Human Sperm Functionality Tests:

Time to Proceed?

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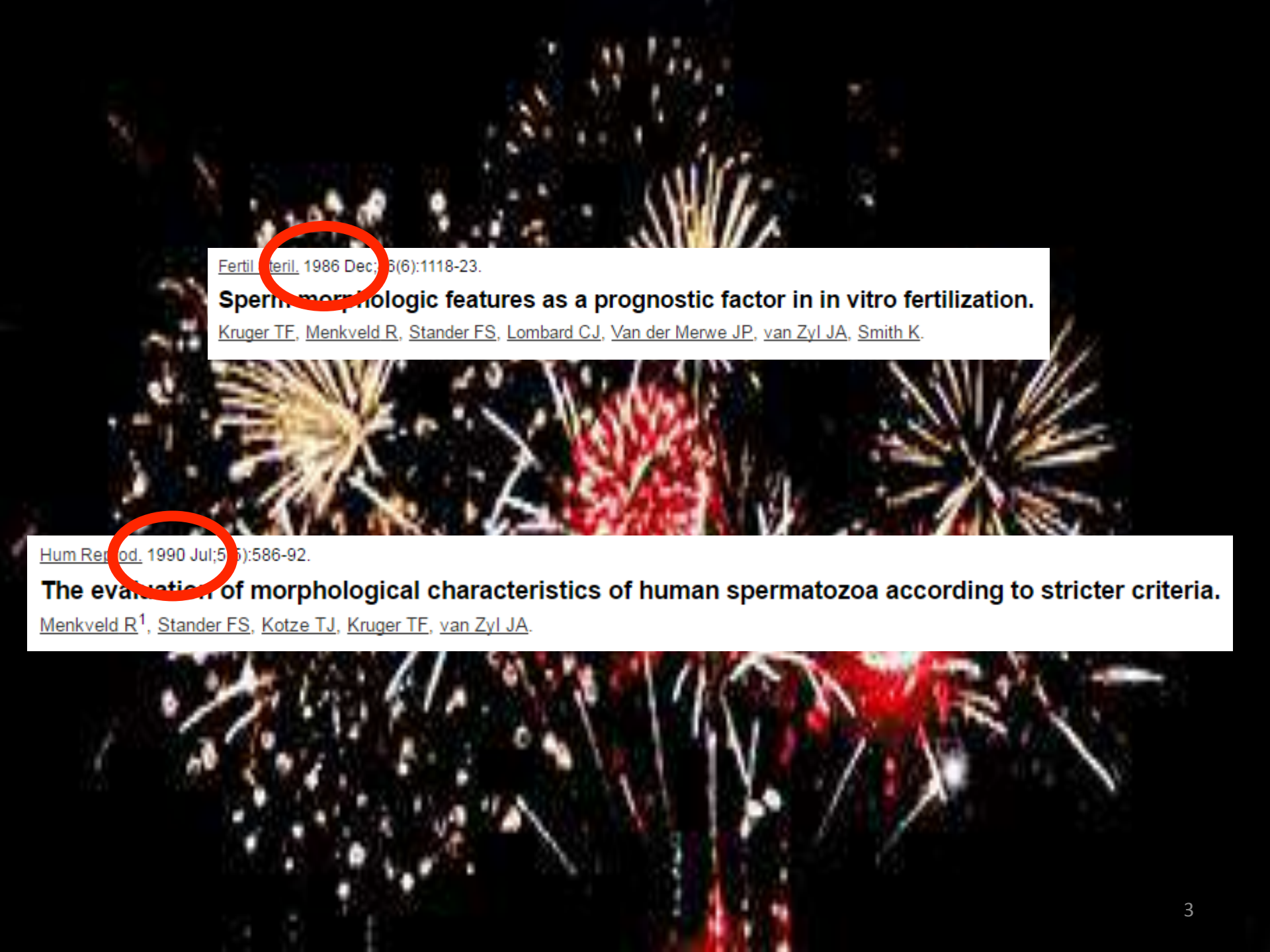
Sperm functionality tests have been a great focus for scientists since decades

- The main purpose of sperm functionality tests were, evaluating the fertility potential of the male
- And, selecting the best sperm for ART
- Three milestones seriously effected this course

1. Morphology
2. ICSI
3. Innovations in Sperm Selection Methods



Fertil Steril. 1986 Dec;46(6):1118-23.

Sperm morphologic features as a prognostic factor in in vitro fertilization.

Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, Smith K.

Hum Reprod. 1990 Jul;5(5):586-92.

The evaluation of morphological characteristics of human spermatozoa according to stricter criteria.

Menkveld R[1], Stander FS, Kotze TJ, Kruger TF, van Zyl JA.

- After the early publications on sperm morphology, almost all scientists focused on the relation between sperm morphology and DNA integrity

- Fortunately (or) unfortunately, ICSI procedure bypassed most of the problems and difficulties

- It was an easy shortcut to reach fertilization.

Globozoospermia: Do Y-chromosome microdeletions play a role in this rare spermatogenic disorder?

- On light and electron microscopy, all the spermatozoa were round headed, with abnormal morphologic features
- Patients had normal 46 XY karyotyping
- No microdeletion of the Y chromosome was detected in any patient.

Taskiran C, Baltaci V, **Gunalp S**. J Reprod Med. 2006 Aug;51(8):628-34.

Isolated Teratozoospermia

- Isolated teratozoospermia was not associated with a statistically significantly decreased probability of pregnancy with assisted reproduction.

Fertil Steril 2011;95:1141–5. 2011 ASRM

Assessment of Reactive Oxygen Species

- The clinical value of semen ROS determination in predicting IVF outcome remains unproved, but identifying oxidative stress as an underlying cause of sperm dysfunction has the advantage that, it suggests possible therapies.

Sperm vacuoles are linked to capacitation and acrosomal status

- Both hyaluronic acid and follicular fluid strongly induce acrosome reaction after 90 min, without significantly modifying sperm nuclear condensation and morphology (Bartoov's criteria)

- We simultaneously observed a highly significant decrease in the presence of vacuoles.

Montjean D, Belloc S, **Benkhalifa M**, Dalleac A, Ménézo Y. Hum Reprod. 2012 Oct; 27(10):2927-32.

Hyaluronan binding assay does not predict pregnancy rates in IUI cycles in couples with unexplained infertility

- Hyaluronan binding assay does not predict pregnancy rates in IUI cycles in couples with unexplained infertility.

Boynukalin FK, Esinler I, Guven S, **Gunalp S.** Arch Gynecol Obstet. 2012 Dec;286(6): 1577-80

CASA

- Results indicate that some of the CASA estimates provide reliable estimation of the fertilizing ability of human sperm

- There were significant differences of the two sperm movement characteristics, including VCL and Rapid (before and after swim-up), indicating that the total distance traveled by rapid sperm movement might be important in human sperm fertilizing abilities.

Counting sperm does not add up any more: time for a new equation?

- Although sperm dysfunction is the single most common cause of infertility, we have poor methods of diagnosis and surprisingly no effective treatment
- We outline the recent progress in the field, for example, in proteomics, which will allow the development of new biomarkers of sperm function.

Lefièvre L, Bedu-Addo K, Conner SJ, Machado-Oliveira GS, Chen Y, Kirkman-Brown JC, Afnan MA, Publicover SJ, Ford WC, **Barratt CL**. Reproduction. 2007 Apr;133(4): 675-84.

Proteomics: a subcellular look at spermatozoa

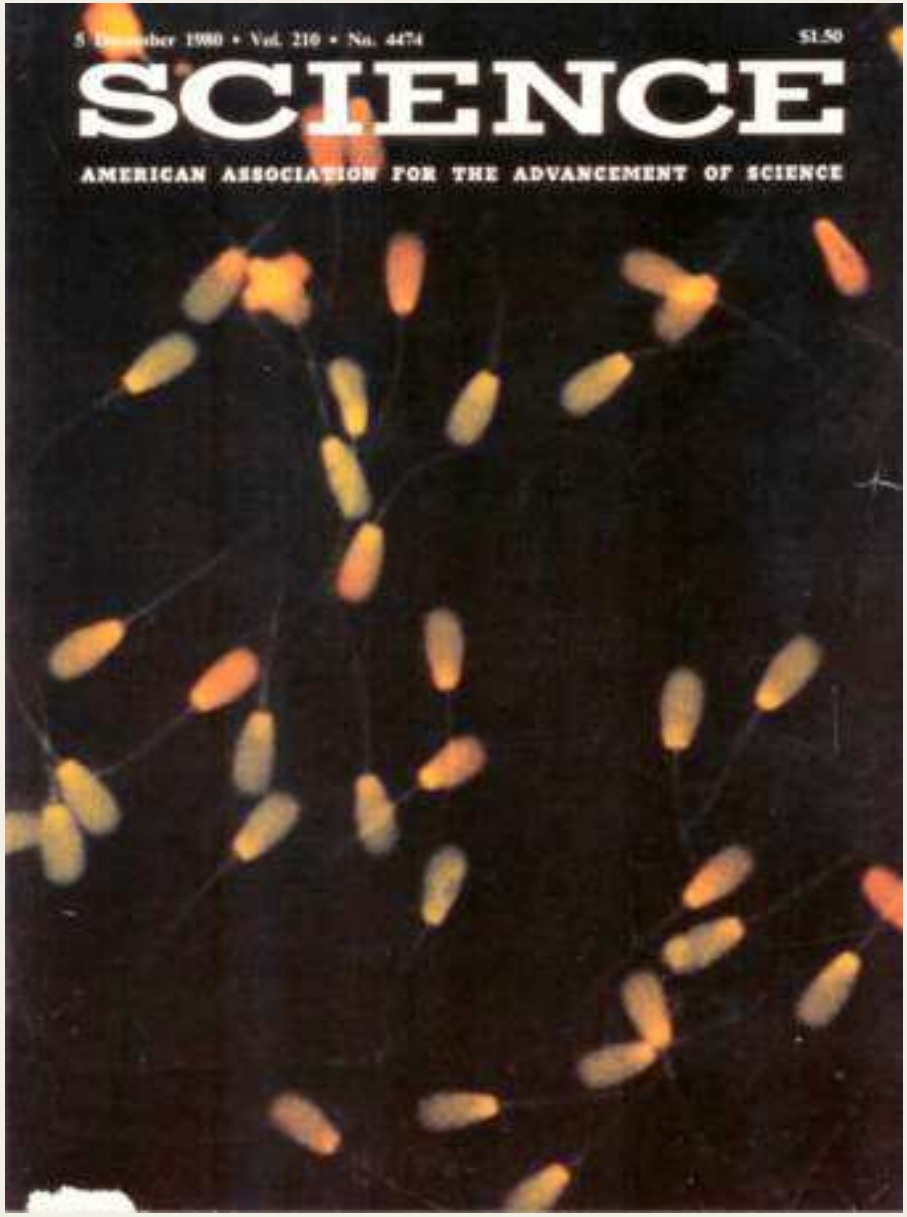
- Advances in proteomics may help to decipher metabolites which can act as biomarkers in the detection of sperm impairments and to potentially develop treatment for infertile couples
- Sperm proteomics allows comparison of protein structure of normal and defective spermatozoa
- Further comprehensive studies on sperm-specific proteome, mechanisms of protein function and its proteolytic regulation, biomarkers and functional pathways, such as oxidative-stress induced mechanisms, will provide better insight into physiological functions of the spermatozoa
- Large-scale proteomic studies using purified protein assays will eventually lead to the development of novel biomarkers that may allow for detection of disease states, genetic abnormalities, and risk factors for male infertility.
  - Plessis et al, *Reproductive Biology and Endocrinology* 2011. 9:36

The clinical utility of sperm DNA integrity testing: a guideline

- Sperm DNA damage is more common in infertile men and may contribute to poor reproductive performance

- However, current methods for assessing sperm DNA integrity do not reliably predict treatment outcomes and cannot be recommended routinely for clinical use.

The Practice Committee of the American Society for Reproductive Medicine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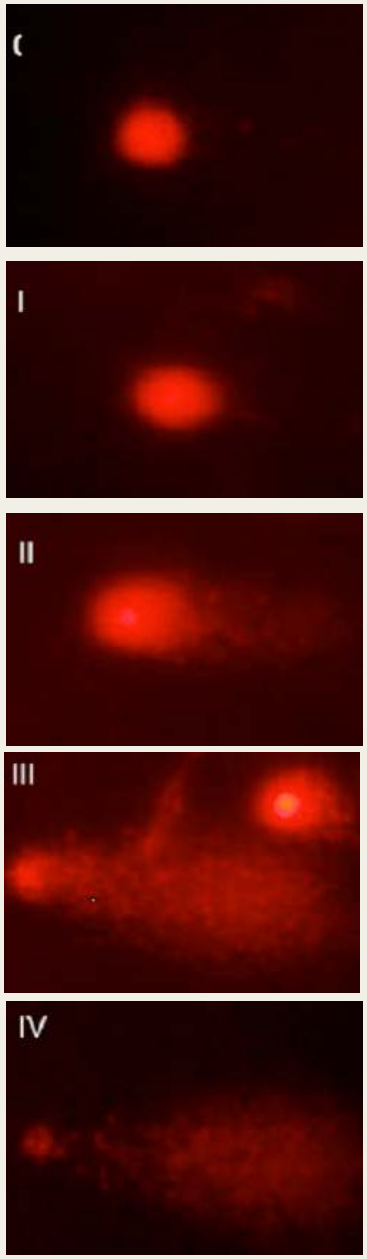


0: Undamaged

1: low damaged

2: moderate damaged

3: damaged

4: high damaged

Evaluating a novel panel of sperm function tests for utility in predicting ICSI outcome

- A panel of three sperm function tests; tests known to assess different aspects of sperm functionality and genomic integrity

1) Sperm DNA Accelerated Decondensation (SDADTM) Test
2) Sperm DNA Decondensation (SDDTM) Test
3) Sperm Penetration Assay (SPA)

Brown et al.

- Determining if positive and negative test scores correlated with failed and successful ICSI outcomes

- SDAD Test scores alone, and SPA and SDD Test scores used together, significantly predicted failed ICSI outcomes

- This indicates that the scores obtained when analyzing patients' sperm using a panel of sperm function tests;

- Specifically, the SPA, and SDAD and SDD Tests, can be used to identify infertile couples who should not be directed to ICSI.

Brown et al.

Which isolated sperm abnormality is most related to sperm DNA damage in men presenting for infertility evaluation

- **CONCLUSIONS:**
- In this large cohort of infertile men with isolated sperm abnormalities, we have found that the sperm DNA fragmentation level is highest in the men with sperm motility defects and that 31 % of these men have high levels of sperm DNA fragmentation
- The data indicate that poor motility is the sperm parameter abnormality most closely related to sperm DNA damage.

Belloc S, **Benkhalifa M**, Cohen-Bacrie M, Dalleac A, Chahine H, Amar E, Zini A. J Assist Reprod Genet. 2014 May;31(5):527-32.

Tests of Sperm DNA Damage

- Although a small percentage of spermatozoa from fertile men also possess detectable levels of DNA damage, which is repaired by oocyte cytoplasm, there is evidence to show that the spermatozoa of infertile men possess substantially more DNA damage and that this damage may adversely affect reproductive outcomes
- **DNA damage - Direct tests**
  - Comet assay, TUNEL assay, DNA oxidation measurement, etc..
- **DNA damage - Indirect tests**
  - SCSA, Sperm chromatin dispersion assay, FISH, etc..

Overall..

- The data suggest that there is no significant relationship between sperm DNA damage and fertilization rate or pregnancy outcomes at IVF or IVF / ICSI

- However, there is evidence to suggest that sperm DNA damage is associated with poor pregnancy outcome after standard IVF.

Indian J Urol. 2011 Jan-Mar; 27(1): 41–48. Semen analysis and sperm function tests: How much to test? Vasan SS.

IMSI

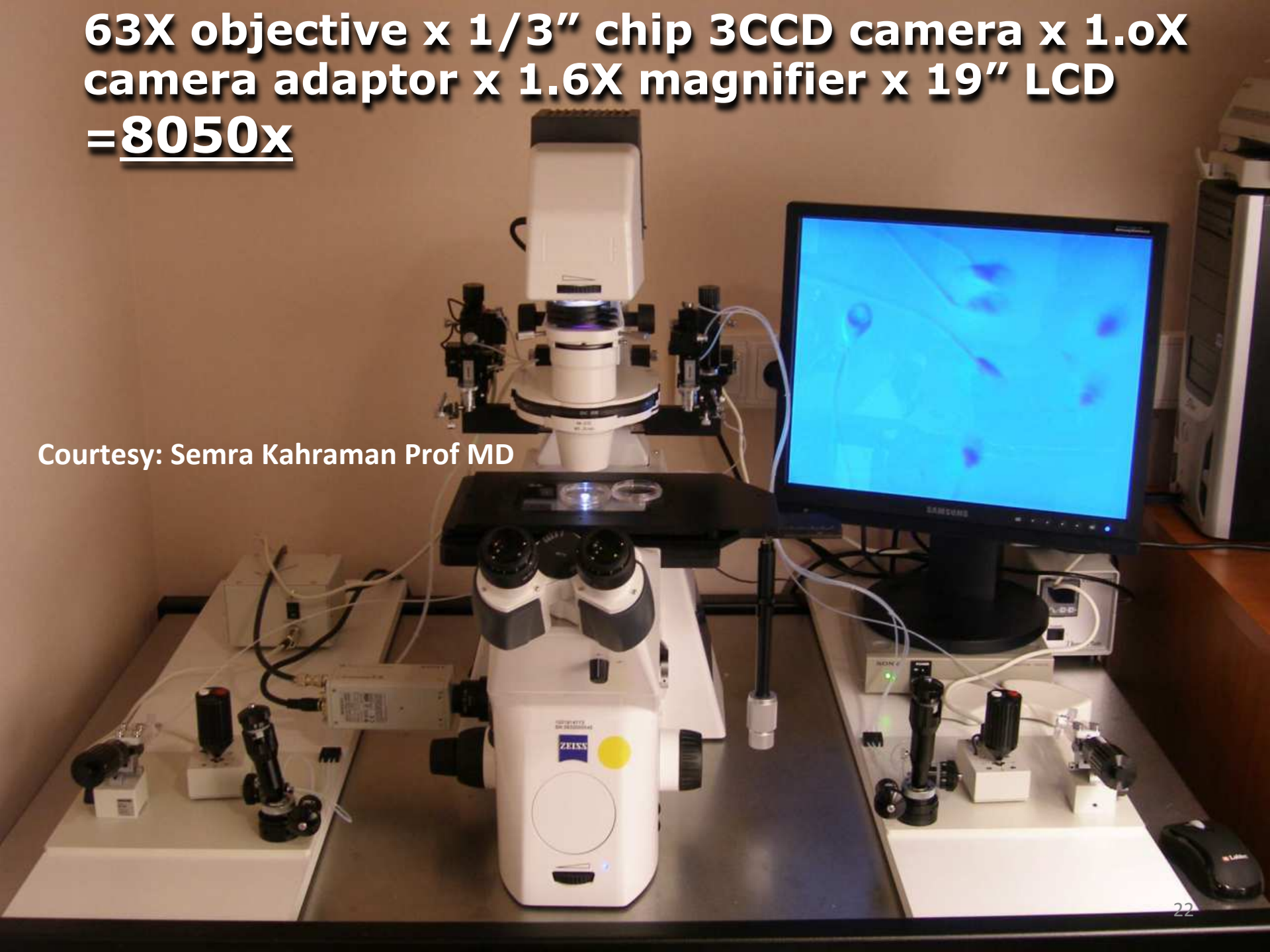
Intra-cytoplasmic Morphologically-selected Sperm Injection

is based on

MSOME

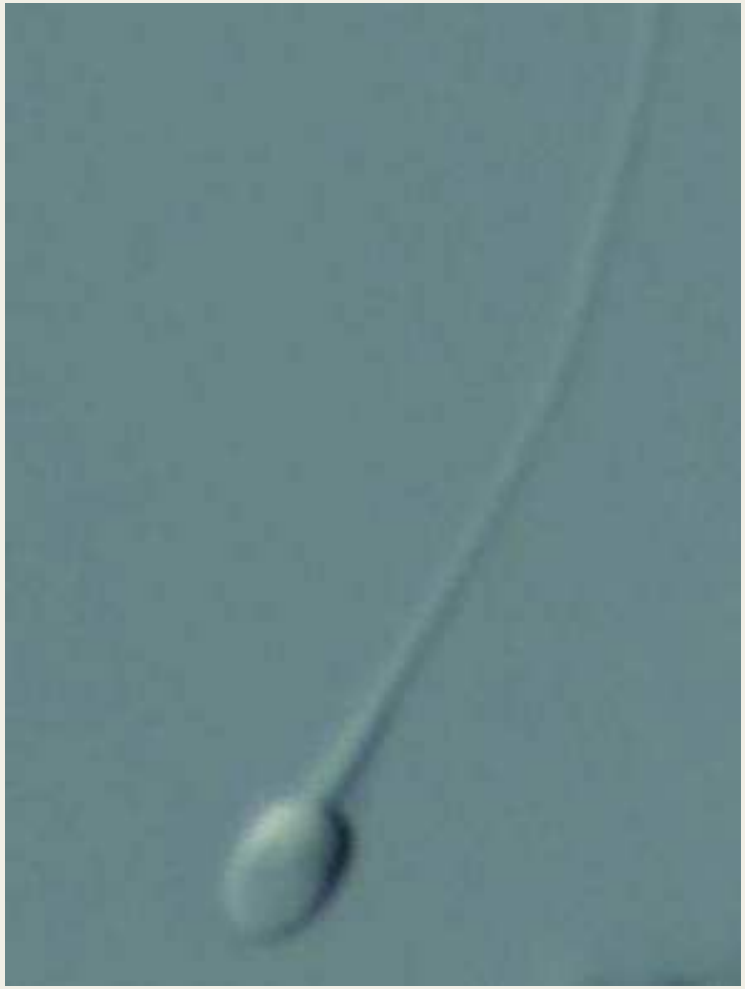
Motile Sperm Organellar Morphology Evaluation

63X objective x 1/3” chip 3CCD camera x 1.oX camera adaptor x 1.6X magnifier x 19” LCD =8050x



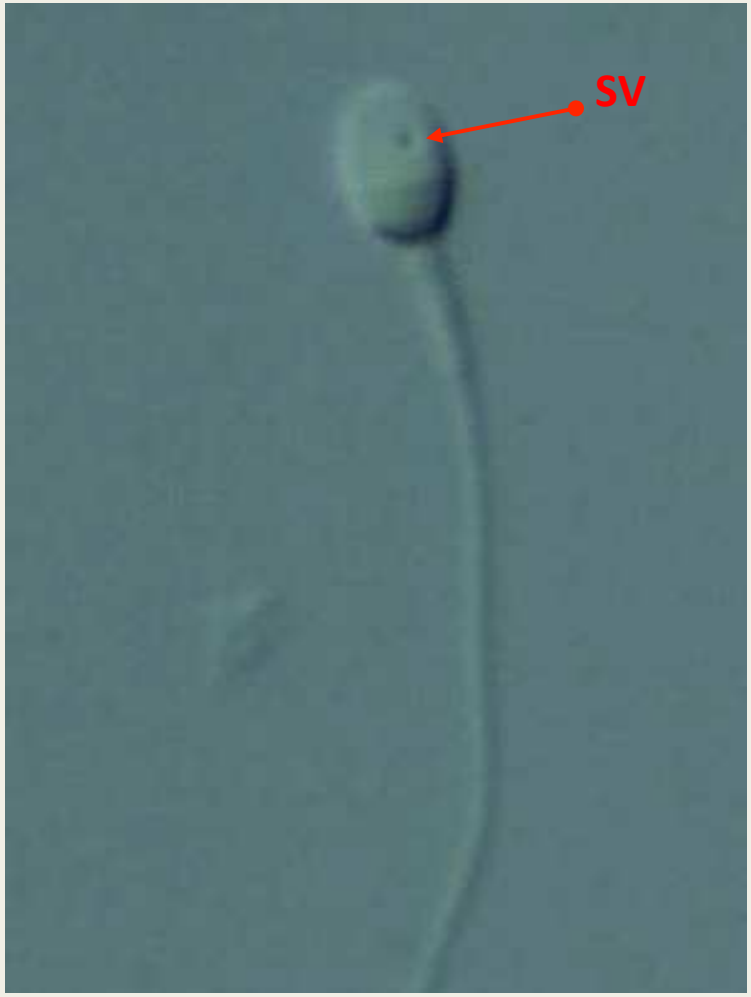
Courtesy: Semra Kahraman Prof MD

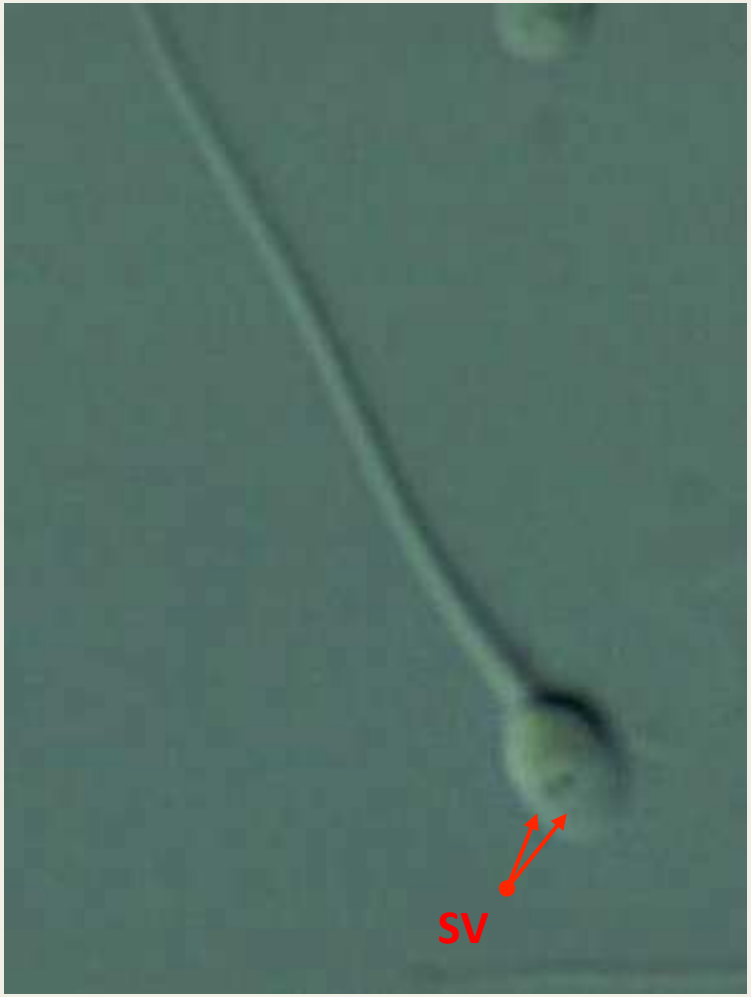
Grade I: Normal form and no vacuoles



Courtesy: Semra Kahraman Prof MD

Grade II: Normal form and ≤ 2 small vacuoles





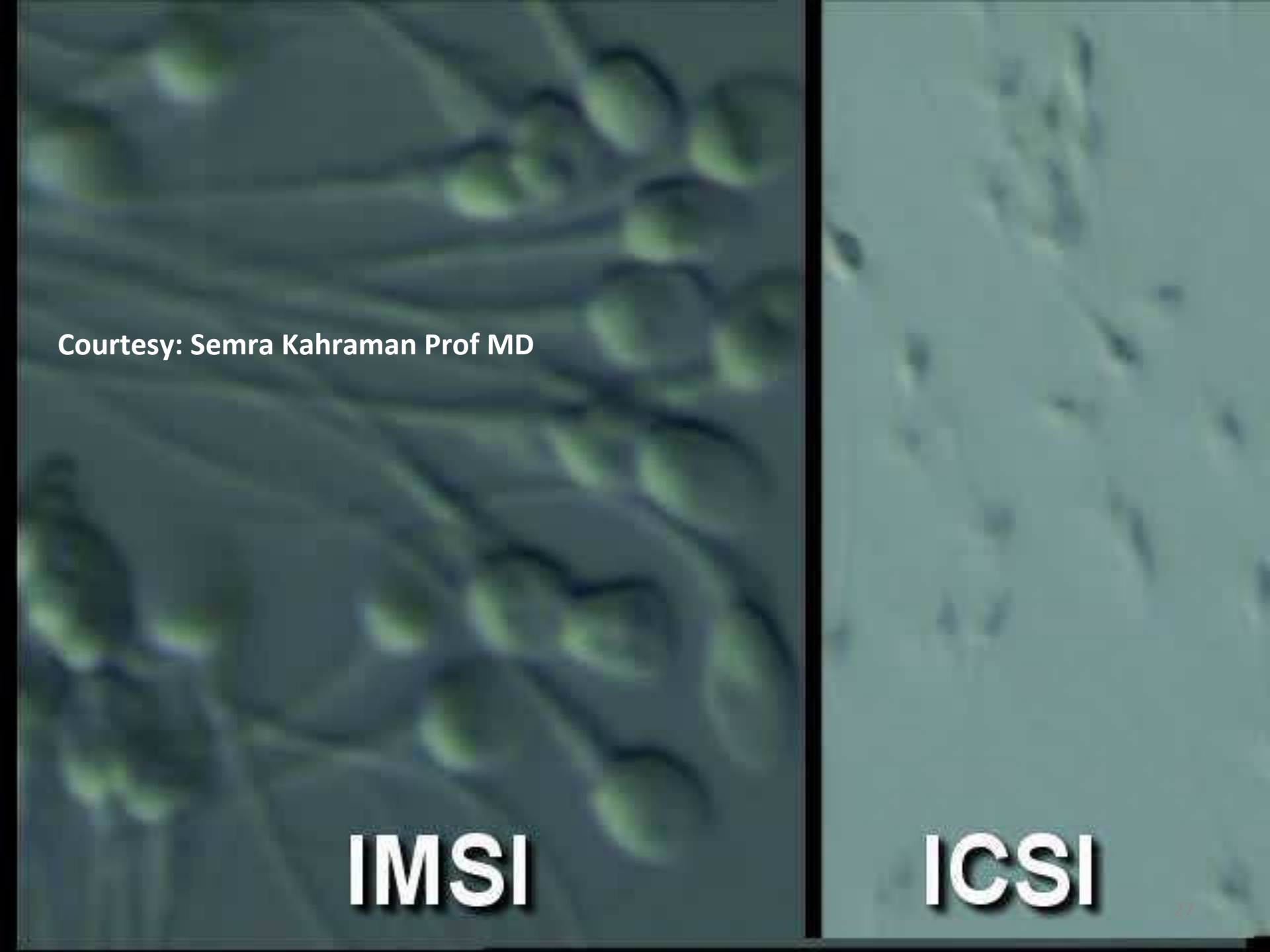
Courtesy: Semra Kahraman Prof MD

Grade III: Normal form, >2 small vacuoles or at least one large vacuole

Courtesy: Semra Kahraman Prof MD

Grade IV: large vacuole and abnormal head shapes or other abnormalities

Courtesy: Semra Kahraman Prof MD


Courtesy: Semra Kahraman Prof MD
IMSI
ICSI

Semen Analysis in 21st Century Medicine: The Need For Sperm Function Testing

- With the advent of intracytoplasmic sperm injection (ICSI), the evaluation and treatment of the infertile male radically changed

- Just a single spermatozoon was required, motility was not necessary, and the normal biological processes of sperm capacitation, the acrosome reaction, cumulus penetration, zona and ova binding, and penetration did not necessarily occur before fertilization.

Asian J Androl. 2010 Jan; 12(1): 64–70. Lamb DJ

Semen Analysis as an integral part of infertility

- The 5th edition of the World Health Organization (WHO) manual is a definitive statement on the first well-defined reference ranges for semen analysis based on the analysis of over 1900 recent fathers
- The methodology used in the assessment of the usual variables in semen analysis is described, as are many of the less common, but very valuable, sperm function tests
- Semen analysis is an imperfect tool but remains the cornerstone of the investigation of male infertility
- Although this reveals useful information for the initial evaluation of the infertile male, it is not a test of fertility
- It provides no insights into the functional potential of the spermatozoon to fertilize an ovum or to undergo the subsequent maturation processes required to achieve fertilization.

Sperm Function Testing

- Once commonly performed for the infertile couple before employing assisted reproductive technology, has fallen out of favour in many reproductive medicine centers throughout the world

- In large part, this reflects the current clinical practice of bypassing the in-depth evaluation of the male partner, while assuming that if a spermatozoon can be found for ICSI

- Nevertheless, sperm function testing can provide valuable clinical insights into defects causing male infertility

- Admittedly, in some cases, functional sperm deficiencies can be overcome using an ART.

- Yet, we know this logic is flawed, as even healthy fertile couples may conceive a child with significant genetic or birth defects

- Sperm function testing may indicate that less expensive technologies may help a couple seeking to conceive a child; ICSI - IVF may not always be required

- The real strength of sperm function testing lies in its ability to identify men with normal semen parameters but who have functionally deficient spermatozoa that will fail to fertilize in routine IVF

- These functional deficiencies will never be observed on a routine semen analysis.

Asian J Androl. 2010 Jan; 12(1): 64–70. Lamb DJ

- The clinical use of these tests decreased significantly with the onset of ICSI, despite the fact that evaluation prior to treatment could prevent over treatment with the most advanced and costly technology, as well as unexpected IVF failure for men with normal semen parameters, but with unrecognized functional sperm deficiencies

- With ICSI, many physicians treating the infertile couple no longer cared to determine the source of the infertility

- All that is needed is a single spermatozoon to achieve a pregnancy with ICSI

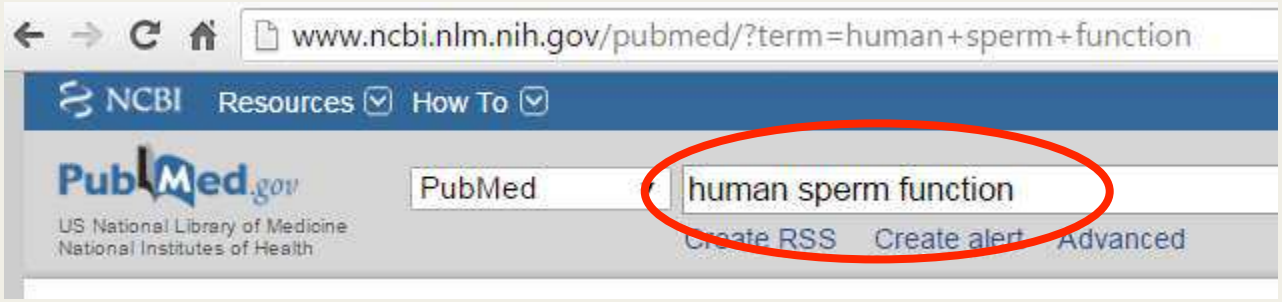
- If a spermatozoon could be found, there was the potential to fertilize an ovum, regardless of the functional deficiency.

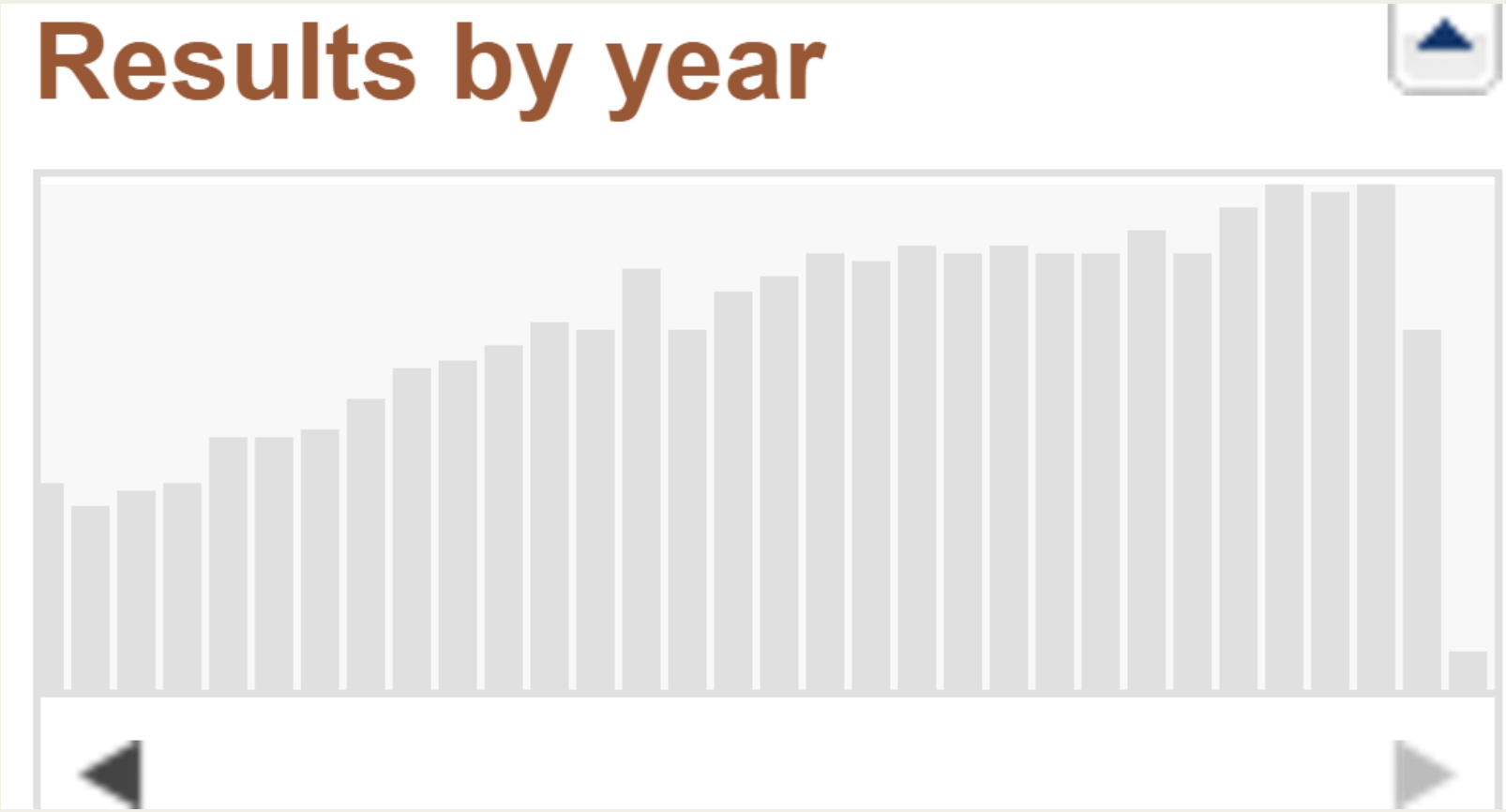
Asian J Androl. 2010 Jan; 12(1): 64–70. Lamb DJ

- And we may also claim that, ICSI was an unlucky innovation from this different point of view
- This fascility offered to scientists by ICSI, rapidly changed the focus
- Nowadays, sperm function tests are not the primary concern of many scientists

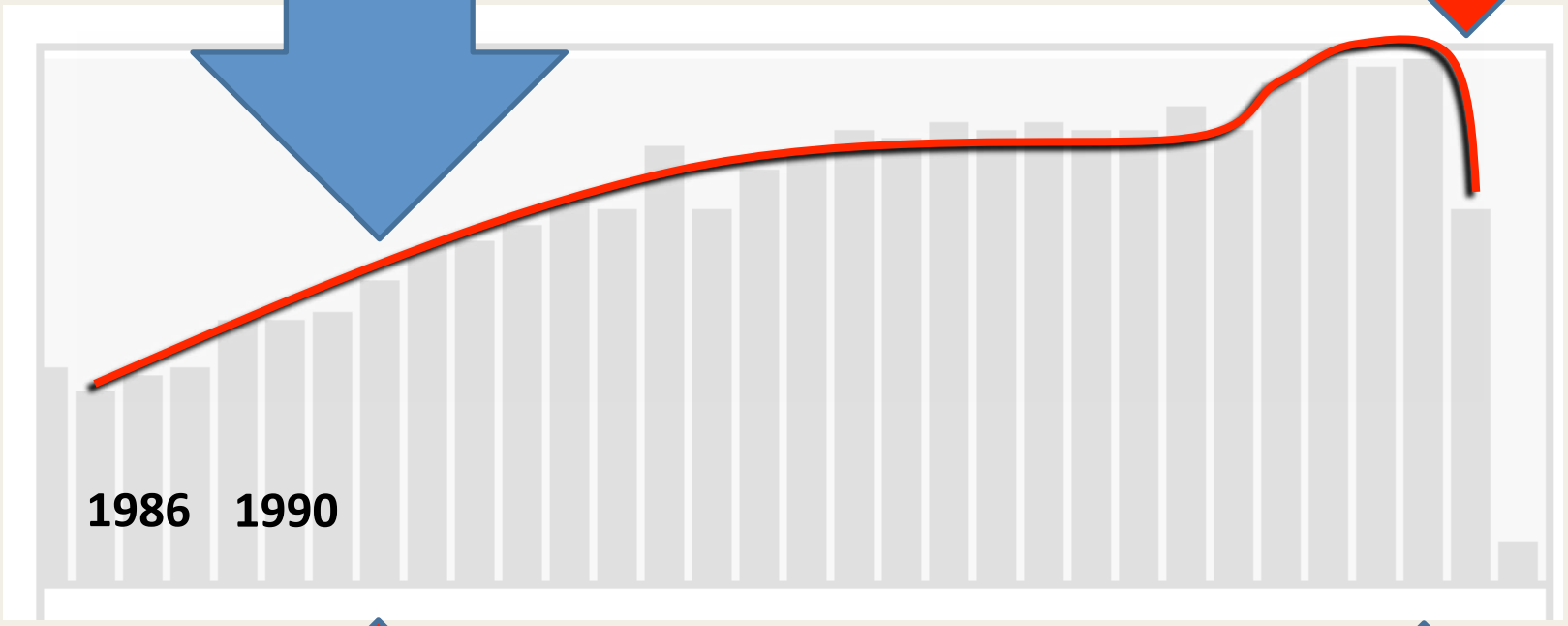
- And also, most funds are directed to easy shortcut methods rather than researches on sperm functions
- Researches on sperm functionality and publications still increase every year
- But the acceleration started to slow down.

Number of Publications in PubMed





Report of the first pregnancy after intracytoplasmic sperm injection (ICSI) by the group in Brussels (Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992;4;340:17-8).



- Diagnostic tests are not currently available for each gene
- Other tests, such as those developed to assess sperm aneuploidy, provide important information to couples planning to use ART
- There is still a place for sperm function testing in the state-of-the-art evaluation of the infertile male.

Asian J Androl. 2010 Jan; 12(1): 64–70. Lamb DJ

Still need to proceed?

- The issues in male infertility will not get resolved till the research finds solutions at molecular level.

Indian J Urol. 2011 Jan-Mar; 27(1): 41–48, Vasan SS

When and how should new technology be introduced into the IVF laboratory?

- If studies are not published, it is possible that technology bringing no clinical benefit or leading to adverse health outcomes in the children born by these practices may be introduced.

Harper J, Magli MC, Lundin K, **Barratt CL**, Brison D. Hum Reprod. 2012 Feb;27(2):303-13.

The Essential Question

Is it possible, or will it be possible to evaluate, diagnose and select the right sperm, when it is still alive and is still in usable condition?

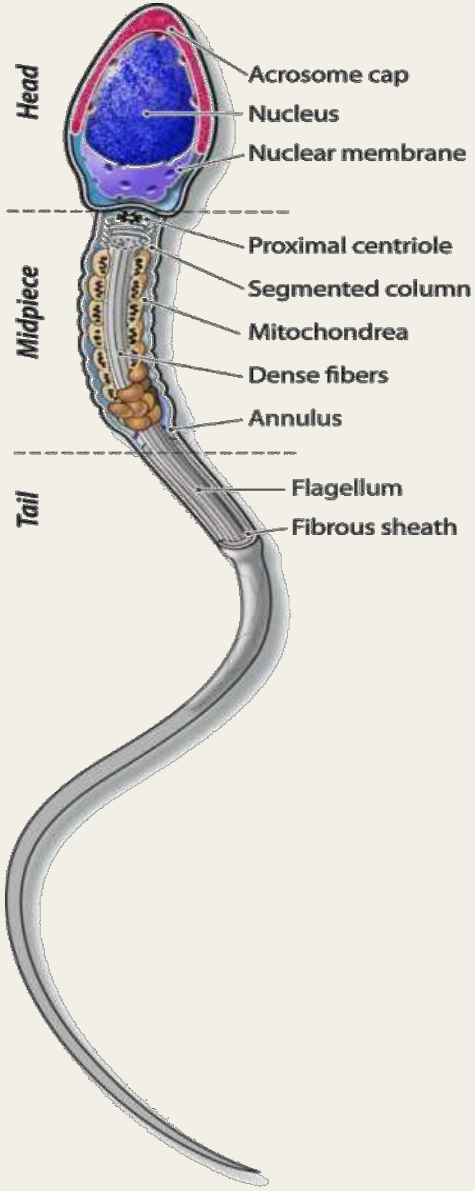
This question arises from this fact:

- Currently there are many evaluation methods that enables us to diagnose and select the right single sperm or a sperm population...
- But unfortunately this is certainly not sufficient, because we evaluate a sperm population...
- And then, we use ANOTHER sperm population and a single sperm for ART
- When we understand the condition of the sperm or sperms, it is too late, because we have already killed the sperm to understand what is going on inside.

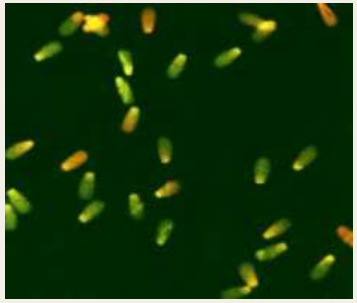
QUESTION
Is it possible, or will it be possible to evaluate, diagnose and select the right sperm, when it is still alive and is still in usable condition?

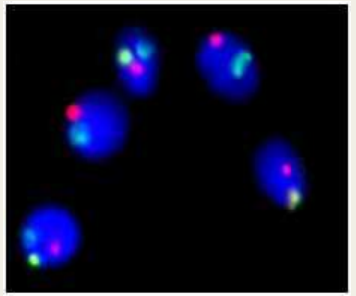
ANSWER
Not in our lifetime..

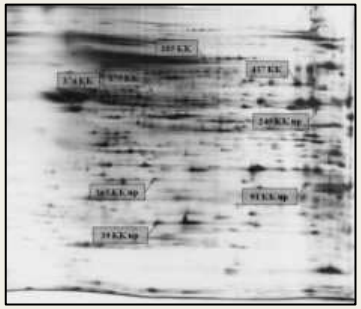
We evaluate this sperm..



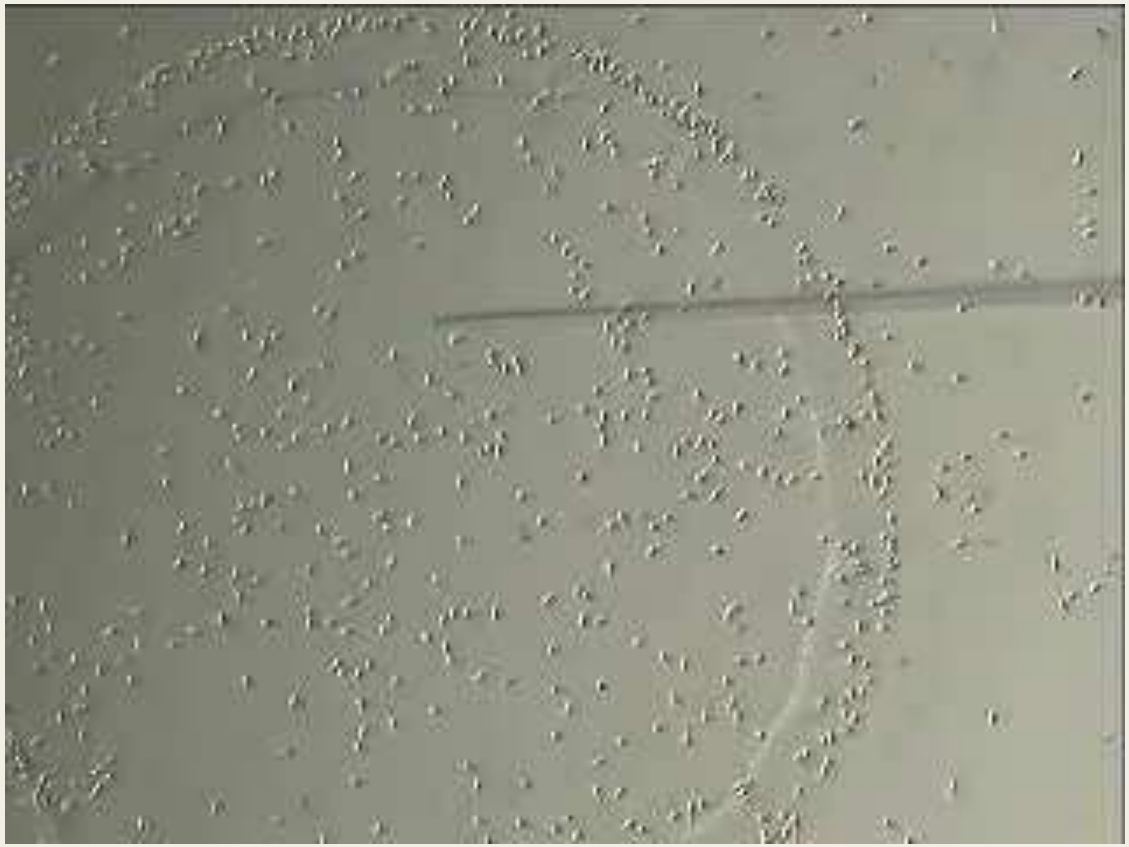
Concentration
Morphology
Motility
SPA
AR
Sperm-ZP binding
ROS
Hyaluronan binding
Hyperactivation
Viability
HOS
CASA
ASA
MAR
Sperm–mucus interaction
DFI
Electron Microscopy
Microarray
Metabolomics







...and we use another sperm



We perform autopsy to evaluate a sperm so cannot be used anymore..

Definitely we need to proceed!