DESIGN: Cross-sectional study of 215 couples undergoing assisted reproductive treatment (ART).

MATERIALS AND METHODS: Sperm from men undergoing ART were analyzed for DNA damage using the alkaline Comet assay and classified into three groups 'Low damage' (0 to 30%), 'Intermediate damage' (31 to 70%) and 'High damage' (71 to 100%). The cause of couples' infertility was categorized into one of the three types (male, female or unexplained). Each embryo was categorized as 'Good', 'Fair' or 'Poor' quality, based on the number and grade of blastomeres. The chi-square statistic was used to test the effect of sperm DNA damage between the patient infertility categories, type of ART and embryo quality. Logistic regression was performed to determine the impact of sperm DNA damage and infertility factors simultaneously to predict the quality of developing embryos, implantation and or pregnancy success.

RESULTS: Result: The total number of embryos analysed in this study was 951 (IVF) and 1259 (ICSI). The percentage of poor quality embryos was significantly lower and the percentage of good quality embryos was significantly higher after ICSI insemination method compared with IVF insemination. Embryo quality was significantly improved after ICSI particularly in couples with increased DNA damage. Implantation was higher after ICSI treatment compared with conventional IVF in couples with high sperm DNA damage group. There were no differences in the clinical pregnancy rates between the IVF and ICSI treatment types.

TABLE 1. Differences (%) and P values comparing embryo quality between ICSI and IVF methods

		Low		Intermediate		High	
Day	Embryo quality	Difference	P value	Difference	P value	Difference	P value
Two	Good	1.5	0.786	17.8	<0.001	8.0	0.010
	Fair	12.5	0.793	2.0	0.503	4.8	0.089
	Poor	-14.0	0.002	-19.8	<0.001	-12.8	<0.001
Three	Good	9.5	0.085	20.5	<0.001	4.2	0.173
	Fair	1.2	0.785	2.8	0.276	0.9	0.663
	Poor	-10.7	0.041	-23.3	<0.001	-5.2	0.096
Five	Good	1.6	0.677	4.5	0.001	1.3	0.485
	Fair	21.4	<0.001	12.9	<0.001	3.9	0.178
	Poor	-23.0	<0.001	-17.4	<0.001	-5.2	0.097

Difference = % ICSI embryos - % IVF embryos

CONCLUSION: ICSI improves prognosis for patients with increased sperm DNA damage.

Supported by: This study was Supported by University of Utah internal funds.

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USE OF ANNEXIN V- MACS IN ICSI INFERTILE COUPLES: EFFECT ON FERTILIZATION RATE (FR), PREGNANCY RATE (PR) AND EMBRYO QUALITY. J. A. Notrica,[a] M. H. Vazquez-Levin,[b] N. M. Bossi,[a] D. E. Notrica,[a] M. C. Granados,[a] E. Polak de Fried.[a] [a]Reproductive Medicine, CER Medical Institute, C.A.B.A, Buenos Aires, Argentina; [b]Reproductive Medicine, IBYME Institute of Biology and Experimental Medicine, C.A.B.A., Buenos Aires, Argentina.

OBJECTIVE: To compare FR, PR and embryo quality in ICSI procedures when density gradient centrifugation (DGC) and MACS is used in teratozoospermic and apoptotic sperm samples vs. DGC applied in teratozoospermic samples.

DESIGN: Retrospective-comparative study.

MATERIALS AND METHODS: Eighty two infertile patients who underwent ICSI procedures in our fertility center. Group A: 54 cycles with severe male factor Kruger morphology $\leq$ 7%, TUNEL >20 %. Sperm was selected by DGC + AnnexinV-MACS and the eluted fraction was used to perform ICSI / embryo transfer (ICSI / ET). Group B: 28 cycles with Kruger morphology $\leq$ 7% and normal TUNEL, underwent ICSI-ET. Statistics: Fisher exact test and Mann – Whitney test ($p<0.05$, significant).

RESULTS: FR was comparable between Group A vs. Group B (77.7±2.7 vs. 83.5±3.0, p=0.21, NS). PR was similar between Group A and B (43.4% vs. 42.8%, p=1, NS). Patients age, number of metaphase II injected oocytes and number of embryos transferred were similar when comparing both groups. Regarding good-quality embryos (grade I and II, Lucinda Veeck criteria) there was a significant difference between Group A and Group B (1.98±0.1 vs. 1.5±0.1, $p<0.05$). Pregnancies from Group A resulted in 22 healthy babies and in Group B 10 healthy babies.

CONCLUSION: Teratozoospermic and apoptotic sperm samples underwent DGC + AnnexinV-MACS have comparable FR and PR to those found with non-apoptotic sperm samples group showing DGC + AnnexinV-MACS is an efficient procedure. All children were healthy suggesting that the use of AnnexinV-MACS could be safe. According to our results we could suggest that this technique may improve the number of good quality embryos. A prospective randomized larger study should be done with long term follow – up of children.

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ABSTRACT WITHDRAWN

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SPERMATOZOA MIR-34C LEVELS ARE RELATED WITH INTRACYTOPLASMIC SPERM INJECTION OUTCOMES. Y. Ye, L. Cui. Women's Hospital, Zhejiang Universtiy School of Medicine, Hangzhou, Zhejiang, China.

OBJECTIVE: Paternal miRNAs, one of the important epigenetic factors, can be delivered to the oocyte during fertilization. It has been reported that sperm-borne miR-34c is important for the first cleavage division of mouse embryo. The objective of our study os to investigate whether the expression of miR-34c in human sperm is related with intracytoplasmic sperm injection (ICSI) outcomes, such as fertilization rate, early cleavage rate, good quality embryo rate, pregnancy rate and implantation rate.

DESIGN: Ejaculated sperm from patients who underwent ICSI for male infertility were collected. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expressions of miR-34c in human sperm.

MATERIALS AND METHODS: One hundred and sixty-two patients with male infertility who underwent ICSI in our center were included in the study. Patients with azoospermia, polycystic ovary syndromes, decreased ovarian reservation, chromosome abnormality, previous fertilization failure were excluded. After ICSI, the spermatozoa were add to RNAlater and stored at -80°C until use. The levels of miR-34c in human sperm were evaluated by qRT-PCR. Differences between two groups were calculated using t-test, comparison of proportions were using c^2 test and Fisher's exact tests.

RESULTS: The levels of miR-34c had no difference between oligospermia (n=18), asthenospermia (n=39), teratospermia (n=15) and Oligoasthenoteratozoospermia (n=90) (F=1.479,P=0.222;F=1.152,P=0.330;respectively). The level of miR-34c was higher in pregnancy group than non pregnancy group ($P<0.001$). If we set 6.82 as a favourable cut-off value of miR-34c, a comparison between the ICSI outcomes of high miR-34c patients versus low miR-34c patients is shown in Table 1.

miR-34c and ICSI outcome

	High miR-34c	low miR34c	p value
cycle	77	85	
fertilization rate	71.34	65.5	0.659
embryo early cleavage rate	14.3	9.0	0.017
good/moderate embryo rate	61.5	47.6	0.304
implantation rate	35.7	15.8	0.001
pregnancy rate	59.7	22.4	0.001

There was no significant difference between the two groups in fertilization rate, good/moderate embryo rate. However, the embryo early cleavage rate, pregnancy rate and implantation rate were significantly higher in high miR-34c patients.

CONCLUSION: High spermatozoa levels of miR-34c are related with better ICSI outcomes. Paternal miRNAs, one of the important epigenetic factors, probably have a role in regulating preimplantation embryo development.