Effect of sperm selection method by cumulus oophorus complexes and conventional sperm preparation method on sperm quality and DNA fragmentation for assisted reproduction techonology

Wasin Naknam, Lingling Salang, Jen Sothornwit, Siriruthai Amnatbuddee, Kanok Seejorn, Thanida Pongsritasana, Sineenart Sookkasame

PII: S0301-2115(19)30446-4

DOI: https://doi.org/10.1016/j.ejogrb.2019.10.004

Reference: EURO 11006

To appear in: *European Journal of Obstetrics & Gynecology and Reproductive Biology*

Received Date: 30 May 2019

Revised Date: 23 September 2019

Accepted Date: 8 October 2019

Please cite this article as: Naknam W, Salang L, Sothornwit J, Amnatbuddee S, Seejorn K, Pongsritasana T, Sookkasame S, Effect of sperm selection method by cumulus oophorus complexes and conventional sperm preparation method on sperm quality and DNA fragmentation for assisted reproduction techonology, *European Journal of Obstetrics and amp; Gynecology and Reproductive Biology* (2019), doi: https://doi.org/10.1016/j.ejogrb.2019.10.004

Effect of sperm selection method by cumulus oophorus complexes and conventional sperm preparation method on sperm quality and DNA fragmentation for assisted reproduction techonology

Wasin Naknam, M.D., Lingling Salang, M.D., Jen Sothornwit, M.D., Siriruthai Amnatbuddee, M.D., Kanok Seejorn, M.D., Thanida Pongsritasana, M.Sc., Sineenart Sookkasame, B.Sc.

Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University,

Khon Kaen, Thailand.

Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.Email: Slingling@kku.ac.th

Corresponding author: Lingling Salang

ABSTRACT

Objectives: To compare the effectiveness of sperm selection using cumulus oophorus complexes (COCs) and conventional sperm preparation methods on sperm quality and DNA fragmentation

Methods: Normal semen samples under the World Health Organization (WHO)'s 2010 eligibility criteria were collected and processed using conventional sperm preparation methods. The prepared sperm were divided into two groups. Spermatozoa in the study group were selected based on their ability to penetrate a layer of COCs. In the control group, spermatozoa were kept in culture medium under similar conditions. The selected-sperm were evaluated based on sperm quality and DNA fragmentation.

Results: Thirty normal semen samples were recruited. Spermatozoa that were able to passthrough the COCs had significantly higher sperm motility parameters than the control group (curvilinear velocity [VCL; 143.5 vs 122.2; P<0.01], average path velocity [VAP; 83.6 vs 69.3; P<0.01], straight-line velocity [VSL; 67.95 vs 60.45; P<0.01]). The percentage of normal spermatozoa morphology in the COCs group was significantly higher than in the control group (21.70 % vs 18.76%). In addition, there was significantly less DNA fragmentation in the COCs group than in the control group (18.83 vs 10.83).

Conclusion: Spermatozoa selected using COCs were likely to be effective in terms of sperm quality and DNA fragmentation.

Key words: cumulus oophorus, human spermatozoa, sperm DNA fragment

Introduction

The intracytoplasmic sperm injection (ICSI) method is generally used in cases of severe oligospermia, asthenospermia, and teratospermia. In addition, this method is also used for failing in standard in vitro fertilization (IVF) cycles. Moreover, ICSI bypasses all-natural barriers that prevent the penetration of abnormal spermatozoa into an oocyte, which can increase the risk of genetic disorders and impact on embryo development[1], [2]. Therefore, selecting the best sperm quality is crucial in order to improve fertilization outcomes in cases of ICSI. Accordingly, there are various techniques that are used to select spermatozoa for use in ICSI and, thus, improve fertilization outcomes[3,4].

Spermatozoa are commonly selected for ICSI visually under an optical magnification microscope based on motility and morphology. However, this method does not reveal the genomic integrity of the spermatozoa.

Cumulus oophorus complexs (COCs) encircle the oocyte and contains cumulus cells and extracellular matrix (ECM). Extracellular matrix is a main component in hyaluronic acid (HA), which is produced by COCs after an LH surge and is a potential indicator of healthy spermatozoa[5,6]. The head of mature spermatozoa have a hyaluronan specific receptor that allows them to bind with hyaluronan[7]. During natural fertilization, only one healthy spermatozoon can pass through the COCs and zona pellucida and penetrate into the ooplasm for fertilization.

By contrast, sperm cannot penetrate the oocyte without the presence of COCs meaning that fertilization[8].

Various studies have confirmed the importance of COCs in fertilization[7,9]. For example, several studies have shown that spermatozoa that are able penetrate through a layer of COCs are more likely to have a normal morphology and be more effective in producing acrosome reactions than spermatozoa that are not able to do. Moreover, these spermatozoa have been shown to have higher chromatin integrity and zona-binding capacity, resulting in increased probability of fertilization[10–12]. However, there have been few studies to confirm these outcomes.

Recently, there have been no studies that have assessed the effect of sperm selection using cumulus oophorus complexes on sperm quality, especially in terms of DNA fragmentation. Accordingly, this study was conducted to compare the effectiveness of sperm selection using cumulus oophorus complexes compared to the conventional sperm preparation method in terms of sperm quality and DNA fragmentation with the goal of improving assisted reproduction, especially in ICSI.

MATERIALS AND METHODS

This was a prospective experimental study conducted in Srinagarind Hospital, a university hospital in Thailand. The study was approved by the institutional review board of The Khon Kaen University Ethics Committee in Human Research (HE 611250).

Thirty Semen samples were obtained from leftover specimens at fertility clinics from June to October 2018 were included prospectively in the study. Normal semen samples according to the World Health Organization's 2010 eligibility criteria were

enrolled[13]. Frozen-thawed semen and semen that was derived from surgical sperm recovery were excluded. Semen was processed using the density gradient and swim-up methods. Following this, the sperm was adjusted to a concentration of 10 x 10^6 spermatozoa/mL. It was divided into two groups: the study group (COCs) and control group.

Collection of cumulus oophorus complexes

Cumulus oophorus complexes (COCs) were collected from women attending the infertility clinic of Khon Kaen univeristy for ICSI treatment. Excess COCs was mechanically dissected using a glass pipette under a 400x optical magnification microscope. The COCs was pooled in bicarbonate buffered culture medium with human serum albumin (G-TL™, Vitrolife, Sweden) and incubated at 37 °C in 5% CO2 until it was used.

***In vitro* cumulus oophorus model**

In each case, we used the patient's own COCs. A cumulus oophorus model was performed (Fig. 1)[14]. The collected COCs was placed the B1 area of the ICSI operation dish with 40 µL of sperm washing medium (FertiCult™, FertiProN.V., Belgium). The B2 area was filled with 40 µL of sperm washing medium alone. After that, 20 µL of spermatozoa was added into areas A1 and A2. We expected that the spermatozoa would be able swim from areas A and B to area C. The spermatozoa that were able transverse the COCs would enter area C1 and those that could not would enter area C2. Accordingly, the sperm in area C1 was selected for analysis as the COCs group (study group), the sperm in area C2 was selected for analysis as the control group. Four COCs models were used for each of the patients. In each

case, we waited one hour for the spermatozoa pass through the COCs. These spermatozoa that were aspirated from area C1 and C2 for several COCs model, pooled into study and control Eppendorf tubes.

Determination of sperm motility

The sperm motility and motility parameters of both the study and control samples were analyzed using the Hamilton-Thorne computer-assisted sperm analyzer [CASA; Hamilton Thorne Research, HTM-IVOS 12.3, Boston, MA., USA]. Five hundred spermatozoa per specimen in randomly selected fields were used to determine curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), linearity (LIN), beat cross frequency (BCF), amplitude of lateral head displacement (ALH), and straightness (STR). All of semen samples were processed in triplicate with a single observer.

Determination of sperm morphology

A 6 µL sperm suspension was smeared on a glass slide and air dried. The slides were fixed in methanol and stained using the Diff-Quik procedure. The researchers who conducted the analysis of sperm morphology were blinded as to whether they were examining the study and control group. For each sample, 200 spermatozoa were evaluated at 1000x optical magnification using a Hamilton-Thorne computer-assisted sperm analyzer [CASA; Hamilton Thorne Research, HTM-IVOS 12.3, Boston, MA., USA].

Determination of sperm DNA fragmentation

Sperm DNA fragmentation was determined using a sperm chromatin dispersion (SCD) test (Halotech™, INDAS laboratories, Madrid, Spain) as previously described using the following process: [15]

Melt agarose gel using a water bath at 95-100 °C until it is completely melted. After that, keep the Eppendorf tube at 37°C for five minutes to prevent gelification. Dilute the sperm sample to a maximum of 10 x 10^6 spermatozoa/mL. Transfer 25 µL of the sperm sample to the Eppendorf tube and mix with a micropipette. Place a 15 µL drop of suspension onto the center of a glass slide. Cover with a coverslip and refrigerate at 4°C for five minutes to solidify the agarose. Prepare the denaturant solution by adding 80 microliters of the contents of the acid denaturation solution to 10 mL of distilled water, mix, and place it in the incubation tray. Remove the coverslip. Immerse the slide into the acid denaturation solution and incubate for seven minutes. Place it in another incubation tray containing 10 mL of tempered lysis solution for 20 minutes. Then, wash the lysis solution for five minutes. Place it on a slide with 70% ethanol (two minutes) and 100% ethanol (two minutes). Then, stain using with the Diff-quick technique. Incubate the slide in Eosin solution for seven minutes. Following this, incubate the slide in Azur B solution for seven minutes. Spermatozoa with fragmented DNA have small halos (approximately 1/3 of the diameter of the core or smaller) or no degraded halos.

The researchers who conducted the analysis of perm DNA fragmentation were blinded as to whether they were examining the study and control group. For each sample, 300 spermatozoa were evaluated under 400x optical magnification using bright field microscopy, and the percentage of sperm with fragmented DNA was calculated.

STATISTICAL ANALYSIS

The baselines characteristics of the sperm were described using the mean ± SD. A Wilcoxon signed-rank test and nonparametric test were used to compare differences in terms of sperm motility parameters, (curvilinear velocity [VCL], average path velocity [VAP], and straight-line velocity [VSL]). Paired Student's *t*- test and parametric tests were used to compare other sperm function parameters.

A statistically significant difference was defined as P ≤ 0.05. A 95% confidence interval (CI) of mean difference (MD) was used to analyze the precision of the data.

Statistical analyses were conducted using SPSS for Windows Statistical Package version 23.0 (SPSS Inc, Chicago, IL., USA).

RESULTS

Thirty normal semen samples were enrolled. The baseline characteristics of the sperm were presented in **Table 1**. The mean age of the participants was 35.7±4.23 years. The mean sperm concentration (10^6/mL) was 64.13±16.33. The Mean percentages of sperm motility and normal morphology were 64.73±10.96 % and 11.76±5.68 %, respectively.

The effects of cumulus oophorous on sperm motility were shown in Table 2. There was no statically significant difference in the percentage of sperm motility between the COCs group and the control group 90.63 vs. 89.53; *P*= 0.283). However, the COCs group showed significantly higher curvilinear velocity movement (VCL; 143.5 vs. 122.2; *P*<0.01), average path velocity (VAP; 83.6 vs. 69.3;*P*<0.01), straight line velocity (VSL; 67.95 vs. 60.45; *P*<0.01), and amplitude of lateral head

displacement (ALH; 6.3 vs. 5.07; *P*<0.01) than the control group. There were no other significant differences between study and control group in terms of motility parameters (P>0.05). Beat cross frequency (BCF) was 26.22 vs. 25.44, Linearity (LIN) was 49.9 vs. 49.4, and Straightness (STR) was 79.9 vs 80.46, respectively.

A significantly higher percentage of spermatozoa that were able to pass through the cumulus complexes had normal sperm morphology than those in the control group (21.70 vs. 18.76; P<0.01). The COC group also had significantly lower sperm DNA fragmentation than the control sperm (10.83 vs 18.83; P<0.01). Sperm morphology and sperm DNA fragmentation data were shown in **Table 2.**

DISCUSSION AND CONCLUSION

This study used an *in vitro* model to evaluate whether selecting spermatozoa using cumulus oophorus could improve sperm quality and reduce DNA fragmentation. Compared with the controls group, spermatozoa that had passed through the culture media within the similar condition. The spermatozoa that were able to penetrate the COCs had significantly higher forward motility (VCL, VAP, VSL, and ALH) compared to control spermatozoa. In addition, there was a significantly higher percentage of normal spermatozoa and a lower percentage of sperm DNA fragmentation among the spermatozoa that were able to penetrate the COCs than in the control group.

There are many advantages to using cumulus cells in COCs during the fertilization process. First, cumulus cells in COCs regulate the fertilizing capability of spermatozoa by increasing their functions after penetration[16]. Second, they increase the zona-binding ability of spermatozoa that have successfully passed through the COCs and reduce the suppressive activity of follicular fluid on sperm-zona

binding[17].Third, the spermatozoa that are able to penetrate the COCs is likely to have more acrosome reaction and higher chromatin integrity[10–12].

The penetrated spermatozoa in our study had increased in sperm motility parameters. This result is consistent with those of other studies. Hong SJ et al., 2004; showed that the spermatozoa that are able to penetrate the layer of COCs had significantly higher of VAL, VSL, LIN, BCF compared to control spermatozoa[10]. A 2009 report by Franken DR et al. reported that spermatozoa that are able to penetrate COCs had significantly higher of ALH, BCF and STR than control spermatozoa[11]. In addition, a 2009 study by Hong SJ et al. revealed significantly higher of VAP, VSL, BCF, LIN, and ALH in spermatozoa that were able to penetrate COCs[12]. Previous reports have demonstrated that the COCs matrix is numerous of HA component, which increased motility, DNA integrity, and membrane maturation of spermatozoa[18]. It can imply that the variation of spermatozoa motility pattern can occur in the viscous medium such as COCs, as a result of mechanical resistance of the COCs from local variety composition[19]. However, a previous study reported that the mechanical characteristic of the COCs cannot induce specific sperm motility patterns as the spermatozoa are able to swim freely in the culture medium.[20]. Therefore, the definite mechanism of COCs should be further investigation.

According to sperm morphology of our study, the percentages of normal sperm morphology in study group was higher than control group. This was consistent with previous reports[10,12]. From basic knowledge of human fertilization, sperm that have severe morphological defects failed to penetrate through the COCs and ooplasm. This is consistent with the results of a previous study, which used an electron microscope to evaluate 36 human sperm in the COCs of a pronuclear stage oocyte that retrieved from a woman after natural sexual intercourse. The outcome revealed

that the spermatozoa with normal morphology were only found in the COCs. It has been proposed that the presence of COCs is a key factor in the selection of morphologically normal spermatozoa[21]. Sperm morphology is associated with their fertilizing competency. Previous studies have found intrauterine insemination (IUI) and IVF outcomes to be closely associated to sperm morphology[22].

In the present study, sperm chromatin dispersion (SCD)test (Halotech™, INDAS laboratories, Madrid, Spain) was performed to evaluate sperm DNA fragment. The accuracy of this test was comparable with the sperm chromatin structure assay, a gold-standard sperm DNA fragmentation test[15]. This is a simple, fast, accurate, and highly reproducible method for the analysis of sperm DNA fragmentation. Sperm DNA fragmentation was significantly lower in spermatozoa that were able to penetrate the COCs than in the control group in our study. The results were similar to those of previous studies. Franken et al. reported that there is a decline in the percentage of chromomycin A3 positive spermatozoa (a chromatin packaging of the spermatozoa) in penetrated spermatozoa when compared to control spermatozoa. This data suggested that the spermatozoa's chromatin condensation quality was dramatically enhanced during passthrough COCs[11].The ECM is an important complex structure with a various cross-linked and reactive molecule. The major component of ECM is HA, which is produced when the LH levels surge. In previous studies, the sperm-HA binding has been used to select sperm maturity in ICSI. Evidence suggests that using HA for spermatozoa selection improves DNA and the integrity of chromosomes and reduces sperm DNA fragmentation [18,23]. Moreover, various studies have revealed that spermatozoa selection by HA substantially increases fertilization rates, top-quality embryo rates, embryo development rates, good blastocyst formation rates, implantation rates, and also decreases embryo

chromosomal aneuploidies[3,18,24]. However, HA-selected spermatozoa may lack glycodelin-C, which converts glycodelin-A and -F into glycodelin-C compared with sperm selected using the COCs model. The glycodelin-C in COCs provided to the male germ cells enable to penetrate the cumulus and regulate sperm functions[12,25].

Strengths and limitations

To our knowledge, this is the first study to use a highly accurate method to compare of spermatozoa that are able to penetrate COCs and control spermatozoa in terms of sperm quality and DNA fragmentation. Using the COCs model for sperm selection is more natural, more convenient, and less costly. In addition, the fact that we used the same of semen sample to compare between the COCs group and control group reduced possible confounding factors and selection bias, making the results of this study accurate and reliable. The limitation of this study, we did not evaluate other sperm functions, such as sperm capacitation, acrosome reaction, and spermatozoa-zona binding. However, these processes are unnecessary in ICSI because the ICSI usually bypass these processes.

Implications for practice and further research

Base on the results of this study, spermatozoa that were able to pass though COCs had higher sperm motility parameters, higher rates of normal morphology, and lower DNA fragmentation. It may be advantageous to implement this process during ICSI, as visual selection under optical magnification alone does not reveal the genomic integrity of the spermatozoa. Choosing the best sperm quality is important for improving the outcomes as well as the safety of ICSI. This study showed that implementing a sperm selection method using an COCs model before ICSI may improve fertility outcomes. Further study is needed to confirm the efficacy of this

method in clinical outcomes such as pregnancy rates and live birth rates. Moreover, a prospective study with a larger sample is required to evaluate whether the use of a COCs selection model before ICSI can improve clinical outcomes.

Conclusion

The results of this study suggest that the using COCs for sperm selection is an effective method that dramatically improves in sperm motile function, sperm morphology and sperm DNA integrity.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author contributions

All of the authors have read and prepared the manuscript.

Acknowledgment

The authors would to thank Mr. Dylan Sarheard for assistance with the English-language presentation of the manuscript.

REFERENCES

[1] Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril 2015;103(3):18-25.

[2] Palermo GD, Neri QV, Takeuchi T, Rosenwaks Z. ICSI: where we have been and where we are going. Semin Reprod Med 2009;27(2):191–201.

[3] Worrilow KC, Eid S, Woodhouse D, Perloe M, Smith S, Witmyer J, et al. Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): significant improvement in clinical outcomes--multicenter, double-blinded and randomized controlled trial. Hum Reprod Oxf Engl 2013;28(2):306–14.

[4] Luna D, Hilario R, Dueñas-Chacón J, Romero R, Zavala P, Villegas L, et al. The IMSI Procedure Improves Laboratory and Clinical Outcomes Without Compromising the Aneuploidy Rate When Compared to the Classical ICSI Procedure. Clin Med Insights Reprod Health 2015;9:29–37.

[5] Zhuo L, Kimata K. Cumulus oophorus extracellular matrix: its construction and regulation. Cell Struct Funct 2001;26(4):189–96.

[6] Parmegiani L, Cognigni GE, Ciampaglia W, Pocognoli P, Marchi F, Filicori M. Efficiency of hyaluronic acid (HA) sperm selection. J Assist Reprod Genet 2010;27(1):13–6.

[7] Huszar G, Ozenci CC, Cayli S, Zavaczki Z, Hansch E, Vigue L. Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. Fertil Steril 2003;79 Suppl 3:1616–24.

[8] Zhuo L, Yoneda M, Zhao M, Yingsung W, Yoshida N, Kitagawa Y, et al. Defect in SHAP-hyaluronan complex causes severe female infertility. A study by inactivation of the bikunin gene in mice. J Biol Chem 2001;276(11):7693–6.

[9] Sousa M, Cremades N, Alves C, Silva J, Barros A. Developmental potential of human spermatogenic cells co-cultured with Sertoli cells. Hum Reprod Oxf Engl 2002;17(1):161–72.

[10] Hong SJ, Chiu PC, Lee KF, Tse JMY, Ho PC, Yeung WSB. Establishment of a capillary-cumulus model to study the selection of sperm for fertilization by the cumulus oophorus. Hum Reprod Oxf Engl 2004;19(7):1562–9.

[11] Franken DR, Bastiaan HS. Can a cumulus cell complex be used to select spermatozoa for assisted reproduction? Andrologia 2009;41(6):369–76.

[12] Hong S-J, Chiu PC-N, Lee K-F, Tse JY-M, Ho P-C, Yeung WS-B. Cumulus cells and their extracellular matrix affect the quality of the spermatozoa penetrating the cumulus mass. Fertil Steril 2009;92(3):971–8.

[13] Cao X-W, Lin K, Li C-Y, Yuan C-W. [A review of WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition)]. Zhonghua Nan Ke Xue Natl J Androl 2011;17(12):1059–63.

[14] Wang C, Feng G, Shu J, Zhou H, Zhang B, Chen H, et al. Cumulus oophorus complexes favor physiologic selection of spermatozoa for intracytoplasmic sperm injection. Fertil Steril 2018;109(5):823–31.

[15] Fernández JL, Muriel L, Goyanes V, Segrelles E, Gosálvez J, Enciso M, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril 2005;84(4):833–42.

[16] Sullivan R, Duchesne C, Fahmy N, Morin N, Dionne P. Protein synthesis and acrosome reaction-inducing activity of human cumulus cells. Hum Reprod Oxf Engl 1990;5(7):830–4.

[17] Hong S, Tse JYM, Ho P, Yeung WS. Cumulus cells reduce the spermatozoa-zona binding inhibitory activity of human follicular fluid. Fertil Steril 2003;79 Suppl 1:802–7.

[18] Parmegiani L, Cognigni GE, Bernardi S, Troilo E, Ciampaglia W, Filicori M. “Physiologic ICSI”: hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality. Fertil Steril 2010;93(2):598–604.

[19] Fulford GR, Katz DF, Powell RL. Swimming of spermatozoa in a linear viscoelastic fluid. Biorheology 1998;35(4):295–309.

[20] Tesarik J, Kopecny V. Assembly of the nucleolar precursor bodies in human male pronuclei is correlated with an early RNA synthetic activity. Exp Cell Res 1990;191(1):153–6.

[21] Pereda J, Coppo M. An electron microscopic study of sperm penetration into the human egg investments. Anat Embryol (Berl) 1985;173(2):247–52.

[22] Van Waart J, Kruger TF, Lombard CJ, Ombelet W. Predictive value of normal sperm morphology in intrauterine insemination (IUI): a structured literature review. Hum Reprod Update 2001;7(5):495–500.

[23] Yagci A, Murk W, Stronk J, Huszar G. Spermatozoa bound to solid state hyaluronic acid show chromatin structure with high DNA chain integrity: an acridine orange fluorescence study. J Androl 2010;31(6):566–72.

[24] Nasr-Esfahani MH, Razavi S, Vahdati AA, Fathi F, Tavalaee M. Evaluation of sperm selection procedure based on hyaluronic acid binding ability on ICSI outcome. J Assist Reprod Genet 2008;25(5):197–203.

[25] Yeung WSB, Lee K-F, Koistinen R, Koistinen H, Seppälä M, Chiu PCN. Effects of glycodelins on functional competence of spermatozoa. J Reprod Immunol 2009;83(1–2):26–30.

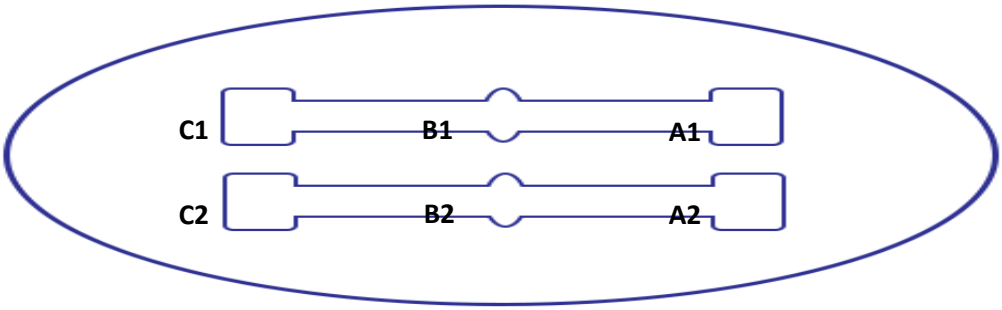

Figure 1. COCs selection model[18]
Area A1, A2 = Place the prepare spermatozoa
Area B1= COCs and sperm washing media
Area B2 = Sperm washing media only
Area C1, C2 = Keep spermatozoa for analysis
(Sperm would swim from area A. through area B. to area C.)

Table 1 Baseline characteristics of sperm characteristics

Sperm characteristics	(Mean ± SD)
Age (years)	35.7±4.23
Volume (mL)	3.35±1.49
Sperm concentration (10^6/mL)	64.13±16.33
Motility (%)	64.73±10.96
VCL (µmol/l/s)	83.54±15.06
VAP (µmol/l/s)	54.33±8.63
VSL (µmol/l/s)	41.07±7.38
BCF (Hz)	23.94±2.99
LIN (%)	47.53±8.81
ALH (µmol/l)	3.85±0.64
STR (%)	75.26±6.82
Normal morphology (%)	11.76±5.68
Vitality (%)	78.56±8.27
pH	8.0

Abbreviations: VCL, Curvilinear velocity; VAP, Average path velocity; VSL, Straight line velocity, LIN, Linearity; BCF, Beat cross frequency; ALH, Amplitude of lateral head displacement; STR, Straightness
Data are presented as mean ± standard deviation

Table 2. Comparison of spermatozoa that were able to penetrate the COCs to the COCs group and control spermatozoa group on sperm quality and DNA fragmentation

	Penetrated spermatozoa	Control spermatozoa	95% CI	p-value
Motility[a] (%)	90.63±7.41	89.53±8.09	-3.160 to 0.960	0.283
Motility parameters				
VCL[b] (µmol/l/s)	143.5 (104-187.7)	122.2 (98.1-186.2)	-21.3 to-11.35	<0.001
VAP[b] (µmol/l/s)	83.6 (73.6-103.8)	69.3 (60.6-105.7)	-15.6 to-8.5	<0.001
VSL[b] (µmol/l/s)	67.95 (58.1-94.2)	60.45 (50-91.9)	-10.3 to -3.69	<0.001
BCF[a] (Hz)	25.44±0.74	26.22±0.82	-0.059 to 2.525	0.365
LIN[a] (%)	49.4±2.54	49.9±1.75	-2.575 to 3.575	0.741
ALH[a] (µmol/l)	6.3±0.29	5.07±0.19	-1.761 to -0.698	<0.001
STR[a] (%)	80.46±1.62	79.9±1.12	-3.056 to 1.923	0.645
Morphology (%)				
Normal form[a]	21.70±1.18	18.76±1.20	-4.326 to –1.539	<0.001
DNA fragmentation[b] (%)	10.83 (4-23.33)	18.83 (5.66-34)	5 to 8.5	<0.001

Abbreviations: VCL, Curvilinear velocity; VAP, Average path velocity; VSL, Straight line velocity, LIN, Linearity; BCF, Beat cross frequency; ALH, Amplitude of lateral head displacement; STR, Straightness; CI, confidence interval

The data are presented as the mean ± standard deviation or median (interquartile range).

The data were compared using a paired Student's *t*- test as a for parametric test and Wilcoxon signed-rank test for as a non-parametric test.

[a] Mean ± standard deviation; [b] Median (interquartile range)