Male infertility
RSA
DFI

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To do or not to do?

- Male infertility is the commonest cause of infertility
- nearly 50% of the 15% of couples of reproductive age
- a marginal improvement in pregnancy and birth rates after 30 years of trying
- 4% more couples seeking ART per year

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- Over the past decades, an increasing number of infertile couples seek medical assistance by ART
  (4% more couples seeking ART per year)

- ART outcome : unpredictable & multifactorial

- SA parameters are not sufficient
- causes of male infertility at the molecular level?

traditional semen analysis tells us little-why do we keep using an inadequate batch of tests?

- SA cannot discriminate between the sperm of fertile and infertile men
- semen analysis can do identify men whose chance of achieving a natural pregnancy is very low i.e. they have few or no sperm.
- only 1% of sperm even reach the oocyte in vivo

Why test sperm DNA?

- impaired fertilization
- slow early embryo development
- reduced implantation
- miscarriage
- in animal studies, birth defects in the offspring.
- Childhood cancers have also been associated with oxidative damage to sperm DNA as a consequence of paternal smoking

DNA damage

- double or single strand breaks

- Any stage:

  spermatogonia to the ejaculated spermatozoa

  post testis >

What couples would benefit from a sperm DNA test?

- IUI
- IVF
- ICSI
- couples with unexplained infertility (men with normal semen)
- prior to embarking on IVF, couples who have had unsuccessful IVF
- couples who have had miscarriages

What couples would not benefit from a sperm DNA test?

- couples with oligoastheno-terato-zoospermia as there is no treatment other than ICSI for them

Available tests

- SCSA
- TUNEL
- SCD Halo
- Comet assay

Background to SDF

- Evanson

- 30 y ago

Sperm chromatin structure assay

- fluorescence cell sorter test
- measure large numbers of cells rapidly
- single stranded fragments
- detect sperm DNA damage in 20% of unexplained couples
- Threshold 30%
- ICSI / IVF

Terminal transferase dUTP nick end labeling (TUNEL)

- ‘nicks’ (free ends of DNA)
- Single and double stranded
- Microscopy or FCM
- its many protocols, which makes comparison between laboratories almost impossible and explains its many clinical thresholds
- Larger numbers of sperm

Sperm chromatin dispersion (Halo) test

- Simple
- Inexpensive
- Measures the absence of damage rather than the damaged DNA in sperm
- Correlations have been observed between DNA and other sperm parameters
- Few correlations between sperm DNA damage and ART outcomes

- Subjective
- Operator dependant
- Different cutoff

Comet Assay

- 2nd generation
- quantifies the actual amount of DNA damage per sperm
- Small sample 100 sperms
- Measure both single and double strand breaks
- 26%
- Clinical utility
- Spermatogenesis & spermiogenesis

Normality

- Natural pregnancy
- SCSA 30-40%
- TUNEL 19%
- Comet 25%

Variation of thresholds

- Age of sperm donor
- Female factor
- Abstinence time
- Oxidative reduction potential
- Leukospermia

Unexplained infertility

- unexplained infertility is a very unsatisfactory diagnosis for couple and clinic alike
- 40-80% SDF
- >25% /sperm
- >50% poor IVF outcome
- Live birth rate < female factor IVF

  15% 20%

Benefits of DFI in IVF

- DFI<25% IVF live birth rate 33%
- DFI>50% IVF live birth rate 13%
- direct these patients straight to ICSI treatment

- avoiding loss of valuable biological time, cost of failed cycles and heartache after repeatedly unsuccessful cycles of IVF treatment

Why sperm with poor DNA are successful in ICSI

- Up to 30% of women having ICSI have no detectable problems. They may be fertile and their oocytes may have more capacity to repair DNA damage
- Gametes are not subjected to prolonged culture so the sperm may have less damage than those exposed to culture media overnight, as in IVF procedures

Mechanism

- much sperm DNA damage is caused by oxidative stress and so these sperm are producing reactive oxygen species. If they are used in IVF, the oocyte may be exposed to oxidative assault during the overnight incubation from these 0.5 million sperm.

Natural pregnancy
high DFI

- Young women
- Total motile count normal
- Single strand

Limitations of sperm DNA testing

- unsuitable for clinical purpose
- Birefringence
- Intracytoplasmic morphologically selected sperm injection (IMSI)
- Hyaluronic acid-selection

Unexpected failure of ART

- IVF
- ICSI

Recurrent spontaneous abortion

- Fetus or embryo loss 20-22w
  under 500g
- 2or more
- 1-5% clinical pregnancy
- 40-50% without answer

- Spontaneous abortion 15%
- Ab2 3^{rd} Ab 30%
- Ab 3 4^{th} Ab 35%

- Chromosomal aberrations are the causal factor in a small percentage of cases experiencing RPL.
- a large number of cases may have sub-microscopic rearrangements or nonspecific DNA damage which is not detected by cytogenetic analysis
- oxidative stress (imbalanced ROS and TAC) in cases of iRPL with both normal and abnormal semen profiles

Sperm DNA damage correlates with:

- patients with DFI > 30% are at higher risk of infertility regardless of whether they conceive via natural conception or ART

- 3-fold increase in the miscarriage rate

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- DFI< 40% 10× greater probability of pregnancy via natural intercourse than those with DFI> 40%
- IUI 8.7× more likely to have a live birth if the DNA fragmentation is ≤ 27%
- a DFI ≥ 30% has been associated with increased spontaneous abortion rates

Sperm DNA

- Nuclear protamines make the DNA highly crystalline and a compact toroid During the later stages of spermatogenesis
- spermatid nucleus is remodelled and condensed,which is associated with the displacement of the majority of histones (85%) by transition proteins and then by protamines

Sperm DNA

- Inter and intra-molecular disulfide cross-links between the cysteine-rich protamines
- the compaction and stabilization of the sperm nucleus
- protect the sperm genome from external stresses such as oxidation or temperature elevation

Sperm DNA

- up to 15% of the DNA remains packaged by histones at specific DNA sequences
- it is peripherally located in the nucleus
- containing genes essential for early embryonic development
- highly susceptible to oxidative damage

Environmental sperm defect factors

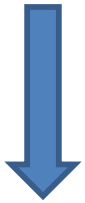
- Oxidative stress
- Chemical
- Toxicants
- Radiation
- Trace element deficiency

High levels of ROS generated by:

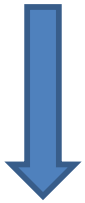
- Immature and abnormal spermatozoa
- Activated leukocytes in semen
- exposure to xenobiotics
- electromagnetic radiation
- varicocele
- infection and inflammation

ROS

- Oxidative stress causes of sperm DNA damage
- Impair oocyte nucleoside excision repair capacity



Sperm DNA damage is not repaired



Higher probability of pre- or post-implantation failure

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During maturation process

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Sperm chromosome karyotype, DNA & genetic mutation

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Abnormal morphology

Abnormal function

- spermatozoa membrane is rich in polyunsaturated fatty acids and it is poor in cytosolic antioxidants
- oxygen induced damage which leads to:

    lipid peroxidation

    mitochondrial damage

    nuclear DNA damage

ROS

- Mitochondrial damage → motility
  infertility
- Aneuploidy → sperm quality
  abortion
- DNA damage → hypospermatogenesis
  embryo development(HOX and HSP genes)
  RSA

Oxygen paradox

- ATP production

- exposure to high ROS levels which damage mitochondrial and nuclear DNA

who conceived from sperm harboring DNA damage

- increased incidences of genetic/epigenetic abnormalities
- genitourinary abnormalities
- musculoskeletal defects
- autosomal disorders and carcinoma in children

SDF & RSA

- Li et al: sperm quality meta-analysis 1690 male
- semen volume
- pH
- Sperm density
- Viability
- Progressive motility
- Nl morphology
- DFI

DFI & RSA & Sperm parameters

- Nazar I india
- 40 patients
- 31-33yo
- Nl partner
- Motility low
- Morphology low
- TAC low
- ROS high
- DFI high 13% vs 23%

DFI(SCSA) & sperm parameters in RSA

- Minmin yuan china
- **DFI was significantly higher in RSA patients**
- **DFI threshold 13.59%.**
- **weak partial correlation between DFI values and conventional sperm analysis parameters**
- weak negative correlation between DFI and viability, motility and normal morphological rate
- **we recommend that each laboratory should establish its own clinical DFI threshold for more precise prediction of RSA**

DFI & ART & sperm parameters & lifestyle

- Hongyi Yang china
- 1185 IUI
- 1221 IVF
- 216 ICSI
- DFI: 15% > 15-30% >30%

…IUI

- Clinical pregnancy

  12.5% 14.3% 13.4%

  no statistical difference between the groups
- early abortion

  27.3% 14.6% 4.9%

…IUI

- DCG-optimization of sperm may minimize the potential effects of high sperm DFI on pregnancy rates following IUI

- it may increase the risk of early abortion

…IVF & ICSI

- No significant differences in the rates of clinical pregnancy, early abortion, oocyte fertilization, or good quality embryos in IVF or ICSI cycles were detected among different DFI groups

…ART

- using preoptimized sperm by DGC, suggesting that appropriate semen pre-treatment removes most high DFI sperms to eliminate the adverse effects of high DFI on oocyte fertilization, embryo development, and the final clinical pregnancy outcome
- DNA repair by oocytes could be another reason why DFI does not influence embryo development or pregnancy outcomes following IVF/ICSI

…Sperm parameters

- Sperm DFI was negatively associated with sperm density, vitality and normal morphology
- It was positively correlated with age, abstinence time and unhealthy lifestyles
- There was no correlation between sperm DFI and male BMI, sperm liquefaction time or semen volume

…conclusions

- DFI is more sensitive than regular semen analysis in detecting latent DNA damage in the sperm
- shorten the male abstinence time
- sperm DFI has proven to be very valuable in male fertility evaluation, but its significance as a predictor of pregnancy outcomes following ART requires further investigation

Sperm DNA fragmentation outcomes after IVF/ICSI

- Katherine A.Green
- 230 patients
- DFI <=15% >15%
- Sperm DNA fragmentation on the day of ICSI is not associated with embryologic or clinical outcomes after euploid blastocyst transfer
- Increasing levels of SDF are associated with low sperm concentration and total motile sperm count.

Two large meta-analyses

- The first of these was a meta-analysis of four studies, with a total of 1962 men, found that men with DFI < 30% were more likely to achieve a pregnancy or live birth either spontaneously or via IUI
- The second found that couples were ~2× more likely to become pregnant via IVF if their DFI was < 30%

Sperm DFI and pregnancy outcome after IVF or ICSI: a meta-analysis

- Zheng zhang
- 20 studies

…DFI & clinical pregnancy

- 3 studies
- cutoff value (>27%, 15–27 %, ≤15 %)
- TUNEL more likely to achieve CP if the DFI was <27 % DFI was not associated with CP in SCSA subgroup
- Higher Cp When DFI was less than the cutoff value (15–27 %)
- The result of ICSI subgroup similar
- if the DFI threshold value was less than 15 % DFI was not associated with CP

…Biochemical Pregnancy

- 3 studies cutoff >27%
- 1 study cutoff 15-27
- no significant difference both for the overall effect

…Pregnancy loss

- 8 studies
- DFI Cutoff above 27 %
- IVF and ICSI
- Non significant results
- DFI cutoff value 15 to 27 %
- Non significant associations between DFI & PL for all the groups

…Pregnant group versus non-pregnant group

- 4 studies
- Subgroup analysis was conducted by IVF and ICSI
- No statistical difference between the two groups

Reasons for the difference of the threshold value

- different DFI detection methods
- different study populations
- different type of fertilization (IVF or ICSI)
- sperm preparation method (raw semen or obtained by density gradient)
- whether or not underwent semen quality control (sperm count, motility, morphology)
- selection criteria was set for couples underwent ART (female partner age, BMI).

…Results

- Infertile couples were more likely to get pregnant if DFI was less than threshold value (For threshold value > 27 % and 15–27 % group
- SCSA : similar CP rate between groups with a high DFI or a lower DFI value (SCSA, For threshold value >27 % and 15–27 % group)
- Meta-analysis based on BP and PL(For DFI >27 %, 15–27 %, ≤15 % group) outcome yielded

  non significant results

…conclusion

- The predication value of DFI for IVF or ICSI outcome is not confirmed in our meta-analysis
- Further better designed studies with larger subjects involved are needed to better address this issue

RSA & DFI

- Leach M Australia

- couples with a history of recurrent miscarriage (≥3 first trimester miscarriages)
- 30% (32/108) of men had sperm with high levels of DNA fragmentation (DFI > 15%)

Sperm chromatin structure assay results in Nigerian men with unexplained infertility

- 404 men
- *unexplained infertility* *fertile sperm*

  27.5%±7.0% *14.1%±5.3%*

  63% DFI > 20% *4% DFI> 20%*

  15.2 DFI > 30% *1% DFI>30%*

…Discussion

- SCSA may be a more reliable predictor of fertility potential than traditional semen analysis in cases of unexplained infertility

Sperm viability and DNA fragmentation rates

- Mary K Samplasky
- 3049 men
- SCSA
- viability ≤ 50% DFI≥ 30% for 95% of the samples

- viability ≥ 75% DFI≤ 30% for 95% of the patients

- Sperm viability correlates strongly with DFI

…Conclusion

- In men with high levels of sperm viability ≥ 75%, or low levels of sperm viability ≤ 30%, DFI testing may be not be routinely necessary.

- Viability test is very less expensive than DFI

ejaculated sperm (EJA-ICSI) or testicular sperm (TESTI-ICSI)

- Esteves SC
- DFI in testicular sperm was 8.3%, compared with 40.7% in ejaculated sperm

	TESTI-ICSI	EJA-ICSI
• pregnancy rate	51.9%	40.2%
• miscarriage rate	10.0%	34.3%,
• live-birth rate	46.7%	26.4%.

Testicular versus ejaculated spermatozoa in ICSI cycles of normozoospermic men with high DFI & previous ART failures.

- Pabuccu EG
- cycles TESA EJ
- Clinical pregnancy 38.7% versus 15%
- miscarriages TESA < EJ

- When high DFI is detected (>30%), ICSI using testicular spermatozoa obtained by TESA seems an effective option particularly for those with repeated ART failures.