Research Article ISSN 2639-9342

Gynecology & Reproductive Health

Negative Impact of Non-Male Factor ICSI can be Alleviated by Capacitated/Acrosome Reacted Spermatozoa

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Received: 25 November 2019; **Accepted:** 12 December 2019

Citation: Wan-Song A Wun, Reeti Mangal, Emma Bukowski, et al. Negative Impact of Non-Male Factor ICSI can be Alleviated by Capacitated/Acrosome Reacted Spermatozoa. Gynecol Reprod Health. 2019; 3(6): 1-4.

ABSTRACT

Non-male factor intra-cytoplasmic sperm injection (ICSI) reported decreasing implantation and live birth rates. While ICSI becomes the major insemination procedure for human assisted reproductive procedure. How to avoid the negative impact of non-male factor ICSI is the question. The hypothesis is the un-needed enzymes in acrosome area responsible for the negative impact. By selection of capacitated/acrosome reacted spermatozoa for ICSI, significant enhance fertilization, blastocyst formation, implantation and fetal heartbeat rates. The results support the hypothesis and provide a way to alleviate/avoid the negative impact of non-male factor ICSI cases.

Keywords

Intra-Cytoplasmic Sperm Injection, Enzymes, Fertilization, Sperm.

Introduction

In the field of human In Vitro Fertilization, Intra-Cytoplasmic Sperm Injection (ICSI) has become the major insemination protocol [1]. For Europe and USA, the ICSI rates is roughly around 70%. For Middle East region the utilization of ICSI is more than 90%. Apparently ICSI procedure is not only used in male factor cases but also applied in non-male factor cases. With big data analysis, non-male factor ICSI cases reported decrease implantation rates and live birth rate [1,2]. Neither ICSI can increase cumulative live birth rate in non-male factor infertility [3]. With this alert, ICSI is still the major protocol for insemination. There are reasons to use ICSI as insemination procedure. This study explores how to avoid/alleviate the negative impact of ICSI procedure to non-male factor cases.

By mouse model, ICSI with more than 1 spermatozoon is detrimental/lethal [4]. By removing acrosomal membrane, detrimental effect disappears [5]. It is known sperm acrosome equips with3 enzymes, hyaluronidase, acrosin, and phospholipase C zeta 1. Hyaluronidase is used for dissociation and pass through hyaluronic acid bind cumulus granulosa layers [6]. Acrosin (trypsin like enzyme) is used to penetrate through the zona pellucida [6]. Phospholipase C Zeta 1 is the only enzyme needed to activate the egg. ICSI is the procedure which bypasses cumulus granulosa layers and zona pellucida "shell". These 2 layers activate acrosomal membrane break down and release/empty hyaluronidase and acrosin. Without cumulus granulosa layers and zona pellucida empty out acrosomal hyaluronidase and acrosin, ICSI procedure may carry over these 2 un-needed enzymes into egg. By mouse model, un-needed acrosomal enzymes (hyaluronidase and acrosin) are responsible for damaging the egg [4,5]. By theory, acrosome-reacted spermatozoa will release hyaluronidase and acrosin. Without these 2 un-needed enzymes injected into ooplasm, the adverse effect of ICSI can be prevented/alleviated.

By tradition ICSI procedure, spermatozoa for ICSI were prepared by swim up or density gradient centrifugation. The process is either to select sperm by motility or by density. These selection procedures do not consider the acrosome status. During ICSI procedure, it is impossible to identify whether sperm goes through acrosome reaction or not. With these considerations, the traditional approach of sperm selection is not ideal. A new way of sperm selection is required. As it is known capacitated spermatozoa gain the capability of chemotaxis [7,8]. The cumulus cell secreted progesterone is the chemoattractant for capacitated sperm [9,10]. This study creates a method to attract capacitated spermatozoa

with cumulus fragments filled straw (0.5ml straw). The plan of this study is to select capacitated spermatozoa for ICSI. The hypothesis is capacitated/acrosome reacted sperm should empty out un-needed enzymes to avoid non-specific metabolic disruption. Avoidance of non-specific enzymatic disturbance should prevent/alleviate the negative effect of ICSI procedure.

Materials and Method

Preparation of sperm

Received semen samples were kept in 37°C incubator for liquefaction. Liquified semen splits into 2 portions with equal volume in each portion. One portion is in 15 ml conical tube for swim up to 0.6ml modified Human Tubal Fluid (mHTF) with albumin. The second portion transfers to another 15 ml conical tube for cumulus contained straw. A 0.5 ml straw was filled with Human Tubal Fluid with albumin (Irvine Scientific) with 6-10 pieces of cumulus fragments close to the tip (about 0.5cm from opening). The straw then directly inserted into the conical tube with semen. Capacitated spermatozoa gain the chemotactic capability to swim into straw [7,9,10]. Both swim up and cumulus straw remained in 37°C incubator for 30 minutes, the straw removed from semen. To prevent contamination, the tip drops from straw expelled and the remained fraction (about 0.3 ml) transferred to 3ml round bottom tube. For swim up fraction, the top 0.3ml of solution transferred to another 3 ml round bottom tube. Both tubes kept in 37°C incubator until ICSI about 4 hours later. Spermatozoa from this portion were used for the acrosome-reaction study and ICSI. Acrosome reaction examined by FITC-PSA protocol [11]. Sperm kinematic parameters are determined by Hamilton Thorn Sperm Analyzer Ceros (version 14).

Patients records

During 9/15/2017–3/31/2018, totally 182 ICSI cases with 2840 mature eggs in the study. The female age range is 22-46 with Median age 34 and mean age at 34.3. Only cases with at least 6 mature oocytes include in the study. For each ICSI case, half number of mature eggs assigned to swim up group and the other half number assigned to cumulus group. About 16-20 hours after ICSI, fertilization is checked. On day 5, Gardner's criteria used to describe blastocyst quality. Blastocyst at 3BB or above is count as fast development with good quality. Total number of blastocysts is the number of full blastocysts, i.e. expansion at 3 or beyond, up to day 7. Some cases had PGT-A request. Trophectoderm biopsies perform at expanded blastocyst stage at day 5, 6, or 7.

Statistical analysis

Statistical analyses are based on Generalized Estimation Equation (GEE) model for binary data or paired t-test for continuous data. Significant level sets at $p < 0.05$ (two-sided).

Results

The results of sperm kinematic attributes are listed in Table 1. Both swim up and cumulus treatments significantly enhance sperm kinematic activities. The recovery of spermatozoa is significantly less in cumulus group than the swim up procedure. The selection of motility subpopulation is significantly less effective by cumulus treatment than the swim up treatment. The spermatozoa kinematic parameters were significantly activated, i.e. VCL, STR, BCF, HA, except ALH.

	Conc.	Mot	VCL	ALH	STR	BCF	HA
Raw semen	54	27	68	3.1	79	17.5	4.3
Swim up	7.6	62	90	4.3	84	20.9	4.8
Cumulus	2.7	36	94	4.2	82	22.3	7.3
Analysis	<0.05	<0.0001	<0.05	n.s.	<0.05	<0.05	<0.001

Table 1: Comparison of sperm kinematic attributes by treatments.
Conc: concentration, million/ml; Mot: motility %; VCL: curve linear velocity, um/second; ALH: amplitude lateral head displacement, um; STR: straightness, %; BCF: beat-cross frequency, Hz; HA: hyperactivation, %.

The percentages of acrosome reacted subpopulations among treatment immediately after treatment (time 0) summarized in Figure 1. By GEE analysis, significant difference among treatments. Both swim up and cumulus treatment significantly enhance acrosome reacted population ($p<0.001$). Cumulus treatment gives significantly higher acrosome reacted population than swim up treatment does ($p<0.05$).

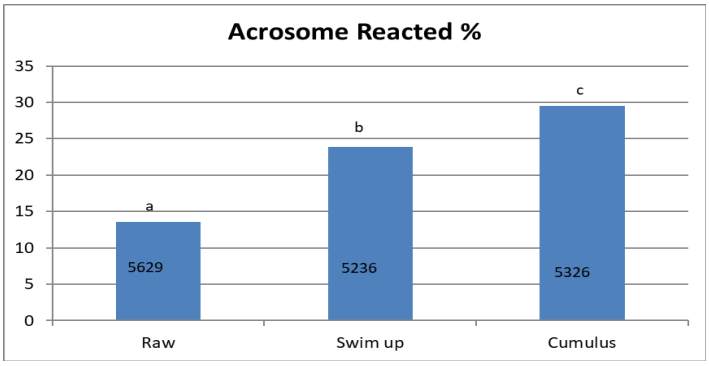

Figure 1: Comparison of acrosome reacted percentage along with treatment immediately after selection procedure (considered as time 0). Number inside each treatment is the number of spermatozoa examined. Different labels indicate significant difference.

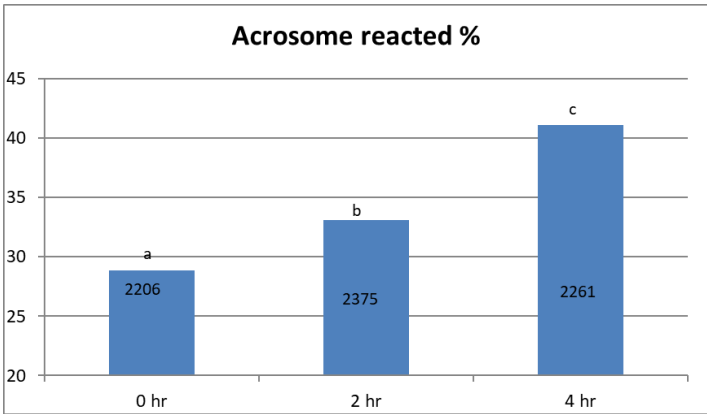

Figure 2: Comparison of acrosome reacted percentage along with culture time. The increasing trend is significant different ($p<0.01$). Different labels indicate significant difference.

Along with incubation time, percentage of acrosome reacted population by cumulus treatment summarized in Figure 2.

	#egg	#fert.	#day5 blast.	Total # blast.	Total # eupl.	# mosaic	I.R.	FHB	SAB
Swim up	1383	957 (69.1%)	113 (11.8%)	473 (49.4%)	176/412 (42.7%)	33/412 (8.0%)	477967 (49.3%)	436/967 (45.0%)	88/477 (18.4%)
Cumulus	1457	1070 (73.4%)	186 (17.3%)	623 (58.2%)	243/548 (44.3%)	48/548 (8.8%)	356/633 (56.2%)	318/633 (50.2%)	56/356 (12.9%)
Analysis	n.a.	< 0.05	<0.0005	<0.0001	n.s.	n.s.	<0.05	<0.05	P=0.07

Table 2: Comparison of sperm treatment along with fertilization, development, and pregnancy attributes. Fert.: Fertilized; blast.: Blastocyst; eupl.: Euploidy; I.R.: Implantation rate; FHB: Fetal heart beat; SAB: Spontaneous abortion.

Capacitated spermatozoa continue engaging acrosome reaction along with incubation before ICSI. ICSI procedure routinely performed about 4 hours after egg retrieval. The acrosome reacted subpopulation is more than 40%. During ICSI procedure, selection of active motile spermatozoa may also increase the utilization of acrosome reacted spermatozoa for ICSI.

The results of comparison of treatment outcomes by fertilization and beyond summarize in Table 2. The fertilization rate, day 5 blastocyst formation rate, and total blastocyst formation rate were significantly increased by cumulus treatment. Euploid rate and mosaic rate were not significant different. The implantation rate and fetal heart beat rate was significantly increased with cumulus treatment. The spontaneous abortion rate was not significant difference. It was marginal at p=0.07.

Discussion

The hypothesis of the study is un-needed enzymes introduce by ICSI may disturb the normal metabolic activity through non-specific enzymatic disruption, i.e. acrosin and hyaluronidase. Un-needed enzymes injected into mouse egg do lethal /damage effect [5]. By removal of acrosomal membrane before ICSI, the damage effect disappears. By that observation [5], this study used acrosome reacted as an indicator of release/empty out of un-needed enzymes.

In order to achieve acrosome reaction, capacitation is a pre-required process. The historical definition of capacitation was "all events that lead to the development of the capacity of mammalian spermatozoa to penetrate eggs" [12]. In this study, in vitro system involves. It may clearer to adapt an in vitro definition "capacitation denotes only those changes undergone by spermatozoa after leaving the male reproductive tract and before the occurrence of acrosome reaction" [13]. It recognizes capacitation is a reversible procedure while acrosome reaction is an irreversible event. Capacitated spermatozoa engage acrosome reaction but may go back through de-capacitation process. This reasoning matches with the observed acrosome reacted status: percent of acrosome reacted spermatozoa were low at time 0 but gradually increase due to accumulation of irreversible of acrosome reacted population (Figure 2). Capacitation is a process to increase metabolism (increase oxygen consumption) and enhance kinematic attributes performance. Swim up procedure is a process to select motile sperm. Swim up process depends on ATP production not capacitation. It is understandable that swim up fraction can enrich some capacitated spermatozoa (by active motility) while cumulus treatment enriches more capacitated spermatozoa (by chemotaxis) (Figure 1). By taking advantage of chemotactic capability of capacitated spermatozoa and progesterone secreted from cumulus cell as chemoattractant [9,10], this study uses cumulus masses to select capacitated/acrosome-reaction engaging spermatozoa for ICSI. The selection did significantly increase kinematic activity as seen in Table 1. The progesterone releasing from cumulus masses in the straw serves as attractant to attract capacitated sperm into straw and enrich the acrosome reacted population (Figure 1).

It is interesting to see the cumulus treatment gives significant higher fertilization rate than the swim up treatment (Table 2). One possible explanation is selection difference. In swim up group, there are more un-capacitated and not acrosome reacted spermatozoa used for ICSI. As previous experience [12], un-capacitated sperm cannot fertilize egg (without acrosomal phospholipase C zeta to activate the egg). Even after fertilization, the developmental ability is not the same. ICSI with swim up sperm showed significant lower implantation and fetal heart beat rates (Table 2). These observations agree with the reports that non-male factor ICSI cases with lower implantation and live birth rates [1,2] when compares with conventional IVF. Day 5 blastocyst (quality ≥ 3BB) number and total blastocyst number are significantly higher in cumulus treated group. It supports the hypothesis that introduce of un-needed enzymes creates un-specific disruption. In cumulus group, blastocysts transferred showing significantly higher implantation rate and fetal heartbeat rate. All these evidences are in agreement with previous report of non-male factor ICSI gives significantly lower implantation and live birth rates [1,2]. The evidence that no significant difference about euploid rate and mosaic rate between treatment suggests the negative impact of un-needed enzymes does not interrupt genetic compartment at chromosome level. The un-specific disruption of un-needed enzymes looks mediate through cytoplasm activities which not involve with genetic making and/or chromosome separation during cell cleavage. The trend of SAB shows no significant different between treatments. This is an interesting phenomenon. Boulet et al. [1] did not find significant difference about SAB between ICSI and IVF group. Grimstad et al. [2] reported a significant difference (p=0.049) for fetal loss or still birth category. The data in this study is marginal (p=0.07). It looks the negative impact of traditional ICSI at SAB phase is subtle. It may depend on study population and waiting for clarification.

In addition to prove the harmful effect of un-needed acrosomal enzymes introduced by ICSI, this study also shows a way to avoid negative impacts of non-male factor ICSI. The novel technique to enrich capacitated spermatozoa through cumulus fragments is simple and can be adapt easily worldwide.

In conclusion, when compared with control group, experimental group (cumulus primed) showed a significant increase in kinematic attributes, i.e. hyperactivation, percentage of acrosome-reacted sperm, percentage of good quality and total number of blastocysts, Implantation rate, and fetal heartbeat. The study demonstrates a way to avoid/alleviate the negative impact of ICSI procedure.

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