Is it necessary to focus on morphologically normal acrosome of sperm during intracytoplasmic sperm injection?

Ziba Zahiri[1] & Fatemeh Ghasemian[2]

[1]Reproductive Health Research Center (IVF Center), Alzahra Educational & Remedial Center, Guilan University of Medical Sciences & [2]Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran

Background & objectives: The detailed assessment of sperm morphology is important in the semen of infertile men because there is a low proportion of normal spermatozoa. One of the parameters of such sperm morphology is the acrosome, and its effect on assisted reproductive outcomes is controversial. This study was undertaken to evaluate the association between different forms of acrosome on the chromatin status and the assisted reproductive outcomes.

Methods: A total of 1587 unstained sperms from 514 infertile men were captured and analyzed for different acrosome forms (normal, large, small, skew, amorphous acrosome and without acrosome) in real time during intracytoplasmic sperm injection into oocytes. The association between the percentage of sperms with atypical acrosome and head shapes and the sperm chromatin status was studied. Fertilization, zygote and embryo quality and clinical pregnancy rates were calculated for different groups of sperms.

Results: The highest frequency of irregular shapes of acrosomes, such as small, large and amorphous, was observed in abnormal ellipticity, anteroposterior symmetry and angularity parameters, respectively ($P<0.05$). The fertilization rate of injected sperms with large ($P<0.01$) and small ($P=0.001$) acrosomes and without acrosome ($P=0.001$) was significantly lower in comparison with normal acrosomes. The quality of zygotes (Z3, $P=0.05$), embryos (grade C, $P<0.05$) and the pregnancy rate ($P=0.001$) from injected sperms with large acrosomes were significantly lower compared with normal acrosomes.

Interpretation & conclusions: Our findings showed that the different sperm acrosome morphologies (*e.g.*, large, small, and without acrosome) might negatively relate with chromatin integrity and decrease the sperm's fertility potential and pregnancy rate during intracytoplasmic sperm injection (ICSI) cycles.

Key words Chromatin status - head shape - ICSI - male infertility - pregnancy - sperm acrosome morphology

The incidence of infertility ranges from 20 to 30 per cent amongst infertile men, and reproductive dysfunction is observed in almost half of all infertile couples[1]. The common causes of male infertility/subfertility are sexual disorders, defects in sperm transportation, primary testicular defects and endocrinopathies[1].

One of the integral techniques to treat couples with male factor infertility is intracytoplasmic sperm injection (ICSI). In such couples, sperm morphology is one of

the important semen parameters for the determination of men's fertility levels[2]. Studies Using different classification systems on the sperm morphology and different preparation and staining techniques have shown that the application of sperm morphology to assay male fertility is still a challenging task[2,3]. One criterion of sperm morphology is the acrosome status. According to the World Health Organization (WHO) criteria[4], the acrosome must be clearly visible, comprising approximately 40-70 per cent of the sperm head. Furthermore, the acrosome biogenesis and nuclear formation are closely associated with each other[5,6].

The evaluation of the motile spermatozoa head, midpiece, acrosome and tail was done based on the motile sperm organelle morphology examination criteria[7]. It was observed in human teratozoospermic semen that the sperm malformation was often accompanied with nuclear deformity[8]. Therefore, the purpose of this study was to quantify human sperm acrosome shape and investigate the association between the different acrosome morphologies of each human sperm in real-time during ICSI and assisted reproductive outcomes. These aims were achieved in two experiments. In the first experiment (Experiment 1), different acrosome shapes and their relationship with different head shapes were evaluated. The chromatin status in different acrosome forms was also studied. In the second experiment (Experiment 2), was examined the ICSI-embryo transfer (ICSI-ET) outcome of each injected sperm was examined based on its head and acrosome shape.

Material & Methods

Semen samples were obtained from 514 men of couples who visited Alzahra Educational and Remedial Center [*in vitro* fertilization (IVF) centre] between May 2012 and March 2016 to treat their infertility. Written informed consent was obtained from these couples for participation in this study. The study protocol was approved by the Guilan University of Medical Sciences Research Ethics Committee (IR.GUMS.REC.1396.523).

Couples with female factor infertility (*e.g.,* ovulatory dysfunction, endometriosis, tubal diseases and under pharmacological treatment) were excluded from this study. Exclusion criteria also covered other abnormalities of sperm motility (asthenozoospermia and oligoasthenoteratozoospermia) and morphology (such as abnormality in the sperm's neck, midpiece and tail morphology). The infertile couples with male factor infertility who were undergoing ICSI treatment were included.

Sperm's head and acrosome assessment: A total of 1587 sperms from 514 men were injected into the related oocytes in accordance with the standard ICSI procedure (Table I): 1093 sperms with normal acrosome from 345 men, 141 sperms with small acrosome from 25 men, 134 sperms with large acrosome from 51 men, 71 sperms with amorphous acrosome from 19 men, 89 sperms with skew acrosome from 47 men and 59 sperms without acrosome from 27 men.

To avoid any possible confounding effect of the fixation and staining processes[2], fresh motile spermatozoa were used to analyze the acrosome shape using sperm morphology analysis (SMA) algorithm[9]. In this way, the image of each sperm was captured in real-time before injection and analyzed using the SMA algorithm[9]. In brief, the SMA method was used to detect and analyze different parts of the human sperm. First of all, image noises were removed, and the image contrast was enhanced by the SMA algorithm. Then, the different sperm parts (*e.g.*, head, acrosome, neck and tail) were recognized, and the size and shape of each part were analyzed. Finally, the sperms (as normal or abnormal) were classified by this algorithm. The SMA method can detect malformations in the sperm's head, midpiece and tail. In contrast to other similar methods, the SMA

Parameter	Normal acrosome	Small acrosome	Large acrosome	Amorphous acrosome	Skew acrosome	Without acrosome
SN/CN	1093/345	141/25	134/51	71/19	89/47	59/27
Men: Age (yr)	36.67±6.9	34.21±5.9	35.17±6.1	36.23±6.2	34.68±5.8	35.47±5.7
BMI (kg/m^2)	25.4±4.8	26.1±4.2	25.5±3.9	26.2±4.1	26.1±5	25.8±3.8
Women: Age (yr)	34.03±7.3	32.3±6.5	32.7±6.8	33.1±6.9	34.5±7.1	33.07±7.2
BMI (kg/m^2)	23.5±4.2	21.7±3.8	22.8±4.2	23.5±3.5	23.7±3.9	21.9±3.7

Table I. The characteristics of patients and cycles of the studied groups

SN/CN, sperm number/cycle number; BMI, body mass index

method can work with low resolution and non-stained images. This algorithm analyses the sperm image in less than nine seconds. After analysis, the sperm was injected into oocyte, and the injected oocyte was incubated. The captured sperm's head shape was classified in accordance with parameters defined by Utsuno *et al*[10] as follows: Ellipticity (length/width ratio), anteroposterior (AP) symmetry, lateral symmetry and angularity parameters (Figure A).

The sperm's acrosome morphology was also categorized regarding size, form and detailed characteristics. The acrosome morphology included the following six classes: normal (N), small (S), amorphous (A), large (L), skew (SK) and without acrosome (W). Figure B shows the different acrosome images of the human sperms. Acrosome evaluation criteria were defined as follows:

Normal acrosomes: The same criteria for a normal sperm head, as regards the acrosome were used to classify the acrosome as normal so that the acrosome would be clearly visible, and well-defined, with a smooth oval configuration, and comprise 40-70 per cent of the sperm head.

Small: Cases with the acrosome covered <40 per cent of the anterior part of a normal head was considered as small[4].

Large: Cases with the acrosome covered more than 70 per cent of the anterior part of a normal head were considered as large[4].

Skew: Cases with the posterior part of an acrosome in an oblique form were considered as skew.

Amorphous: All other abnormalities, including the acrosomes that were seen irregularly, were classified as amorphous.
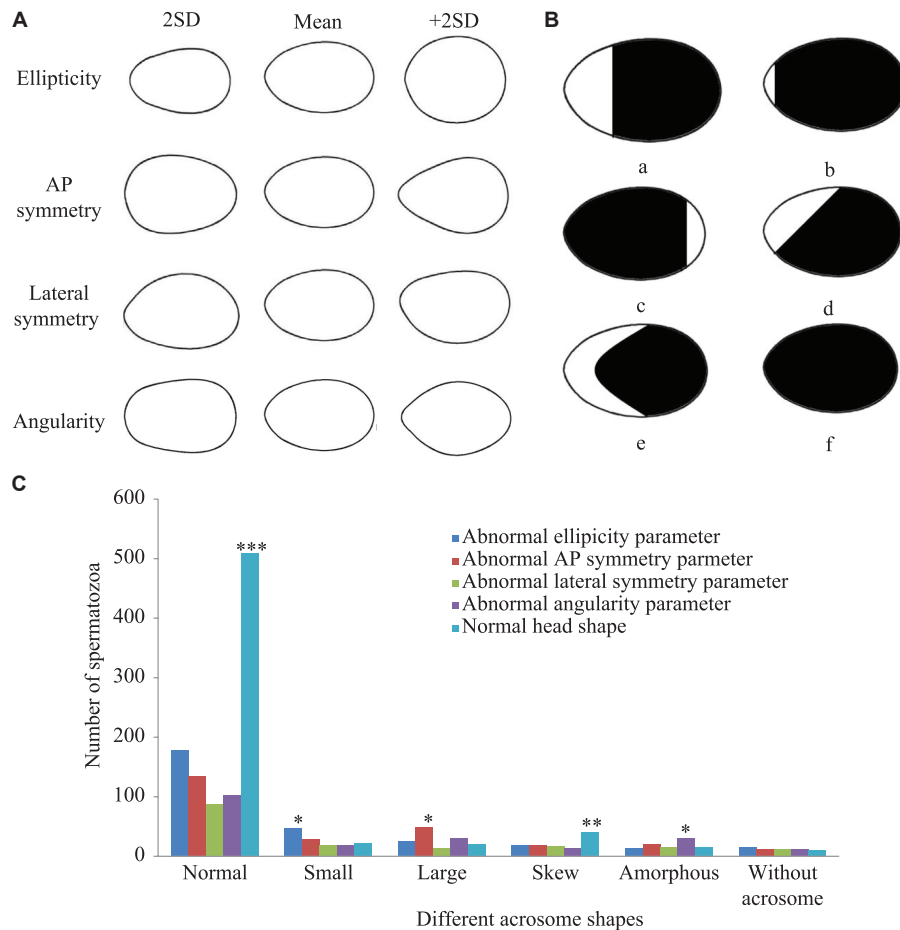

Figure. Different head and acrosome images of sperm. (**A**) The four numeric parameters of head shape. SD, standard deviation; AP, anteroposterior. (**B**) Schematic images of different acrosomes: (a) Normal acrosome, (b) small acrosome, (c) large acrosome, (d) skew acrosome, (e) amorphous acrosome, and (f) without acrosome. (**C**) Distribution of different acrosome morphologies amongst spermatozoa with different head shapes in comparison to normal head shape. $P^*<0.05$, $^{**}<0.01$, $^{***}<0.001$.

Without acrosome: In such cases, there was no acrosome in the sperm head.

It is expected that acrosome abnormality would proceed in parallel with head abnormality. Therefore, the distribution of acrosome abnormality is shown in four head shapes, with the normal shape of the head (Figure C).

Sperm chromatin assays: After the injection of all the oocytes of each patient, the remaining sperm samples were used for the chromatin assay. This assay was conducted to determine the frequency of chromatin damage in each sperm sample.

Toluidine blue stain: Thin smears were prepared on silane-coated slides to assay chromatin integrity. The air-dried smears were fixed in 96 per cent ethanol-acetone medium (1:1 v/v) at 4°C for one hour and were then put in 0.1 N HCl at 4°C for five minutes for hydrolysis. After three-times rinsing with distilled water for two minutes, these were stained with 0.05 per cent toluidine blue (TB, in 50% Mcilvaine's citrate phosphate buffer, *p*H 3.5) (Sigma-Aldrich, USA) for five minutes at room temperature (RT). Under light microscopic evaluation, a minimum of 200 spermatozoa were seen in each slide and evaluated using oil immersion with ×1000 magnification. Sperm's head with intact and fragmented chromatin was seen as light blue and deep violet (purple), respectively.

Aniline blue (AB) stain: To assay the chromatin condensation of sperms, aniline blue (AB) stain was used. In brief, the fixation of smear was performed in four per cent formalin, rinsed in water and stained in five per cent AB (Sigma-Aldrich) in a solution of four per cent acetic acid (*p*H 3.5) for five minutes at RT. The slides were rinsed with water, dried and evaluated under a light microscope. Under light microscopic evaluation, a minimum of 200 spermatozoa were counted in each slide and evaluated using oil immersion with ×1000 magnification. Immature sperm with excessive histone and abnormal sperm chromatin was indicated as stained dark sperm.

In vitro fertilization (IVF) laboratory procedures: The semen samples were collected on the day of follicle aspiration. The semen samples were liquefied for at least 15 min at RT. An aliquot of each semen sample was used for a standard semen analysis in accordance with the WHO criteria[4]. To calculate the percentage of normal sperm morphology in fresh semen samples,

more than 200 sperms were assessed at a magnification of ×1000 after Diff-Quik staining[4]. The density-gradient centrifugation method was done to process the semen samples. In brief, 45 per cent SpermGrade medium (45% SpermGrade and 55% GIVF[PLUS], vitrolife, Sweden) was overloaded on top of 90 per cent SpermGrade (90% SpermGrade and 10% GIVF[PLUS]) medium. After incubation for one hour, liquefied fresh semen was layered on top of it and centrifuged at $300{\times}g$ for 15 min. The pellet was transferred to new tube and re-suspended with 5 ml equilibrated GIVF[PLUS] medium, and centrifuged again. The centrifugation was repeated twice. The pellets were finally resuspended in 0.5-1 ml of equilibrated GIVF[PLUS] depending on the sample quality. Then, the washed samples were incubated at a temperature of 37°C with six per cent CO_2, five per cent O_2 and 89 per cent N_2 in GIVF[PLUS] medium.

The sperms with different head and acrosome forms were injected during each ICSI cycle, and the immotile sperms and those with other abnormalities (*e.g.*, tail and neck abnormality and the presence of vacuole) were excluded for injection. The only difference amongst injected sperms was in head and acrosome morphology. The sperm selection based on skew, amorphous and normal acrosome forms was done randomly. However, some semen samples only had sperm with large or small acrosomes or without acrosomes. Hence, there was no choice for injection. Therefore, recovered oocytes were injected in accordance with the ICSI procedure, using an inverted microscope (Olympus IX70, Tokyo, Japan). In this way, the selected sperm was captured (×600 magnification) and the shape and size of the sperm's acrosome and head were analyzed in real-time during ICSI, using SMA algorithm[9].

The injected oocyte was cultured in a 25 µl droplet of G1[PLUS] medium to be analyzed in more detail. About 16-18 h after microinjection, the fertilization was evaluated with the observation of the two-pronucleus stage. The two pronuclei zygote assessment was performed on the basis of the scoring system of Scott *et al*[11]. The evaluation of the embryos was also performed on the basis of the scoring system of Ebner *et al*[12] on day 3 after injection. The embryos were classified as grade A: regular cells and without fragmentation; grade B: cells with lower than 25 per cent fragmentation; grade C: cells with fragmentation between 25 and 50 per cent; and grade D: cells with fragmentation

Table II. The relationship between different acrosome forms of the human sperm and *in vitro* fertilization-embryo transfer outcomes

Parameter	Normal acrosome	Small acrosome	Large acrosome	Amorphous acrosome	Skew acrosome	Without acrosome
			OR (95% CI)			
Fertilization (%)	71.72	52.48***	59.7**	69.01	69.66	3.33***
	1.0	0.435 (0.305-0.621)	0.584 (0.404-0.845)	0.878 (0.522-1.476)	0.905 (0.565-1.449)	0.013 (0.002-0.097)
Zygote degree (%)						
Z1	21.3	16.21	32.56	28.57	32.25	0
	1.0	1.0	1.0	1.0	1.0	
Z2	40.07	43.24	29.75**	30.63	35.48	0
	1.0	1.419 (0.65-3.08)	0.47 (0.22-0.973)	0.57 (0.27-1.18)	0.585 (0.304-1.08)	
Z3	26.65	33.78	23.75**	22.44	24.19	0
	1.0	1.66 (0.732-3.78)	0.461 (0.206-1.03)	0.628 (0.284-1.388)	0.599 (0.276-1.303)	
Z4	11.98	6.77	13.94	18.36	8.08	0
	1.0	0.740 (0.23-2.32)	0.653 (0.248-1.72)	1.143 (0.456-2.86)	0.444 (0.156-1.266)	
Embryo degree (%)						
A	51.21	54.42	51.25	31.25	62.29	0
	1.0	1.0	1.0	1.0	1.0	
B	29.32	39.72	28.75	35.41	22.95	0
	1.0	1.236 (0.748-2.043)	0.98 (0.574-1.675)	1.95 (0.996-3.833)	0.644 (0.342-1.214)	
C	12.41	1.35[†]	16.25[†]	22.91[††]	4.93	0
	1.0	0.100 (0.014-0.736)	1.301 (0.671-2.521)	3.137 (1.474-6.675)	0.324 (0.098-1.071)	
D	7.06	4.5	3.75	10.43	9.83	0
	1.0	0.525 (0.157-1.753)	0.525 (0.157-1.753)	2.111 (0.75-5.947)	1.133 (0.458-2.803)	
TE/total cycles	714/345	63/25	80/51	45/19	61/47	0
TE per cycle	2.06±0.91	2.52±0.58	1.56±0.72	2.36±0.59	1.29±0.54	0
IE/total cycles	497/240	33/12	13/10	18/8	18/14	0
Implantation rate (%)	69.6	52.38	16.25***	40	29.5	0
	1.0	0.698 (0.468-1.04)	0.426 (0.26-0.691)	1.583 (0.96-2.585)	0.917 (0.571-1.471)	
C+/IE	448/497	27/33	8/13	13/18	15/18	0
Clinical pregnancy rate (%)	90.1	81.81	61.53	72.22	83.33	
	1.0	0.49 (0.21-1.13)	0.17*** (0.08-0.38)	0.28** (0.13-0.62)	0.54 (0.23-1.27)	0
One embryo (n)	55	1	1	1	3	0
Two embryos (n)	63	7	2	3	3	0
Three embryos (n)	89	4	1	2	2	0

Contd...

Parameters	OR (95% CI)					
	Normal acrosome	Small acrosome	Large acrosome	Amorphous acrosome	Skew acrosome	Without acrosome
A/IE	26/497	2/33	2/13	2/18	1/18	0
Miscarriage rate (%)	5.23	6.06	15.3*	11.1	5.5	0
	1.0	1.213 (0.35-4.11)	3.35 (1.16-9.61)	2.34 (0.78-7.02)	1.00 (0.28-3.56)	
LB/IE	422/497	25/33	6/13	11/18	14/18	0
LB (%)	84.9	75.7	46.1	61.1	77.7	
	1.0	0.55 (0.27-1.13)	0.152*** (0.07-0.29)	0.27*** (0.142-0.551)	0.62 (0.301-1.27)	

P *<0.05, **<0.01, ***<0.001 compared to normal acrosome; P+<0.05, ++<0.01 compared to normal and grade A acrosome. The reference category is expressed as 1.0. TE, transferred embryo number; IE, implanted embryo number; C+, number of embryo in clinical pregnant; A, aborted fetus; LB, live birth; OR, odds ratio; CI, confidence interval

higher than 50 per cent.

Three days after microinjection, an ET was performed using soft catheter as intrauterine. The implantation and clinical pregnancy were determined with increasing serum beta-human chorionic gonadotropin (B-HCG) concentration and observation of foetal heartbeat, respectively, at two and four weeks after ET. Abortion was defined as pregnancy loss spontaneously after observation of pregnancy by ultrasound[13].

The captured sperms were used for further analysis and for the evaluation of their relationship with zygote and embryo quality. The implantation fate of embryos related to each injected sperm was considered as follows: in some cases, a single embryo from sperm injection with distinct acrosome was transferred (200 transfers), and its implantation fate was followed. In other treatments, two or three embryos were transferred (196 embryos in 98 cycles and 567 embryos in 189 cycles, respectively). If all the transferred embryos had the same fate (had implantation or did not have implantation), these cycles with known implantation data were included to analyze in more detail. In cases with multiple ETs, when some transferred embryos were implanted, the recognition of the implanted embryo type result from injected acrosome type was not possible. Such cases were excluded to analyze implantation rate.

The number of transferred embryos is related with the number of available embryos and their quality. Therefore, the number of transferred embryos may be different between one to three embryos. The clinical pregnancy rate was also calculated based on all embryos implanted that were included in the analysis of the implantation rate.

Statistical analysis: Statistical analysis was performed using SPSS version 20 (IBM, Armonk, NY, USA). Binary and multinomial logistic regressions were used to test the differences amongst the groups.

Results

The characteristics of the studied couples are summarized in Table I. The women and men had a mean age between 28.0±5.6 (range: 20 to 39 yr) and 32.0±5.9 (range: 26 to 38 yr) yr, respectively. There was no significant difference in age and body mass index (BMI) of men and women amongst groups with different acrosome forms.

Table II shows the assisted reproductive outcomes obtained from injected sperms with different acrosome forms (*e.g.* normal, small, large, amorphous, skew and without acrosome). The fertilization rate was significantly different between normal acrosome group and small, large and without acrosome groups. Thus, the odds of having successful fertilization were 0.435 (0.305-0.621; *P*<0.001) and 0.584 (0.404-0.845; *P*<0.01) times lower for sperms with small and large acrosomes, as opposed to the normal acrosome. This rate was decreased to 0.013 (0.002-0.097; *P*<0.001) times for sperm without acrosome. The normal acrosome and unsuccessful fertilization are the reference categories. It should be mentioned that the sperms without acrosome were excluded (not considered) in analyzing the zygote and embryo quality and the pregnancy rate, since fertilized oocytes did not develop in this group. The zygote degree assessment shows that the odds ratio for Z2 and Z3 degrees was 0.47 (0.22-0.973; *P*<0.01) and 0.461 (0.206-1.03; *P*<0.01) for spermatozoa with large acrosome compared to those with normal acrosome. The normal acrosome and Z1 zygote were considered the reference categories.

In addition, the odds of having grade C of embryos was 3.137 (1.474-6.675; *P*<0.01) times higher in the sperms with amorphous acrosome, as opposed to the normal acrosome and grade A embryos. This grade of embryo also significantly increased in groups with large acrosome (1.301: 0.671-2.521, *P*<0.05) while the grade C rate decreased in the sperms with small acrosome (0.100: 0.014-0.736, *P*<0.05) compared to grade A and the normal acrosome. The normal acrosome and grade A were considered the reference categories.

There was a significant difference in the implantation rate amongst different groups. Thus, the odds ratio for implantation for spermatozoa with large acrosomes was 0.426 (0.26-0.691; *P*<0.001) compared to those with normal acrosomes. The odds ratio for miscarriage percentage was significantly higher (3.35: 1.16-9.61; *P*<0.05) in this group in comparison with the normal acrosome group. The live birth rate per transferred embryo was 0.152 (0.07-0.29; *P*<0.001) times lower in the large acrosome group in comparison with the normal acrosome. This decline was observed for spermatozoa with amorphous acrosome (0.27: 0.142-0.551; *P*<0.001) compared to normal acrosome. The normal acrosome was the reference category.

As shown in Figure C, there was a significant association between the frequency of different acrosome shapes compared to the abnormal shape. The highest frequencies of normal (*P*<0.001) and skew (*P*<0.01) acrosome shapes were observed in the normal head shape in comparison to other acrosome morphologies. The small, large and amorphous acrosome shapes were significantly observed in abnormal ellipticity, AP symmetry and angularity parameters, respectively, compared to other acrosome morphologies (*P*<0.05).

For the evaluation of the sperm chromatin status in relationship with the sperm acrosome morphology, TB and AB staining were used and the percentage of abnormal sperm chromatin structure and condensation was compared in sperms with different acrosome forms (Table III). The results showed a significant association between the sperm acrosome morphology and the semen normal morphology. The normal semen morphology percentage of group without acrosome was lower (0.34: 0.12-0.99, *P*<0.05) than sperms with the normal acrosome. There were significant differences in the abnormal chromatin condensation of sperms with the acrosomes of small (2.9: 1.68-5.15, *P*<0.001), large (9.89: 4.28-22.81, *P*<0.001), amorphous (3.29: 1.62-6.69, *P*<0.001), and without acrosome (24.2: 10.2-57.7, *P*<0.001). For the sperms with damaged chromatin, there was also a positive relationship between the acrosomes of small (6.84: 4.07-11.5), large (17.2: 7.52-39.46), amorphous (5.35: 2.72-10.52), and without acrosome (18.25: 7.33-45.44) and the increasing rate of damaged sperm chromatin. This increase was observed in the sperms without acrosome and with large acrosome (*P*<0.001).

Discussion

The results of this study indicated that the frequency of each acrosome shape varied in different parameters of the head shape. The different acrosome forms (*i.e.* small, large, amorphous, normal, skew or without acrosome) influenced the chromatin integrity and its condensation. Therefore, the change in head and acrosome shapes and chromatin quality may influence the sperm fertility potential. Consequently, IVF-ET outcomes were changed by considering atypical acrosome forms of injected sperm. As a result, the fertilization rate was decreased in the groups of small, large, and without acrosome sperms. The pregnancy rate of embryos from injected oocytes with spermatozoa having large acrosome was also

Table III. Relationship between the acrosome status and human sperm chromatin status

Parameter	OR (95% CI)					
	Normal acrosome	Small acrosome	Large acrosome	Amorphous acrosome	Skew acrosome	Without acrosome
n (%)	543 (33.21)	305 (24.15)	176 (10.76)	209 (12.78)	224 (13.7)	178 (10.88)
	4.0 (1.8-8.4)***	2.5 (1.1-5.5)	0.9 (0.4-2.4)	1.2 (0.5-2.8)	1.3 (0.5-3.05)	1.0
Normal semen morphology (%)	13.59±5.34	8.02±4.82	7.94±5.13	9.07±5.61	8.88±5.09	5.14±4.82*
	1.0	0.55 (0.22-1.39)	0.54 (0.21-1.38)	0.63 (0.26-1.54)	0.61 (0.25-1.5)	0.34 (0.12-0.99)
Abnormal chromatin condensation AB (%)	13.46	31.42***	60.6***	33.89***	32.77	79.06***
	1.0	2.9 (1.68-5.15)	9.89 (4.28-22.81)	3.29 (1.62-6.69)	1.13 (0.72-5.7)	24.2 (10.2-57.7)
Damaged chromatin TB (%)	17.97	60***	79.06***	54***	48.57	80***
	1.0	6.84 (4.07-11.5)	17.23 (7.52-39.46)	5.35 (2.72-10.52)	1.3 (0.5-7.4)	18.25 (7.331-45.44)

P *<0.05, ***<0.001 compared to normal acrosome. The reference category is expressed as 1.0. n, number of studied sperms; AB, aniline blue; TB, toluidine blue

significantly decreased. However, the miscarriage rate of this group was higher than other groups.

It has been documented that the sperm morphology is related closely to the sperm-zona pellucida binding[14] and the clinical outcome[15]. It has been reported that an unusual chromatin pattern correlates with an apoptotic process in the round-headed spermatozoa[16]. In the present study, the zygote and embryo quality were decreased in the groups of injected oocytes with spermatozoa having large acrosome and without acrosome. A strong positive correlation has been reported between sperm and acrosomal morphology[17]. It was found that morphologically normal acrosomes played an important role during the interaction of sperm and zona pellucida[17]. It has also been reported that the binding ability to zona pellucida for small, oval-formed and pyriform sperms and morphologically normal sperms are the same[18]. It has been reported that the presence of globozoospermia and sperms with abnormal acrosomes shows the greater percentage of DNA fragmentation and sex chromosome aneuploidy and disomy 8 in the sperms[19]. In this study, the correlation between the abnormal acrosome and chromosomal aneuploidy was established. It has been indicated that using a machine learning-based decision support system during the sperm selection can predict male fertility potential and assisted reproductive oucomes[20]. It has been observed that DNA damage is caused by the excess reactive oxygen species (ROS) and appears in the sperm morphology defects[21,22].

Our results suggested that the evaluation of acrosome morphology in real time during ICSI could be a simple and effective way of gaining more information on the sperm fertility potential and predicting ICSI-ET outcomes in couples with the male factor infertility. One of the main benefits of using acrosome morphology is the fertility prognosis in men with severe teratozoospermia. The acrosome evaluation along with other sperm parameters can be performed during injection and it can help to improve the ART outcomes. However, one of the main limitations of this study was that male infertility/subfertility could be due to idiopathic spermatogenic defects or genetic mutations that was not considered.

In conclusion, the results of this study show that the acrosome morphology can be regarded as a valid additional parameter for predicting ICSI rates. Another important fact was that acrosome evaluation of unstained and captured sperms was performed in real time during ICSI using the SMA algorithm[9]. Therefore, the acrosome morphology evaluation can be repeated and confirmed using the sperm images. The results of this may can be helpful in deciding the effectiveness of acrosome morphology for ICSI-ET outcomes and the potential of sperm fertility, because these atypical acrosome forms may be associated with nuclear formation and integrity.

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For correspondence: Dr Fatemeh Ghasemian, Department of Biology, Faculty of Sciences, University of Guilan,
P.O. Box: 41335-1914, Rasht, Iran
e-mail: ghasemian@guilan.ac.ir