

**EMBRYO QUALITY AND REPRODUCTIVE OUTCOMES OF SPERMATOZOA SELECTED BY PHYSIOLOGIC-ICSI OR CONVENTIONAL ICSI IN PATIENTS WITH KRUGER <4% AND >4% NORMO-MORPHOLOGY.** J. Castillo-Baso, G. Garcia-Villafaña, R. Santos-Haliscac, P. Diaz, J. Sepluvada-Gonzalez, S. Hernandez-Ayup. Instituto para el Estudio de la Concepción Humana, Monterrey, Nuevo Leon, Mexico.

**OBJECTIVE:** Spermatozoa bound to HA show significant reduction of DNA fragmentation and aneuploidy and could offer better results in IVF cycles. The aim of this study was to determine differences in embryo quality and reproductive outcomes in ICSI cycles with the sperm selected using Physiologic -ICSI (PICS).

**DESIGN:** Prospective comparative analysis.  
**MATERIALS AND METHODS:** A total of 120 consecutive fresh non donor ICSI cycles were analyzed. Patients were divided into two groups according to the strict Kruger sperm morphology criteria of <4% and >4%. For each group and for each patient were randomly injected with sperm selected by PICS (*Mid Atlantic Diag. Inc.*) or conventional ICSI.

**RESULTS:** Both groups were homogeneous for demographic, length of infertility and stimulation protocols: Female age (35.1 vs 35.2); FSH day 3 (8.7 vs .8.8 IU/l); LHday3 (6.6vs.6.1 IU/l); total doses of rFSH (2161.5 vs 2490.2 IU) and HMG (750 vs 900 IU); Agonist (35% vs. 27%) or antagonist (65% vs. 73%) cycles and endometrial thickness Day 10 (9.16vs. 9.15mm); male age (37 vs 36 years) and sperm parameters. Reproductive outcomes are in table 1.

Embryo Quality and Reproductive Outcomes.

	Kruger < 4%			Kruger >4%		
	PICSI (n:30)	ICSI (n:30)	P	PICSI (n:30)	ICSI (n:30)	P
Total Oocyte $\alpha$	12.40	11.07	NS	9.30	10.27	NS
Oocyte MII $\alpha$	9.70	8.76	NS	8.21	8.78	NS
MI Fertilization Rate	49%	51%	NS	57%	62%	NS
Day2 GI	35%	33%	NS	32%	32%	NS
GII	25%	32%	NS	24%	26%	NS
GIII-GIV	38%	35%	NS	44%	42%	NS
Day 3 GI	35%	34%	NS	34%	27%	NS
GII	26%	29%	NS	41%	34%	NS
GIII-GIV	37%	36%	NS	25%	39%	NS
Number of Embryos in Day 6	6	1	0.001	3	1	NS
Embryo Transfer GI	47%	36%	0.037	39%	25%	NS
GII	29%	32%	NS	38%	28%	NS
GIII-GIV	24%	32%	0.040	23%	47%	NS
Implantation Rate	34%	24%	0.035	25%	22%	NS
Pregnancy Rate	53%	40%	0.041	47%	42%	NS

$\alpha$  Mean; G: Grade

**CONCLUSION:** In the group of patients with Kruger <4%, embryos generated with PICS-selected sperm are of better morphological quality, development and reproductive outcomes. This benefit is not maintained in patients with Kruger sperm morphology >4%.

**SPERM VACUOLE IMPROVEMENT AFTER ANTIOXIDANT THERAPY.** M. Brassesco, G. López, A. Monqaut, R. Lafuente. Medical Department, CIRH, Barcelona, Spain; Andrology Laboratory, CIRH, Barcelona, Spain.

**OBJECTIVE:** It has been observed a relation between sperm nuclear vacuoles and oxidative stress. Oxidative stress occurs when the production of potentially destructive reactive oxygen species (ROS) exceeds the natural antioxidant defenses, resulting in cellular damage. Embryos derived from a spermatozoa with a high content of vacuoles could involve abortions or implantation failures. Our aim in this study was to assess the efficacy of antiox-

idants by determining the presence of vacuoles in the spermatozoa head at 8000x.

**DESIGN:** Assessment of sperm nuclear vacuoles after antioxidant therapy.  
**MATERIALS AND METHODS:** Sperm semen samples from 33 patients were analyzed, before and after antioxidant treatment, using a high magnification Leica AM6000 inverted microscopy. Sperm morphology was assessed *in vivo* at 8000x magnification and spermatozoa were classified into four grades according to the presence and size of the vacuoles. The antioxidant treatment is based on the administration of L-carnitine, oligoelements and vitamins for at least one month. Statistical Analysis was performed by comparing the four grades of magnification between pre and post treated semen and using the Wilcoxon matched pairs test. Data were analyzed using R software v 2.9.0.

**RESULTS:** Less vacuolated spermatozoa (grade I and II) showed a significant increase after the antioxidant treatment and the spermatozoa grade IV (with large vacuoles in conjunction with abnormal head shapes or other abnormalities) showed a significant decrease.

**CONCLUSION:** Antioxidant treatment reduces the presence of vacuoles in the spermatozoa nucleus, most probably, because the antioxidant therapy decreases ROS production. In addition, high magnification is a good diagnostic tool that can help allows the identifications of nuclear vacuole-free spermatozoa for assisted reproductive techniques.

**NUCLEAR VACUOLES AND APOPTOSIS MARKERS AFTER ANNEXIN V COLUMNS IN PATIENTS WITH SEVERE TERATOZOOSPERMIA.** C. Alvarez Sedó, M. Lavolpe, H. Uriondo, S. Papier, F. Nodar, C. Chillik. Centro de Estudios en Ginecología y Reproducción (CEGYR), Capital Federal, Buenos Aires, Argentina.

**OBJECTIVE:** Annexin V columns (AnV-C) has been proposed as a tool to reduce levels of sperm apoptosis. The aim of this study was to determine the presence of nuclear vacuoles and apoptotic markers after using AnV-C and assess the relation between the size and number of nuclear vacuoles with sperm apoptosis.

**DESIGN:** Double-blinded prospective cohort study.  
**MATERIALS AND METHODS:** Twelve semen samples, with a previously altered DNA fragmentation (TUNEL) assay (>20%), were analyzed. After two-layers discontinuous gradient, the pellet was eluted (filtered) through the AnV-C. Three sperm populations were studied: after gradient (Initial), filtered fraction (Negative) and the fraction retained in the column (Positive). It was evaluated the number and size of vacuoles (counting 200 spermatozoa) and apoptosis markers (500 spermatozoa/ marker): phosphatidylserine externalization (EPS), cleaved caspase-3 (CC3) and TUNEL.

Number and size of sperm vacuoles were assessed by MSOME, considering the following patterns: A) Without vacuole, B) 1 vacuole lower than 25% of sperm head, C) 2 vacuoles lower than 25%, D) 1 vacuole larger than 25% and E) Others.

**Statistical analysis:** ANOVA  
**RESULTS:** A significant decrease of apoptotic sperm markers levels were observed after using AnV-C (negative fraction) ( $^*P<0.05$ ). However, the number and size of nuclear vacuoles did not show a significant difference between fractions. The fraction with high levels of sperm apoptosis (positive) did not show a prevalence of vacuolated spermatozoa in relation to vacuole size (table 1).

TABLE 1

	INITIAL	NEGATIVE	POSITIVE
Levels of apoptotic markers			
EPS (%)	28.7	2.3 <sup>a</sup>	57.2
CC3 (%)	14.8	4.0 <sup>a</sup>	28.4
TUNEL (%)	32.3	9.6 <sup>a</sup>	53.7
Levels and types of sperm vacuoles			
A (%)	3.6	2.6	3.6
B (%)	18.1	19.6	20.6
C (%)	14.8	12.3	14.9
D (%)	24.0	24.1	25.5
E (%)	39.6	41.4	35.4