

In the name of God

Teratozoospermia&PGT

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Introduction

- While in vitro fertilization (IVF) is most often employed as a remedy for infertility, a discussion of the field would not be complete if it did not address the application of IVF to avoid genetic disorders.
- IVF makes it possible to assess the genetic status of the embryo before establishing a pregnancy when couples are at risk for an affected child.
- Physicians in the field will benefit from being informed about the diverse set of molecular and cytogenetic technologies employed in preimplantation genetic diagnosis (PGD) and screening (PGS), and from understanding their relative power and limitations as tools for genetic counseling.

- PGD and PGS refer to two distinct types of clinical procedure that help a couple to have a healthy child:
- PGD determines the embryo's genotype, while PGS assesses an embryo's karyotype and has been used in screening chromosomal aneuploidy.
- PGD and PGS require the use of the IVF technique. These technologies, initiated in the late 1980s as an alternative to prenatal diagnosis (PND), allows a couple at risk of a genetic disorder to give birth to an unaffected child by avoiding selective termination of an affected pregnancy.
- Genetic disorders could be due to either a single gene disorder, or an abnormal number or structure of chromosomes

- For years, sperm morphology has been a debated indicator of male fertility and success with assisted reproductive technologies (ARTs) .
- While subfertile men have a lower percentage of normal forms when compared with men with proven fertility, the question of “Does form impact function?” remains.
- While an assessment of sperm morphology is a component of the standard semen analysis, the clinical utility of this is debated.

WHO percentages for normal sperm decreased dramatically.

In the 1st edition, the average normal morphology was 80.5% , which decreased to 50% in the 2nd edition, 30% in the 3rd edition, 14% in the 4th edition, and is currently 4% for the 5th edition.

While it is commonly believed that the decline in reference values are mostly due to the introduction of strict criteria, there are some that believe there is an actual decline in the number of morphologically normal sperm due to environmental factors .

Normal sperm

- The 2010 WHO manual defines a morphologically “normal” sperm as having a head (with acrosome), midpiece, and tail.
- Specifically, a “normal” head has an oval shape with smooth contours. The acrosome is clearly visible, well-defined, exhibits a homogenous light-blue staining, and covers 30–60% of the anterior portion of the sperm head.
- A “normal” midpiece lacks cytoplasmic residues and is axially attached to the head, without forming a definite angle with respect to the head, $\leq 1 \mu\text{m}$ in width and approximately 1.5 times the head length.
- The tail should also lack cytoplasmic residues, be apically inserted to the post-acrosomal end of the midpiece, have a length of approximately 45–50 μm long, and be lacking any sharp bends. Sperm should be analyzed after being stained via a modified Papanicolaou method. The analyzer should assess at least 200 spermatozoa per sample

CASA

- The assessment of sperm morphology is still highly subjective and prone to inter- and intra-laboratory differences.
- In an attempt to circumvent the subjective nature of a visual assessment, computer-aided sperm analysis was developed. This system analyzes sperm kinetics in an attempt to provide more objective sperm parameters.
- While this computer generated analysis attempts may minimize observer bias, it does not provide the detailed morphological assessment necessary to accurately define normal versus abnormal sperm, and therefore, may not be as useful for determining sperm morphology .

Of note, there are specific sperm morphology anomalies that do necessitate in vitro fertilization (IVF). These include globozoospermia, primary ciliary dyskinesia, and significant tail defects. These are sperm without the capacity to swim to the egg, or penetrate the egg.

Conversely, macrocephalic heads have been associated with sperm aneuploidies, where intracytoplasmic sperm injection (ICSI) is ineffective and contraindicated .

Natural Conception

Kovac et al. conducted a retrospective chart review investigating the likelihood of achieving pregnancy without the use of ART in men with 0% normal forms, as per strict Kruger criteria .

Twenty-four men with 0% normal forms were compared to 27 randomly selected men with $\geq 4\%$ normal forms over a 3-year period. While the natural conception rate was higher in men with $\geq 4\%$ normal forms compared to the 0% group (51.8% vs 25%, $p \leq 0.05$), **men with 0% normal forms were still able to conceive naturally in 25% of cases.**

Additionally, in cases where men with 0% normal forms conceived naturally, 100% of these men had another child via natural conception. The authors concluded that strict morphology should not be used to predict fertilization, pregnancy, or live birth potential, and in men with 0% normal forms, alternative reproductivemodalities should be considered before immediate IVF.

IUI

- More recently, Kohn et al. conducted a meta-analysis of 41,018 IUI cycles looking at the impact of sperm morphology ($> 4\%$ and $\leq 4\%$, and $\geq 1\%$ and $< 1\%$ normal forms) on ultrasound verified pregnancy outcome .
- when using the WHO 4th or 5th guidelines (4% normal morphology threshold), no difference in pregnancy rates was seen (14.2% versus 12.1% versus 13.9% for normal forms $> 4\%$, $\leq 4\%$ or $< 1\%$, respectively).
- The most recent data do not seem to show a strong correlation between sperm morphology and IUI success rates.

Does teratozoospermia impact ICSI success rates?

- The existing data on the impact of sperm morphology on IVF and ICSI outcomes is conflicting.
- While there are studies showing that morphology affects reproductive outcomes, more recent data show that fertilization and clinical pregnancies have been reported in couples even with normal sperm morphology of 0% .
- More prospective data is needed in this area to define the true impact and the role for IVF and ICSI in affected couples.

Embryo Quality and Development

Parinaud et al. found that sperm with morphologic abnormalities of the post-acrosomal region and sperm with cytoplasmic droplets resulted in embryos of a lower quality .

Similarly, a 2018 study by Coban et al. found a relationship between sperm morphology and embryo aneuploidy. Donor oocytes were used to minimize the impact of the maternal factor on aneuploidy .

A total of 1165 embryos were divided by sperm morphology according to Kruger's strict criteria (score groups 1–5, where a higher score indicated better morphology). While fertilization rates improved with increasing morphology score, this was not statistically significant. However, **mean incidence of aneuploidy was lower in group 5 compared to the other groups with lower morphology scores** ($p < 0.003$).

The true impact of sperm morphology on embryo quality and development is unclear based on the very little evidence in this area .

- Genetic factors are however likely to be very frequent as it has been estimated that a genetic origin of male infertility could be found in nearly 1 in 40 men .Chromosomal aberrations (mainly 47,XXY—Klinefelter syndrome),microdeletions of the Y chromosome and cystic fibrosis transmembrane conductance regulator (CTFR) mutations have been unambiguously demonstrated to be recurrent genetic causes of male .
- Despite this, routine screening for these well-established genetic causes (in principle justified only for patients presenting with certain specific phenotypes) results in a diagnosis in ,5% of all phenotypes .
- We can therefore safely assume that the bulk of the genetic causes of male infertility is still uncharacterized, likely due to the large number of genes involved and the lack of ambitious studies carried out on large cohorts of affected men .

European Academy of Andrology guideline

- We recommend karyotype analysis of infertile men with a sperm concentration $\leq 5 / 10^6/\text{mL}$ to assess the risk for an unbalanced karyotype of embryos .
- We recommend assessment of Yq microdeletions of infertile men with a sperm concentration $\leq 5 / 10^6/\text{mL}$, which will be inherited to male offspring .
- We recommend cystic fibrosis transmembrane conductance regulator (CFTR) gene evaluation in case of suspicion for incomplete congenital obstruction of the genital tract.

- The cases with severe teratozoospermia undergoing ICSI treatment can display a higher rate of sex chromosome aneuploidies in their embryos (threefold) than cases with moderate teratozoospermia.

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The current agreed upon indications for PGD and PGS include:

- 1. Screening for embryo chromosomal aneuploidy in cases of advanced maternal age or known parental translocation
- 2. Family history indicating risk for known autosomal Mendelian genetic disorders
- 3. Sex selection with family history indicating risk for X-linked disorders
- 4. Sex selection for family balancing, e.g. parental preference for a male or female
- 5. Human leukocyte antigen (HLA) matching to achieve a child to provide hematopoietic progenitor cells from cord blood to an existing sibling who requires bone marrow transplantation

WHEN TO CONSIDER PGD IN ADDITION TO IVF?

- Recurrent miscarriages
- One child already affected with a genetic disease
- Family history of inherited disease
- Maternal age older than 38
- Couples with >3 IVF failures
- Epididymal or Testicular sperm aspiration with >1 IVF failures
- **Family “balancing” for sex**



INDICATIONS FOR PGD

- When there is suspicion of Chromosomal Disorders
 - Chromosomal rearrangements
 - Inversions
 - Translocations
 - Chromosome Deletions
- Severe monogenic diseases
 - cystic fibrosis,
 - β thalassaemia,
 - sickle cell anemia,
 - fragile X syndrome,
 - myopathies



- Infertile couples due to severe male factor can be treated with ICSI.
- In order to generate normally fertilized oocyte safety ICSI, a spermatozoon containing a functional genome and centriole is required .
- A study in cases of macrocephalic spermatozoa demonstrated an increased incidence of chromosomal abnormalities, and the majority of the abnormalities were aneuploidy .Due to the high incidence of aneuploidy these patients might benefit from PGS owing to its effect of eliminating chromosomally abnormal embryos.

- Several groups have reported increases in sex chromosome disomies and diploid spermatozoa in patients with OAT undergoing ICSI cycles.
- Nevertheless, taking into account the risk of production of chromosomally abnormal spermatozoa (aneuploid, diploid), application of PGD could be useful in some patients showing meiotic abnormalities.
- Cytogenetic analysis of embryos derived from these couples and further replacement of those evaluated as normal could result in an increase in the implantation and pregnancy rate, as well as in a decrease in the rate of chromosomal abnormalities in the offspring.

Studies with & without Beneficial Outcome of PGS


Kahraman et al. compared the implantation and ongoing-pregnancy rates of PGS cycles with non-PGS cycles in cases with predominantly macrocephalic spermatozoa and absolute teratozoospermia. A statistically higher implantation rate as well as a significantly reduced missed abortion rate were found in PGS group (25.0% and 14.3%) compared with non-PGS group (12.3% and 46.7%).

Although severe male-factor infertility is one of the PGS indications that have been put forward, current reports of PGS in severe male-factor infertility are rare. There is a lack of scientific evidence to prove whether PGS is effective in these patients.


Reasons for Lack of Benefit in PGS

- Technical reasons for lack of benefit in PGS include **both biopsy damage** to the remaining embryo that reduces its developmental potential and **limitations of current FISH technology** that allows only a few chromosomes .
- **Mosaicism**, a difference of the chromosomal constitution among individual cells in an embryo, is another possible reason for confusion.
- A single blastomere that had been biopsied might thus be classified as abnormal, whereas the remaining blastomeres in the embryo are normal. Thus, the test results from the biopsied cell may not be an accurate indication of the embryo's genetic status
- **contamination** and laboratory mistakes can also result in inaccurate diagnoses. For example, DNA from sources other than the biopsied cell may be read as part of the genetic analysis, mixup, or mislabeling of a sample or embryo from clinic or laboratory mistakes in handling samples or embryos.

PGD - LIMITATIONS

- PGD is expensive, time and labor intensive to develop and work with single-cell diagnostic techniques.
 - PGD can only detect a specific genetic disease in an embryo.
 - It cannot detect many genetic disorders at a time and cannot guarantee that the fetus will not have an unrelated birth defect.
 - Removal of a single cell without breaking it or causing serious damage is technically difficult and requires skill and experience. Damage to the embryo (projected to be 0.1%) may accidentally occur during removal of the cell.
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PGD - LIMITATIONS

- For aneuploidy screening, not all chromosomal or genetic abnormalities can be diagnosed with PGD because only a restricted number of chromosomes can be examined at one time during the course of a single procedure.
 - Currently, FISH offers evaluation of less than half of the 23 chromosomes; usually 9-11 are analyzed.
 - Studies using comparative genetic hybridization (CGH) and FISH demonstrate that as many as 25% of aneuploid embryos are characterized as normal because the abnormal chromosomes were not analyzed.
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PGD - LIMITATIONS

- Current recommendations from the Society for Assisted Reproductive Technology (SART) and American Society for Reproductive Medicine (ASRM) state that
 - Available evidence does not support the use of PGS to improve live-birth rates for advanced maternal age, recurrent pregnancy loss, or implantation failure
 - Also recommends that patients be counseled about the limitations of the technique and should not make future treatment decisions based solely on PGD result.



PGD - POTENTIAL

- In the future, genetic links to common diseases (eg, diabetes, hypertension, cardiovascular diseases, endometriosis, cancers) may be identified, and PGD will become available to control the transmission of these diseases to future generations.



Conclusions

- The use of PGS applied for severe male factor infertility needs more scientific data from clinical trials.
- The routine use of PGS to avert the birth of an aneuploidy infant is still in question.
- Application of micro-CGH and blastocyst biopsy might be new approaches for improvement of the efficacy of PGS. Furthermore, the cost-effectiveness of PGS for the IVF patients should be considered.
- Nowadays, PGD is used to improve IVF success rate in **certain** groups of patients

THE END

