# Chromatin structure abnormalities of human spermatozoa. Implications for infertility

# Anomalies de structure de la chromatine des spermatozoïdes humains Implications pour l'infertilité

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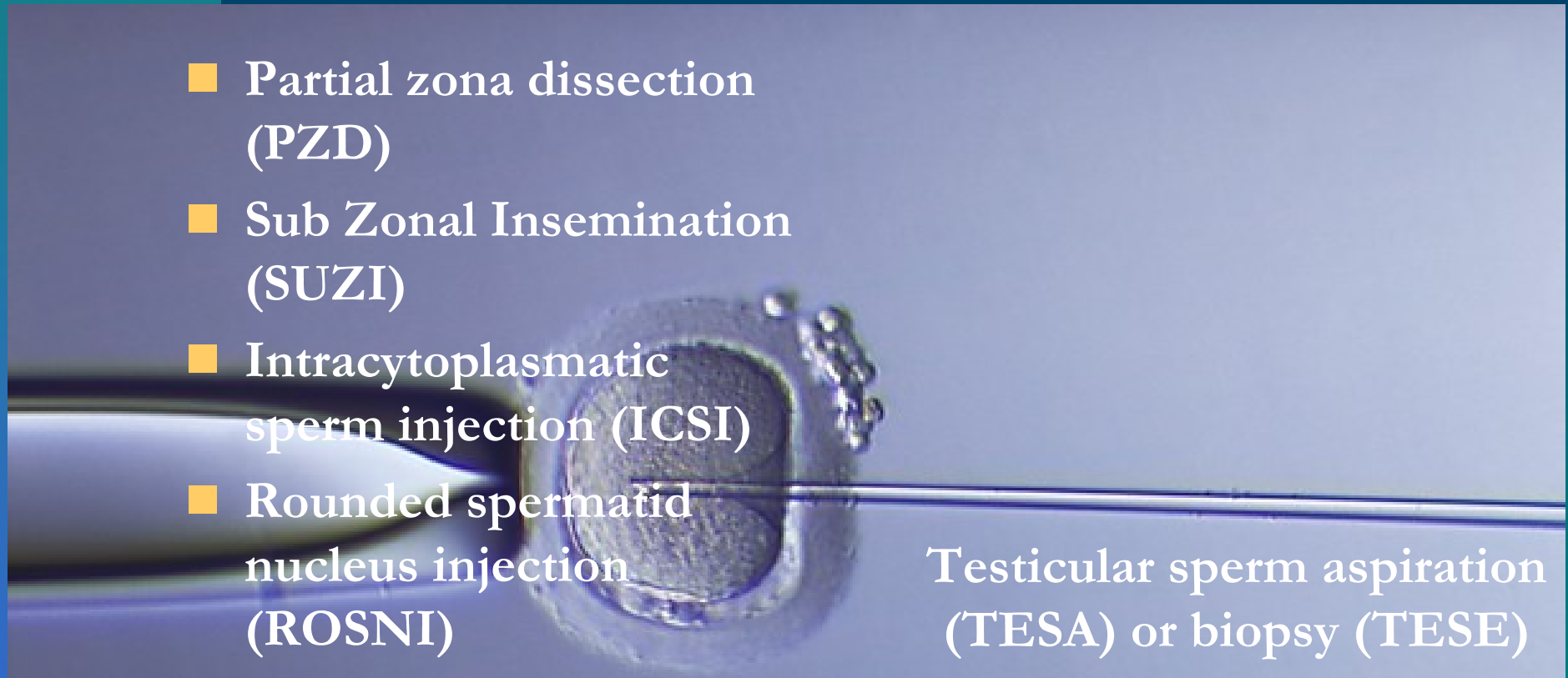
***Genève 22 Septembre 2003***

# Landmarks of in vitro fertilisation technology (IVF)

- 1878 First attempts at in vitro fertilisation in mammalian eggs
- 1880 First successful embryo culture
- 1930 First successful ivf of mammalian eggs resulting in a live birth
- 1935 First successfully fertilised human eggs in vitro
- 1978 Birth of Louise Brown
- 1990 Preimplantation diagnosis
- 1992 Intracytoplasmatic sperm injection developed in humans

# Landmarks II

- Partial zona dissection (PZD)
- Sub Zonal Insemination (SUZI)
- Intracytoplasmatic sperm injection (ICSI)
- Rounded spermatid nucleus injection (ROSNI)



Testicular sperm aspiration (TESA) or biopsy (TESE)

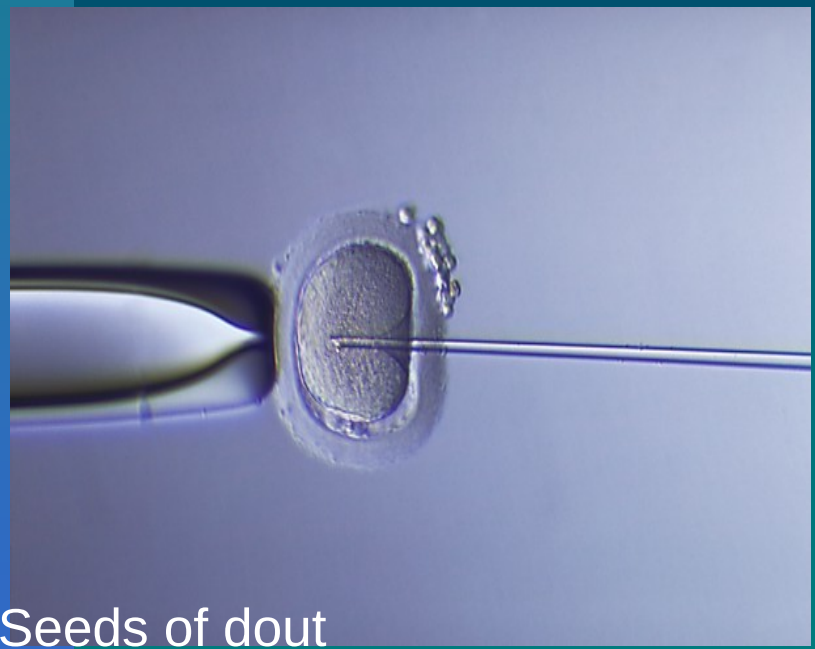
- Microsurgical Epididymal Sperm Aspiration (MESA)

# Skipping evolutionary barriers? Is it going to affect the future?

"Bad genes tend to end up in bodies that die young or without reproducing (R.Dawkins,1995)

"What form of male infertility are we left to cure? (Silber, 1995)

# Risks for off-spring and assisted reproductive technology. Questions are now being asked… What do we know?



Seeds of dout

*Powell K : Nature , 17 April 2003*

Twice as high a risk of a major birth defect as naturally conceived infants?

*Hansen et al. NEJM: 346, 725. 2002*

*The course of pregnancy or the outcome after ICSI are not affected by origin or number of sperm in the ejaculate*

*Ludwig M et al. Hum. Reprod: 18, 351. 2003*

*ART is associated with a human overgrowth syndrome (Beckwith-Wiedemann)*

*DeBaun MR et al. Am. J. Hum. Genet. 72, 156. 2003*

# Effects of sperm damage

- Fertilisation
- Cell division
- Embryo development
- Pregnancy outcome
- Congenital anomalies in babies
- Future health issues

# Sperm characteristics

Human sperm quality is usually  defined to by standard WHO semen analysis parameters: number, motility morphology

*WHO laboratory manual for the examination of human semen and sperm –cervical mucus interaction. 1999 Cambridge University Press*

# Morphology I

Normal forms

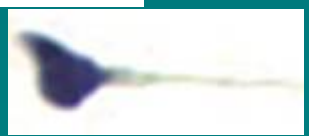
Pyriform

Macrocephalic

Microcephalic

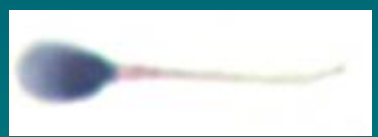
Round

Amorphous



# Morphology II

**Mid piece anomalies**



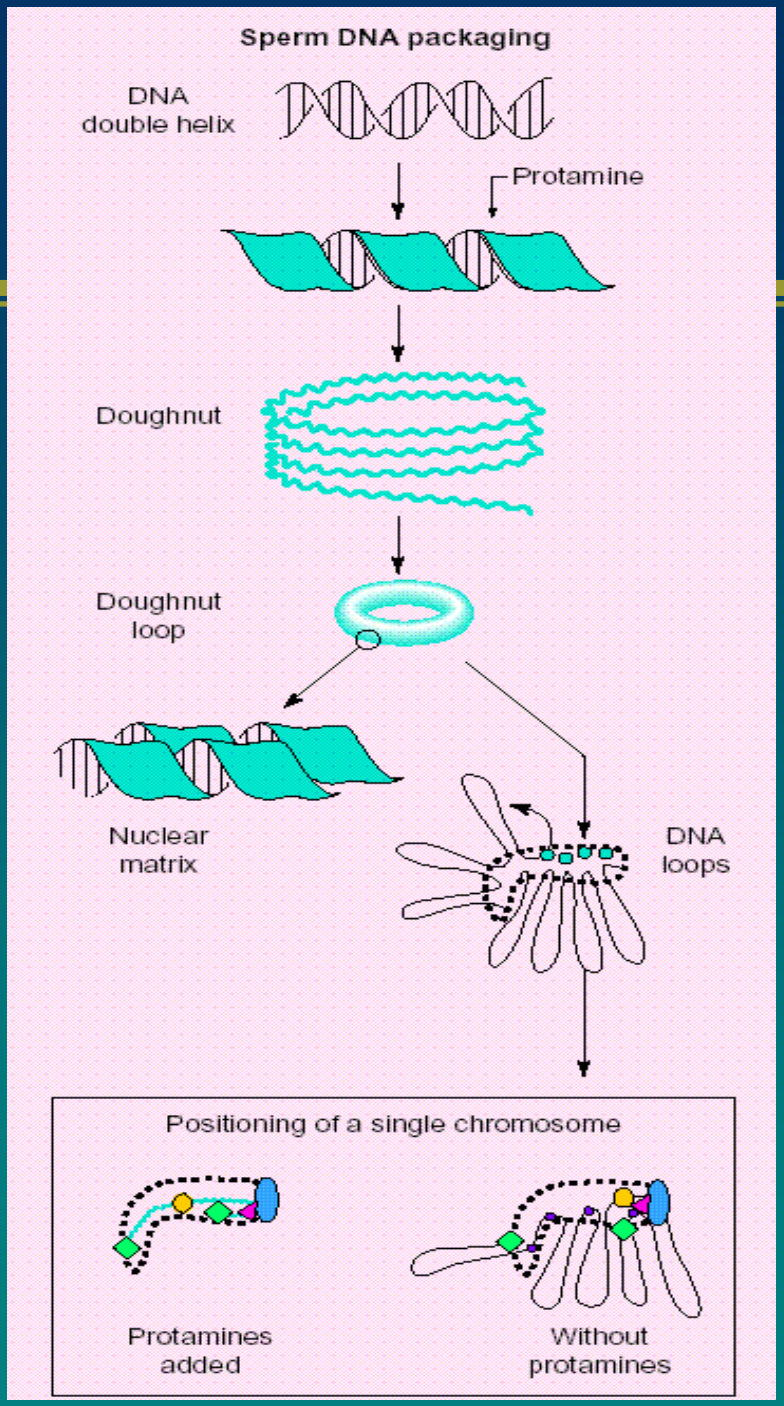
**Tail anomalies**

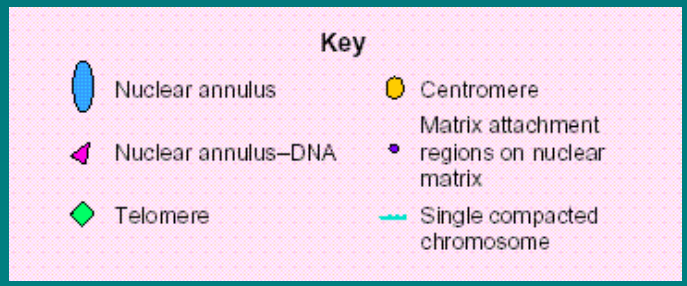
# Chromatine structure in spermatozoa

*Reviews of Reproduction (1999)* ***4****, 31–37*

***Origin of DNA damage in ejaculated human spermatozoa***

*Denny Sakkas, Ewa Mariethoz, Giancarlo Manicardi, Davide Bizzaro, Patrizia G. Bianchi and Umberto Bianchi*



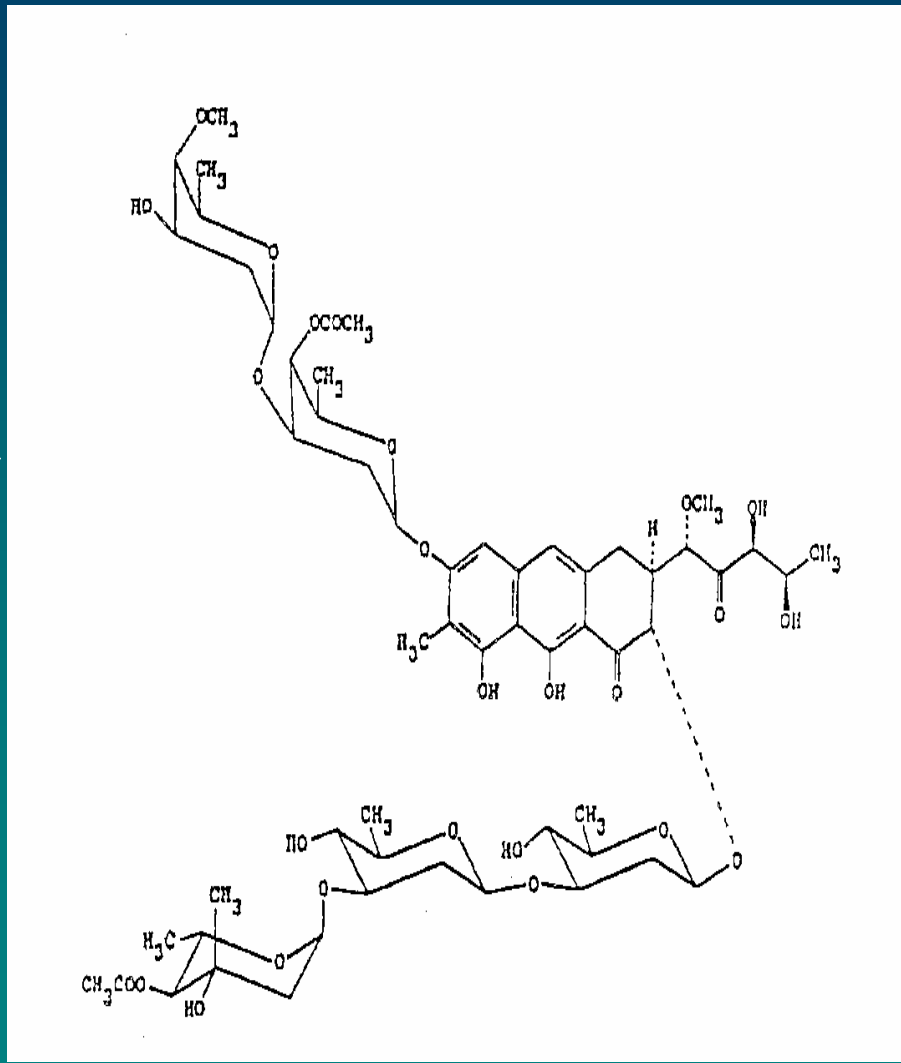



# Aim of the research project

- Study chromatin structure and DNA organisation in spermatozoa
- Relate nuclear anomalies to the classical descriptive parametres of sperm quality
- Relate these anomalies to success rates of in vitro fertilisation and intracytoplasmatic sperm injection
- **Isolate spermatozoa of a better nuclear consistency to increase the likehood of a normal offspring of infertile couples**

# Chromomycin $A_3$ ($CMA_3$)

- Fluorochrome specific for GC sequences
- Reciprocal inhibitory competitive process between protamines and $CMA_3$
- Chromomycin ($CMA_3$) is a useful tool to for assessing the packaging quality of the chromatin in spermatozoa and allows indirect visualisation of protamin deficiency

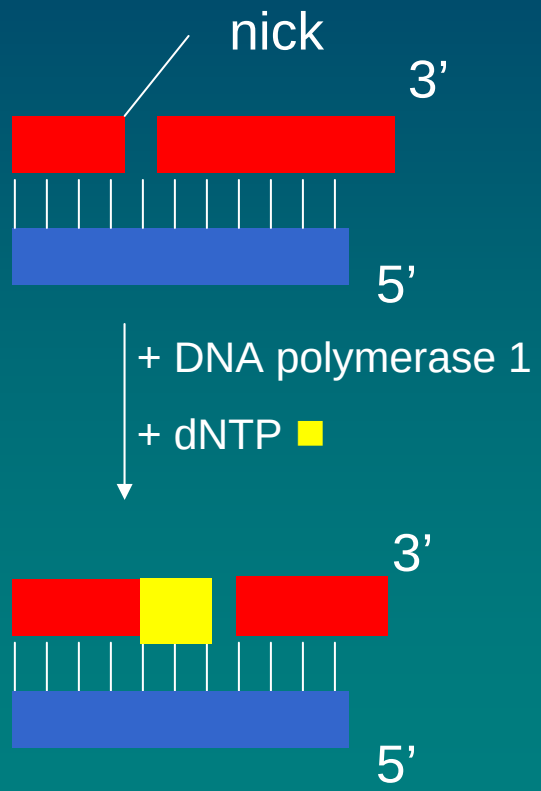


*Bianchi et al. Biol. Reprod. 49, 1993*

# Endogenous nick translation

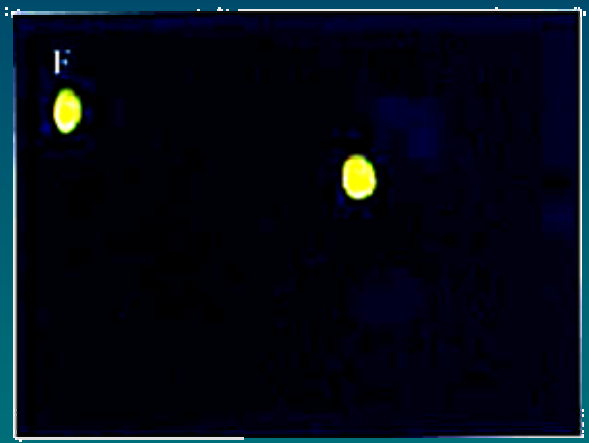
No endonuclease treatment prior to nick translation
Detection system: streptoavidin fluorescein isothiocynate( FICT) or digoxigenin-11-dUTP

*Manicardi et al. Biol. Reprod. 52, 1995*

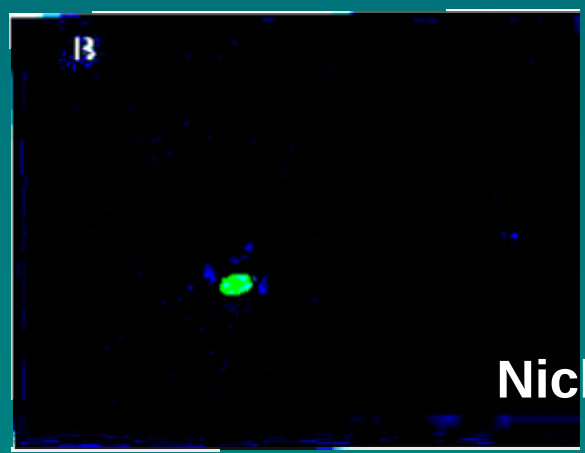


# Techniques for the evaluation of sperm chromatin damage I

Phase contrast

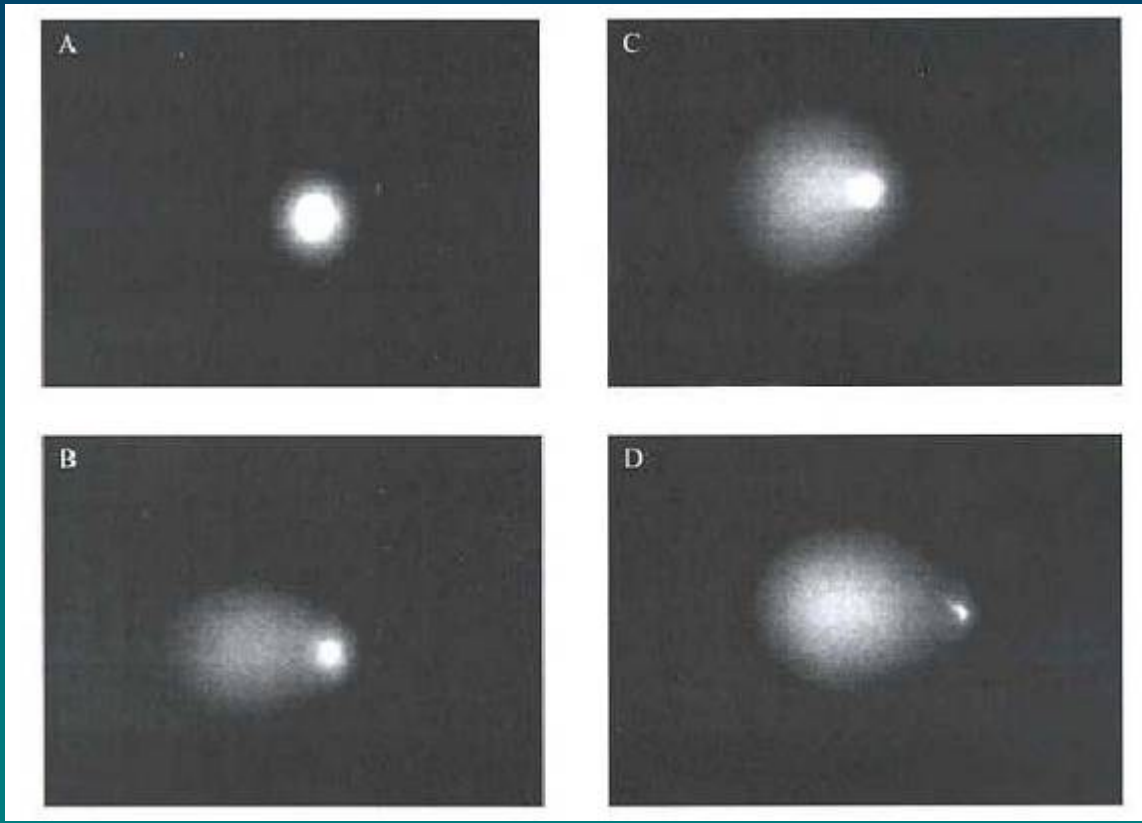


Chromomycin $A_3$ ($CMA_3$)



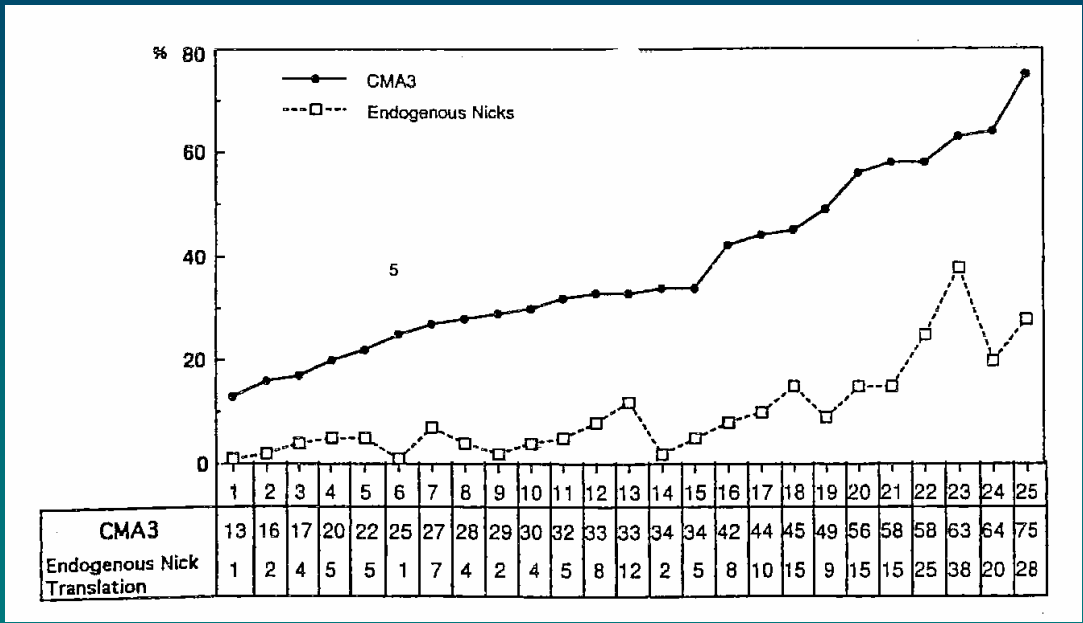
Nick translation

# Different patterns of comets obtained from single spermatozoa.




The morphology and dimension of the tails are related to the amount of DNA fragments.

# Percentage of human spermatozoa positive after treatment with $CMA_3$ and in situ nick translation

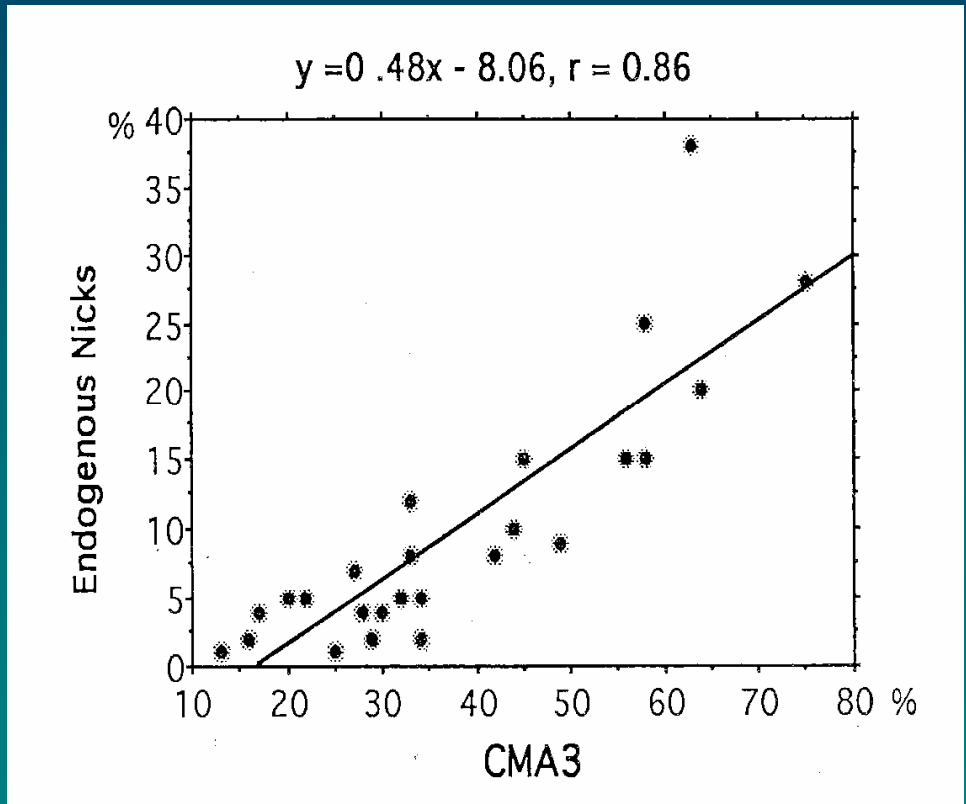



| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CMA3 | 13 | 16 | 17 | 20 | 22 | 25 | 27 | 28 | 29 | 30 | 32 | 33 | 33 | 34 | 34 | 42 | 44 | 45 | 49 | 56 | 58 | 58 | 63 | 64 | 75 |
| Endogenous Nick Translation | 1 | 2 | 4 | 5 | 5 | 1 | 7 | 4 | 2 | 4 | 5 | 8 | 12 | 2 | 5 | 8 | 10 | 15 | 9 | 15 | 15 | 25 | 38 | 20 | 28 |

Values represent assessment of 500 sperm on each slide.

*Manicardi et al. Biol. Reprod. 49, 1995*

# Correlation between $CMA_3$ fluorescence and presence of endogenous nicks in human spermatozoa collected from 25 patients




*Manicardi et al. Biol. Reprod. 49 , 1995*

# Relationship between $CMA_3$ positivity, presence of endogenous nicks and sperm morphology

In individuals presenting less than 20% of morphologically normal spermatozoa both $CMA_3$ and endogenous nick-translation positivity were high

- ***Bianchi et al. J Assist. Reprod.Genet. 13, 1996***

# Evidence of hidden anomalies in normal spermatozoa

Infertile males undergoing sperm microinjection treatment do also show fluorescence in spermatozoa judged as morphologically normal thus suggesting the presence of hidden structural chromatin defects even in those gametes that have more chance to be selected for the procedure

*Bianchi et al. Mol . Hum. Reprod. 2, 1996*

# Mean fertilisation rates in relation to $CMA_3$ positivity of morphologically normal spermatozoa

| % OF MORPHOLOGICALLY NORMAL SPERM FLUORESCING | No of cases (a) | Mean fertilisation rate (b) |
|---|---|---|
| 0-50 | 10 | 56.4± 25.2 |
| 50-70 | 7 | 53.6±30.2 |
| >70 | 10 | 24±15.9 (c) |

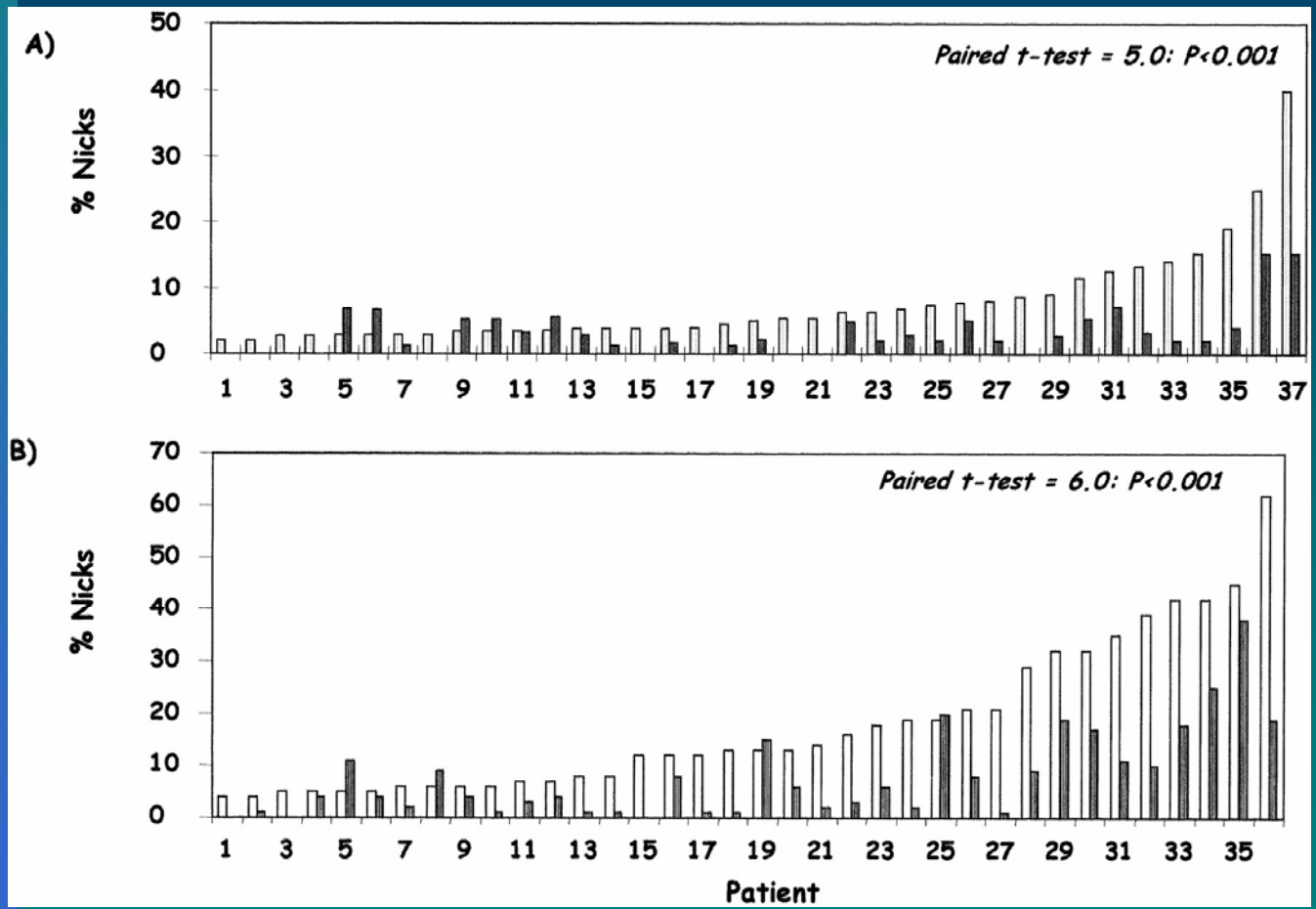
*Bianchi et al. Mol . Hum. Reprod. 2, 1996*

# Percentage of $CMA_3$ positive spermatozoa after isolation using swim-up, Percoll and PureSperm techniques

| N° of samples | Fraction of sperm preparation | | | Paired t-test (wash vs s-u or 90%) |
|---|---|---|---|---|
| | Swim-up | | | |
| 31 | Wash<br>21.5±9.5 | Sediment<br>19.6±9.7 | Swim-up<br>22.0±9.5 | P=0.6 |
| | Percoll | | | |
| 39 | Wash<br>29.9±15.5 | 45%<br>30.72±18.3 | 90%<br>18.1±12.1 | P<0.001 |
| | Puresperm | | | |
| 45 | Wash<br>33.9±21.2 | 45%<br>25.0±19.8 | 90%<br>12.4±12.6 | P<0.001 |

*Sakkas et al. Hum. Reprod: 15, 2000*

# Percentage of spermatozoa exibiting endogenous Dna nicks after preparation with Percoll (A) and Puresperm (B)




*Sakkas et al. Hum. Reprod. 15, 2000*

# Cervical mucus characteristics I

- A heterogenous secretion containing 90% water
- Reological properties
  - Consistency
  - Spinnbarkeit
  - Ferning

A hydrogel comprising a high viscosity component  and low viscosity

# Cervical mucus characteristics II

- High viscosity component:
  - Macromolecular network of mucins
    - A fibrillar system of subunits with a peptide core and oligosaccharide side chains
- Low viscosity component:
  - Electrolytes, organic coumpounds, soluble proteins

# Cervical mucus properties

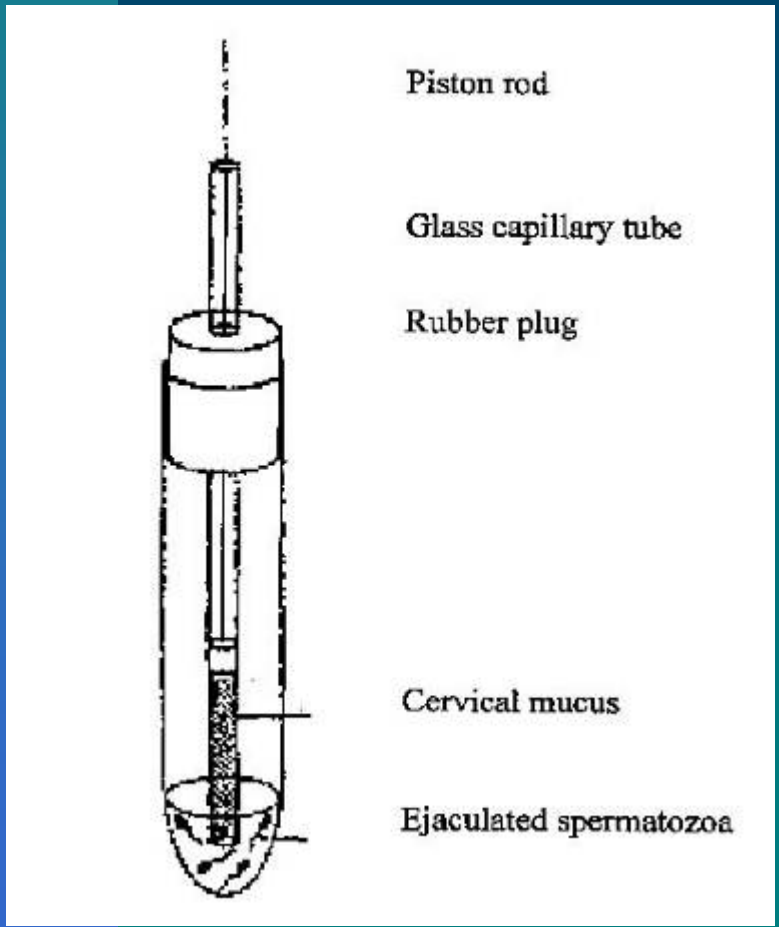
- *Receptivity to sperm*
- *Protection of sperm from the hostile envirronment of the vagina*
- *Supplementation of energy requirements of sperm*
- *Short term sperm reservoir*
- *Initiation of sperm capacitation*
- ***Filtering effect***
  - *Mortimer et al., 1982; Katz et al.,1990; Eggert-Krause et al., 1995*

***WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction , 1992***

# Patient selection for the cervical mucus study

- Patients were deliberately chosen on a random basis among the men consulting for couple infertility at the Sterility Clinic of the University Hospital of Geneva.
- The aim was to evaluate selection power of cervical mucus on sperm independently from classical sperm parameters.
- The controls were intra-individual as the ejaculate of each individual was separated in two and analysed.

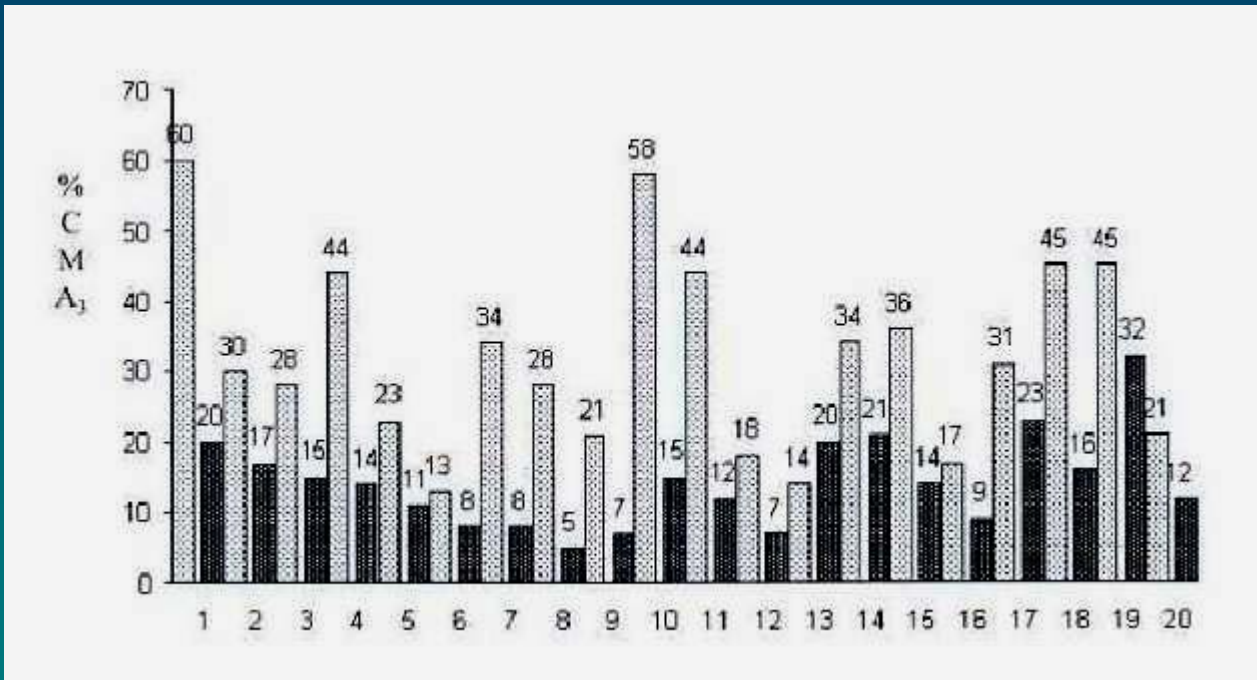
# Cervical mucus as a selective barrier against spermatozoa carring fragmented DNA and chromatin structural anomalies




T6 medium was used at the end of the capilary tube to recover the sperm crossing successfully the mucus

Recovered sperm was then fixed in Carnoy, spead on slides and treated according to the chromomycin and the nick translation technique protocol

# Cervical mucus as a selective barrier against spermatozoa carring fragmented DNA and chromatin structural anomalies




**Percentage of positivity to $CMA_3$ in raw spermatozoa (white) and in spermatozoa selected by cervical mucus (black).**
**T-test = 5.2256, $p<0.05$ = 2.024, $p>0.001$ = 3.566**

*Bianchi et al. submitted for publication*

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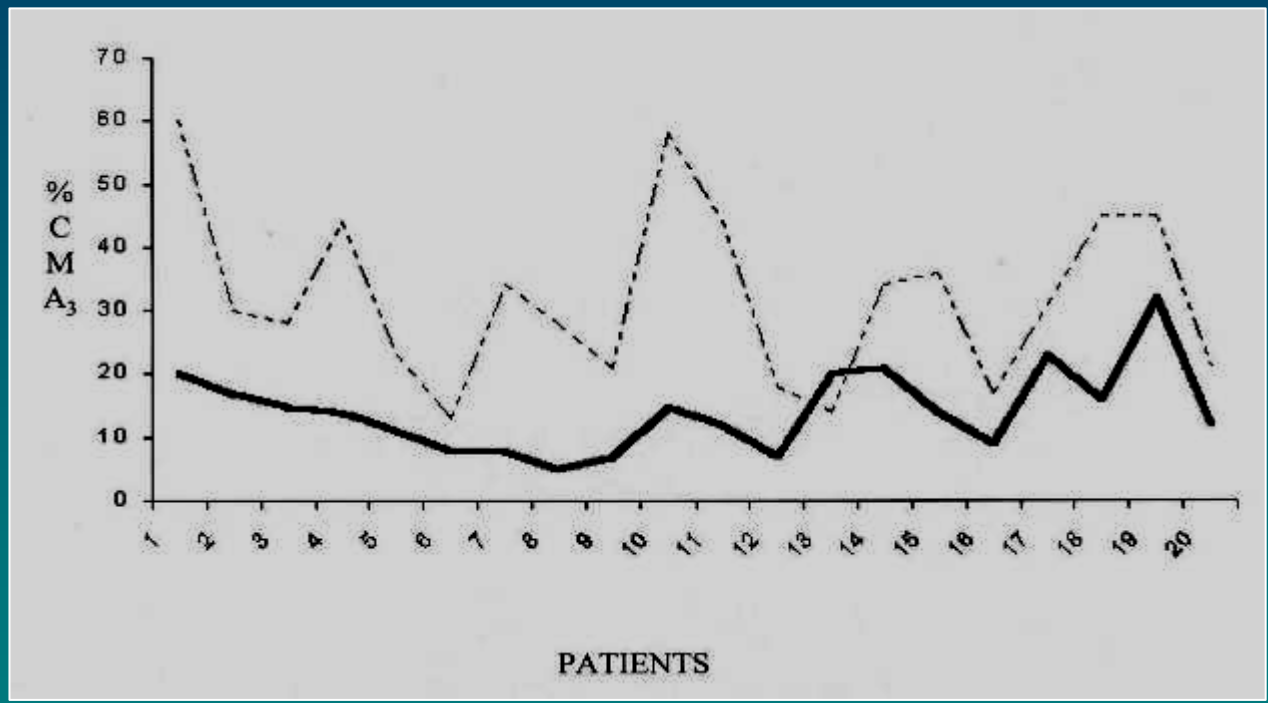
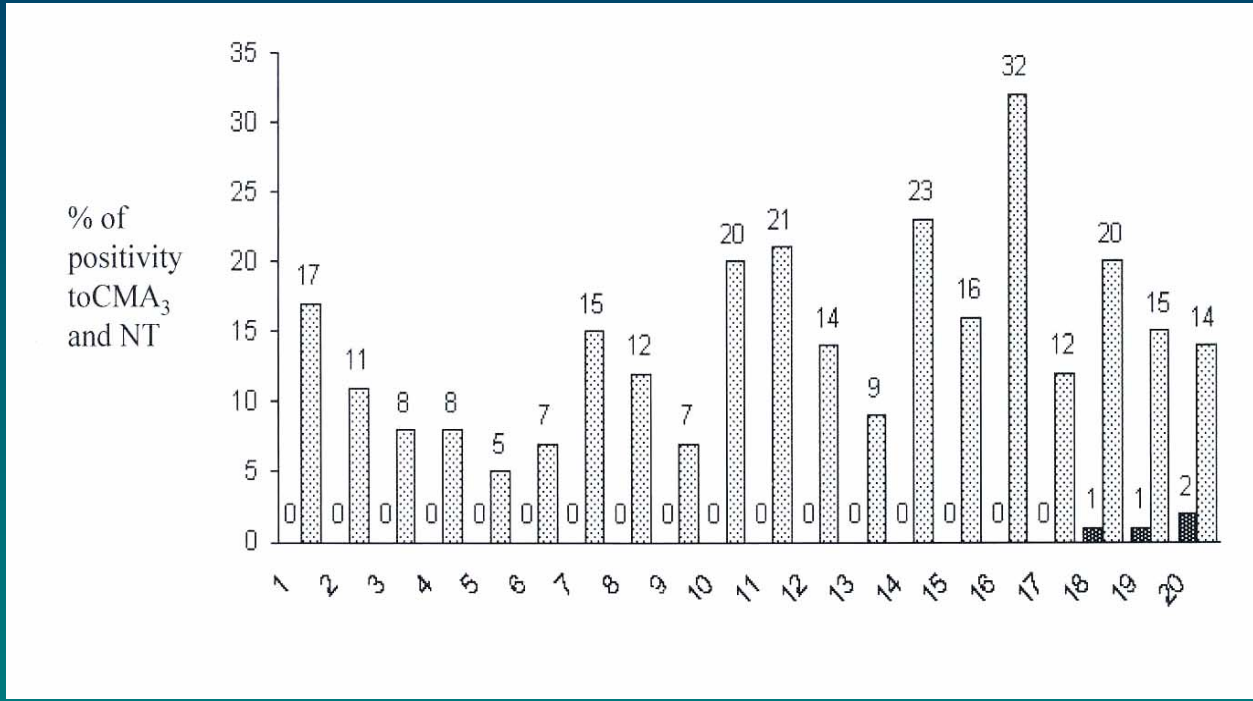



**Comparison between percentage of positive to $CMA_3$ of raw (-.-) and selected spermatozoa (-)**

*Bianchi et al. submitted for publication*

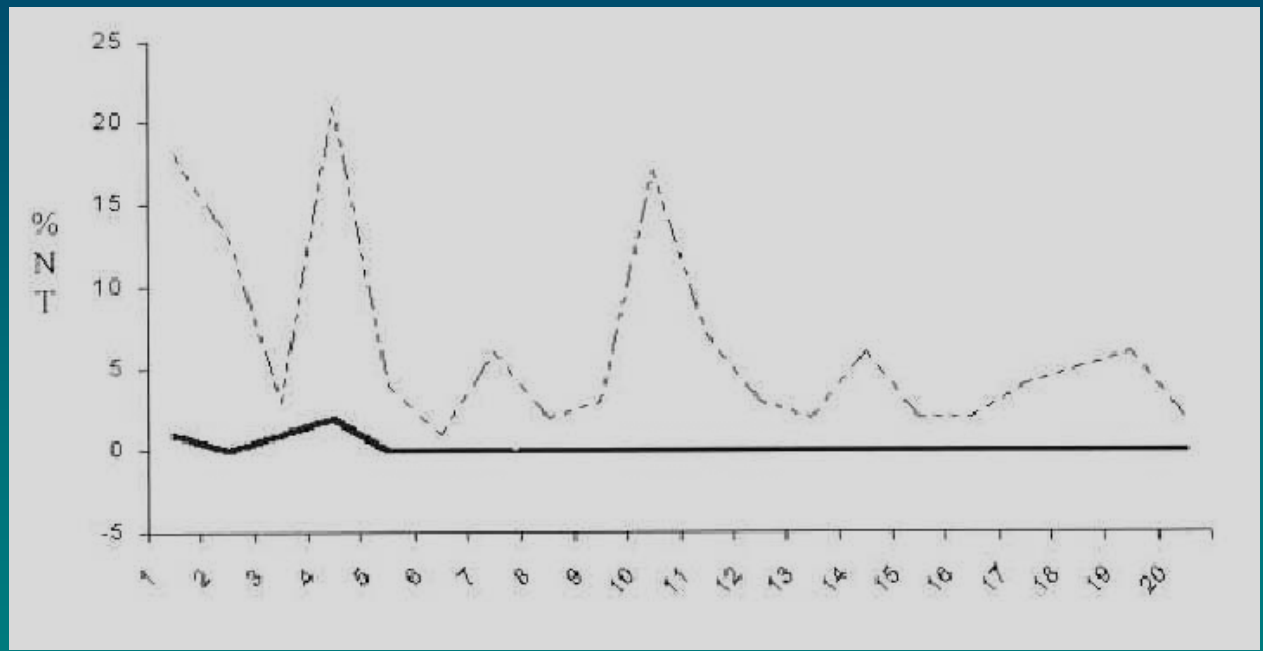
# Cervical mucus as a selective barrier against spermatozoa carring fragmented DNA and chromatin structural anomalies




**There is no correlation between percentage of positivity to in situ nick translation (black columns) and $CMA_3$ (white columns) estimated in spermatozoa selected by cervical mucus - r = 0.08817 - p= 0.717**

*Bianchi et al. submitted for publication*

# Cervical mucus as a selective barrier against spermatozoa carring fragmented DNA and chromatin structural anomalies




Comparison between percentage of positivity to in situ nick translation of raw (-.-) and selected spermatozoa (-)

*Bianchi et al. submitted for publication*

# Conclusions

Number, motility and morphology only partially define the genetic quality of human spermatozoa

Evaluation of sperm quality also partially benefits from the use of techniques such as nick translation and $CMA_3$

Cervical mucus, in vitro, seems to be also capable of selecting structurally normal spermatozoa

Identifing sperm with physical and/or chemical integrity may allow treatment of the most severe cases of male infertility with less risks for progeny

# Thank you


- *Denny Sakkas*
- *Davide Bizzaro*
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