Abstract title:
Lack of consistency in the execution and reporting of sperm FISH poses potential problems for clinical interpretation and patient counselling

Study question:
To what extent are strategies for sperm aneuploidy testing and the reporting of results standardized?

Summary answer:
Comparison of FISH reports from multiple laboratories in nine different countries confirms a remarkable lack of consistency in technical, analytical and reporting methods.

What is known already:
Improper segregation of chromosomes during meiosis results in the generation of genetically unbalanced sperm or oocytes. If these gametes participate in fertilization, the resulting embryo will be aneuploid and will consequently be at greatly elevated risk of implantation failure, miscarriage or the birth of a chromosomally abnormal child. Multiple studies have indicated that some infertile men with normal karyotypes, and in many cases normal semen parameters, display increased sperm aneuploidy. Fluorescence in situ hybridization (FISH) analysis with chromosome-specific DNA probes is capable of evaluating aneuploidy rates in human sperm and has been employed for this purpose by many laboratories.

Study design, size, duration:
This is a descriptive study comparing a large number of FISH sperm reports issued by 46 different laboratories, located in nine countries. The patients that provided the sperm samples attended 11 ART clinics (part of a single network) between 2017 and 2018. Sperm-FISH was undertaken for specific couples when physicians wished to obtain a more complete picture of the male factor, using molecular methods to supplement routine semen analysis.

Participants/materials, setting, methods:
The reports analysed were generated in laboratories that offer sperm FISH, located in Spain (n=28), France (n=6), Italy (n=6), Argentina (n=1), Norway (n=1), Portugal (n=1), Switzerland (n=1), Australia (n=1) and Turkey (n=1). Information from the reports was extracted, and a data sheet was created to analyse all the variables.

Main results and the role of chance:
The data reported varied between laboratories. Reported parameters included disomy rate (50.0% of laboratories), X and Y bearing sperm ratio (41.3%), diploidy (45.6%), nulisomy (29.8%) and monosomy rates (21.7%). The number of sperm analysed was reported by 84.8% of laboratories (mean 1914.8, range 200-104887). In 82.6% of the cases five chromosomes were examined (13,18,21,X,Y), 2.1% screened a different set of five (12,18,21,X,Y), 8.7% analysed seven chromosomes (13,15,16,17,18,21,22,X,Y), and 6.5% examined only three chromosomes (18,X,Y).

The test result was clear in 41 (89.1%) reports, with about half (56.1%) yielding an abnormal outcome. Reference values were given in 58.7% of the reports, but the control population was only well-defined in
one laboratory (normozoospermic fertile men). In 10.9% of reports, it was stated that the reference values were obtained from publications, whereas no information about the control population was given for the rest. The test was considered normal when the percentage of abnormalities was lower than the control group (89.1%) or when the difference in abnormality rates reached statistical significance (10.9%). When disomy was assessed, gonosomes showed higher abnormality rates (17.6%) followed by chromosomes 13 (11.8%), 18 (8.8%) and 21 (5.9%). A high percentage of reports (33.3%) indicated an increased diploidy rate.

Limitations, reasons for caution:
While we were able to carry out a full assessment of the way clinical data was reported, we were only able to make inferences about certain aspects of the laboratory method (eg, number of sperm analysed, chromosomes tested, etc). We were unable to examine the accuracy of the FISH protocol.

Wider implications of the findings:
Variability in FISH methodology and reporting is challenging for physicians who must interpret results to counsel patients. The creation of a Best Practice Guideline by a panel of experts and the participation in pilot schemes for sperm FISH should be encouraged, in order to maximize diagnostic accuracy and consistent reporting.

Trial registration number:
Not applicable

Keywords:
Spermatozoa
Aneuploidy
Fluorescence in situ hybridization
Diploidy
Male factor