2 Randomization and masking

Eligible men were requested to produce two semen samples within two weeks, one at home and the other in a room near the laboratory of the andrology clinic. Participants were randomly assigned into either home or clinic groups for the site of first sample collection, at a 1:1 ratio in blocks of ten. The randomization sequence was computer-generated (www.randomization.com) and concealed in sequentially numbered, opaque, sealed envelopes by a research nurse who was not involved in the clinical care. Men were enrolled by the andrologists and were informed of the sequence of semen collection sites by the research nurse when they booked the dates for semen analysis. As a result, participants were not blinded to assigned groups.

All semen samples were delivered to the laboratory by the research nurse approximately one hour after semen collection, and the information on sample collection sites was concealed. The doctors, laboratory technicians, and statisticians

were blinded to assigned groups. Randomization codes were only revealed after completion of the whole study and statistical analysis.

2.3 Semen sample collection

Men were instructed to collect the semen samples by masturbation after an abstinence period of 2-7 days, to keep the samples close to the body, and to submit the samples to the research nurse within one hour after sample collection.

2.4 Semen analysis

Conventional semen analysis, including semen volume, sperm concentration, motility, viability, and morphology was performed in accordance with WHO guidelines (World Health Organization, 2010). As part of our routine clinical services, analysis was initiated approximately one hour after ejaculation. Sperm motility was assessed at room temperature. The Diff-Quik staining method was used to assess sperm morphology.

2.5 Assessment of sperm DNA fragmentation

Sperm DNA fragmentation was detected by the TUNEL assay using the In Situ Cell Death Detection Kit, Fluorescence (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. Briefly, an aliquot of 4×10^6 of spermatozoa from the raw sample was centrifuged at 600 g for 4 min, washed twice with phosphate-buffered saline (PBS), fixed in 2% paraformaldehyde at 4°C for 15

min and washed with PBS. A smear was prepared and air-dried, and the spermatozoa were permeabilized with 0.1% Triton X-100 (EMD Millipore, Billerica, MA USA) and incubated at 37°C for one hour with the reaction solution, which contained fluorescein-labelled dUTPs and the terminal deoxynucleotidyl transferase (TdT) enzyme to label DNA breaks. Spermatozoa were then incubated with 4',6-diamidino-2-phenylindole (DAPI) for nuclear staining. Positive control samples were pre-treated with deoxyribonuclease I (Affymetrix, CA, USA) (1 mg/ml) for 15 min at 37°C before labelling. Negative control samples were incubated in the reaction solution free of TdT. The fluorescent signal was captured by the fluorescence microscopy and analyzed using the Image-Pro Plus software (Version 6.0, Media Cybernetics Inc., USA). At least 300 spermatozoa were assessed for each sample (Henkel et al., 2003). The level of sperm DNA fragmentation was determined by the percentage of the TUNEL-positive spermatozoa.

2.6 Assessment of the satisfaction level

Satisfaction level on semen sample collection by masturbation was assessed using a previously published questionnaire (van Roijen et al., 1996). This questionnaire contains six questions regarding relaxation, sexual arousal, quality of erection, intensity of orgasm, ease of achieving orgasm, and satisfaction level after orgasm. Men scored each question on a 10 cm visual analogue scale, with 0 indicating the worst and 10 indicating the best. In order to reduce recall bias, men were asked to complete the satisfaction questionnaire immediately after collection of each sample.

2.7 Statistical analysis

The primary outcome measure was sperm concentration. The secondary outcome measures included semen volume, total sperm count, percentage of progressive motile spermatozoa, percentage of spermatozoa with normal morphology, sperm viability, percentage of spermatozoa with DNA fragmentation and satisfaction level.

The sample size was calculated for paired comparison between home-collected samples and clinic-collected samples within individuals. Based on the database for semen samples submitted to our andrology laboratory, the mean sperm concentration was 84.6 $\times 10^6$/ml with a standard deviation of 73.3×10^6/ml. We assumed that the sperm concentration would be 30% higher in home-collected samples than clinic-collected samples, based on previously reported differences (Elzanaty & Malm, 2008). As there were no published data on the standard deviation of change, we used the standard deviation (73.3×10^6/ml) instead. Based on these parameters, 90 men were needed for a test of significance of 0.05 and a power of 0.9. We anticipated that 20% of men could withdraw from the study, and therefore a total of 110 men were recruited into the study, with 55 men in each arm.

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL) according to intention-to-treat (ITT) and per protocol (PP) analyses. Continuous variables were expressed as median and range for non-parametric data. A comparison of first semen collection between the two arms was performed using Mann-Whitney rank sum test or Chi-square test. Paired comparisons between home-collected samples and clinic-collected samples within individuals were made by Wilcoxon signed rank

test or McNemar's test. *P*-values less than 0.05 were considered as statistically significant.

3 RESULTS

3.1 Participant flow

We recruited 110 infertile men from January to September 2018, and 55 were randomly assigned into one of the two groups (Figure 1). Eight men withdrew from the study. The loss to follow-up rate was comparable between the two groups (10.9% in the clinic first group and 3.6% in the home clinic group, $p > 0.05$).

3.2 Baseline characteristics

For the first semen collection, the baseline data were comparable except that the collection-to-analysis time was longer in the home-first group than the clinic-first group in the ITT analysis (Table 1) and PP analysis (Supplementary Table 1).

Within individuals, there were no significant differences in the baseline data between samples collected at home and at the clinic, according to the ITT analysis (Table 2) and PP analysis (Supplementary Table 2).

3.3 Primary outcome

For the first semen collection, sperm concentrations were comparable between home-collected and clinic-collected samples, according to the ITT analysis [44.4 (0-239.5) versus 51.8 (0-239.5), $p = 0.441$; Table 1] and PP analysis [Supplementary

Table 1].

There was no significant change in sperm concentration between home-collected and clinic-collected samples within individuals, according to the ITT analysis [48.0 (0-603.8) versus 42.2 (0-239.5), $p = 0.543$; Table 2]. Results were similar with the PP analysis [Supplementary Table 2].

3.4 Secondary outcomes

In the ITT analysis, no significant differences were detected in semen volume, total sperm count, percentage of progressive motility, percentage of normal morphology, sperm viability, or the percentage of spermatozoa with fragmented DNA between home-collected and clinic-collected samples for the first semen sample (Table 1) or within individuals (Table 2).

Satisfaction levels were significantly higher during the first semen sample collection at home than at the clinic, for all items of the satisfaction questionnaire: feeling at ease, degree of sexual arousal, rigidity of erection, intensity of orgasm, how easily orgasm was achieved, and satisfaction level after orgasm (Table 1). In a paired comparison within individuals, satisfaction levels were also significantly higher when samples were collected at home compared to at the clinic (Table 2).

Similar results were obtained for all the secondary outcomes with the PP analysis (Supplementary Table 1; Supplementary Table 2).

4 DISCUSSION

To our knowledge, this is the first randomized trial investigating the effect of semen collection site on semen parameters and satisfaction levels of infertile men. Our results were consistent with the findings of a retrospective study that found no significant differences in the sperm concentration or other conventional semen parameters between samples collected at home and at a clinic (Licht, Handel, & Sigman, 2008). In Licht's study, samples from the same individual were collected at the two sites and compared, but men were allowed to determine the sequence of the collection site. In a different prospective but non-randomized study, no differences were observed in sperm concentration, percentage of motile sperm, progressive velocity, or ongoing pregnancy rate of intrauterine insemination (Song, Herko, & Lewis, 2007). On the contrary, a cross-sectional study revealed that sperm concentration and rapid progressive motility were higher in samples collected at home than at the clinic (Elzanaty & Malm, 2008). The discrepancy may be due to differences in the study design, as well as to the the semen collection environment, which includes factors such as the presence of others individuals, a hospital atmosphere, space limitations, and noise.

We randomized the sequence of the collection sites to avoid bias. We also conducted paired comparison for each man to reduce inter-personal variability, because semen parameters vary greatly among individuals, and using two separate groups would have introduced even more variability (Licht et al., 2008). We also took additional measures to reduce intra-personal variability: First, men were requested to

produce two samples within two weeks with similar duration of abstinence. Second, the semen samples collected at the clinic were not delivered to the andrology laboratory until one hour after collection, in order to minimize the difference in collection-to-analysis time between semen samples collected at home and at the clinic.

In addition, this study has provided the first demonstration of sperm DNA fragmentation levels being unaffected by the site of semen collection. The recruited men produced comparable volumes of the ejaculate, suggesting no change in the secretory function of the reproductive glands, which was supported by a previous study that showed similar levels of biochemical markers including of neutral α-glucosidase, prostate-specific antigen, zinc, and fructose in clinic-collected and home-collected samples (Elzanaty & Malm, 2008).

There are several assays targeting different aspects of DNA damage in the spermatozoa. Our choice for the TUNEL assay was based on three considerations. First, the TUNEL assay and the alkaline comet assay directly measure the level of DNA damage, and thus have more predictive power for male infertility than the SCSA and SCD assays (Cissen et al., 2016; Cui et al., 2015; Ribas-Maynou et al., 2013). Second, the TUNEL assay is more feasible for the clinical services than the alkaline comet assay due to time constraints, and because it remains questionable whether pretreatment with thiol pre-treatment in the comet assay induces DNA damage (Barratt et al., 2010). Third, the reproducibility of the TUNEL assay has been proven (Ribeiro et al., 2017). The limitation of the TUNEL assay is its moderate to low

sensitivity (Cui et al., 2015; Sharma, Ahmad, Esteves, & Agarwal, 2016), due to the enzyme's difficulty in accessing the compacted sperm DNA (Barratt et al., 2010; Mitchell, De Iuliis, & Aitken, 2011).

In our study, men reported a higher level of sexual arousal and less difficulty in producing semen samples at home compared to at the clinic. Home collection may benefit men with difficulty in producing a semen sample for examination or assisted reproduction treatment. However, the subjective level of sexual arousal was not correlated with semen quality or sperm DNA integrity. This is in line with a previous study that found a higher satisfaction score with erotic materials provided for sample collection than without, but no improvement in semen parameters (van Roijen et al., 1996).

In our study, ITT and PP analysis produced similar results. The ITT approach is based on the original allocation of trial participants, regardless of protocol deviation or non-adherence. The PP analysis only includes data from participants who complied with the protocol of intervention that they were original allocated to. The ITT analysis is recommended by the CONSORT guidelines as standard practice, while a supplementary PP analysis can be performed to evaluate the influence of any missing data (Sedgwick, 2015).

One limitation of our study is that only infertile men were assessed. It remains to be validated whether the conclusions can be applied to the men undergoing a fertility check-up before attempting pregnancy. Another limitation is that, our results on DNA fragmentation are preliminary and need further investigation. Finally, we did not

record whether erotic materials were used for semen samples collected at home, as these materials are not usually provided on-site by hospitals in our country.

In order to reduce the risk of sample contamination and exposure to extreme temperatures, clear instructions on sample collection and transportation should be given, especially for men who collect samples at home. As there is no guarantee that the semen sample obtained at home is from a male partner himself, a tiny portion of semen sample can be saved on a filter paper for future identity check if the semen sample is for the purpose of fertility treatment.

As semen analysis in our study was incorporated into routine clinical services, sperm motility was assessed at room temperature and the Diff-Quik stain was used according to our unit protocol. These methods comply with WHO guidelines, but the absolute values for sperm motility and morphology may differ from those assessed at 37^{o}C and with the Papanicolaou stain. We did not exclude samples with collection-to-analysis time of more than one hour because the primary outcome sperm concentration would not have been affected. When we performed exploratory subgroup analyses that excluded samples assessed more than one hour after collection, the sperm motility and viability remained similar between semen samples collected at home and at the clinic.

To conclude, conventional semen parameters and sperm DNA fragmentation levels of infertile men were comparable for semen samples collected at home or at the clinic, while men were more satisfied when semen samples were collected at home. Men can be offered the option to collect semen samples at home for examination or

assisted reproduction without compromising semen quality, especially for those who have difficulty in producing a semen sample at the clinic.

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