

## **Assessment of sperm ploidy status by flow cytometry correlates to embryo quality**

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**Study question.** To assess, by using flow cytometry, the effect of sperm ploidy status on embryo quality following an *in vitro* fertilization treatment

**Summary answer.** Sperm ploidy condition constitutes a critical parameter for the evaluation of semen samples before an assisted reproductive treatment

**What is known already?** Male infertility is a contributing factor in up to 50% of all infertility cases. New and improved diagnostic methods that reduce inter/intra variability should be evaluated. Flow cytometry has been an outstanding tool to evaluate sperm cells for achieving a better reproductive condition. The implementation of this technique in order to evaluate sperm ploidy, is contributing to the discrimination of spermatozoa from subfertile men in an objective way, leading to a reproducible assessment of male infertility, especially in candidates for an assisted reproductive treatment.

**Study design, size, duration.** Prospective and observational study. Sixty seminal samples coming from sperm donors (n=30) and subfertile patients (n=30) were analyzed by flow cytometry in IVI Madrid from June to December 2016. Furthermore, in order to analyze if there were any correlation between sperm ploidy and embryo quality, we evaluated clinical outcomes from 30 couples included in our oocyte donation program; all these couples underwent an ICSI procedure and a fresh embryo transfer.

**Participants/materials, setting, methods.** Semen analysis was performed according to World Health Organization guidelines. Ploidy determination was performed by using propidium iodide (PI) staining in combination with sperm flow cytometry; the resulting cell suspension (n=20000 cells/sample) was examined. For describing the correlation between ploidy and other sperm and embryo parameters, linear functions were approximated and the Pearson's correlation coefficient (r) was determined. Differences were considered to be significant if the probability of their occurrence by chance was <0.05.

**Main results and the role of chance.** We found significant differences in some of the analyzed variables between patients and donors respectively. From semen analysis, data were as follows: ejaculation volume (ml) 2.8 vs. 3.5,  $p=0.027$ ; sperm concentration (million/ml) 43.6 vs. 70.9,  $p<0.001$ ; total sperm number (million) 122.4 vs. 234.6,  $p<0.001$ ; progressive motility (%) 36.7 vs. 58.5,  $p<0.001$ ; total motility (%) 47.8 vs. 139.0  $p=0.001$ . Data derived from sperm ploidy showed significant differences in the percentage of subploid cells between patients (20.4%) and donors (8.1%),  $p<0.001$ ; similar results were obtained for haploid cells (75.6 % vs. 84.3%,  $p=0.011$ )

According to the correlation between sperm ploidy and some embryo features, we observed a positive correlation in the fertilization rate ( $r=0.398$ ) and in the number of viable embryos ( $r=0.454$ ) as well as the percentage of haploid cells increased in the seminal sample. On its behalf, seminal samples with a higher rate of subploid cells were significantly negatively correlated with both variables,  $r=0.265$  and  $r=0.459$  for the fertilization rate and viable embryos respectively.

**Limitations** Flow cytometry is a screening method in which it is not possible to perform a precise determination of aneuploidies of specific chromosomes; moreover, we cannot quantify the degree of DNA damage within a single cell.

**Wider implications of the findings.** Although conventional semen analysis maintains its central role in assessing male fertility, it is often insufficient to provide a definitive diagnosis. Implementing flow cytometry in the daily practice due to its higher sensibility and lower cost could help many couples undergoing unexplained infertility to improve clinical outcomes within an assisted reproductive treatment.

**Study finding/competing interest.** None

**Trial registration number.** It does not apply

