

Ο ρόλος της δοκιμασίας κατακερματισμού DNA σπερματοζωαρίων στην πρόγνωση της γονιμοποιητικής ικανότητας του σπέρματος: Είναι μία σημαντική δοκιμασία



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Some Tips...

Ο κατακερματισμός του DNA των σπερματοζωαρίων είναι μία φυσιολογική διαδικασία στα πλαίσια της απόπτωσης που σκοπό έχει:

-Ιδανικό λόγο σπερματικών κυττάρων – κυττάρων Sertoli

-Έλεγχο και απομάκρυνση των σπερματικών κυττάρων με αλλοιωμένο DNA και διασφάλιση μεταφορά υγιούς DNA

Στα σωματικά κύτταρα ο κατακερματισμός ξεκινάει από το μιτοχονδριακό DNA που πυροδοτεί τελικά την απόπτωση του κυττάρου

Some Tips...

Στα σπερματικά κύτταρα λόγω της συμπυκνωμένης μορφής τους ο κατακερματισμός ξεκινάει από το **μιτοχονδριακό DNA** του μέσου τμήματος με άμεση επίδραση στην **κινητικότητα** του

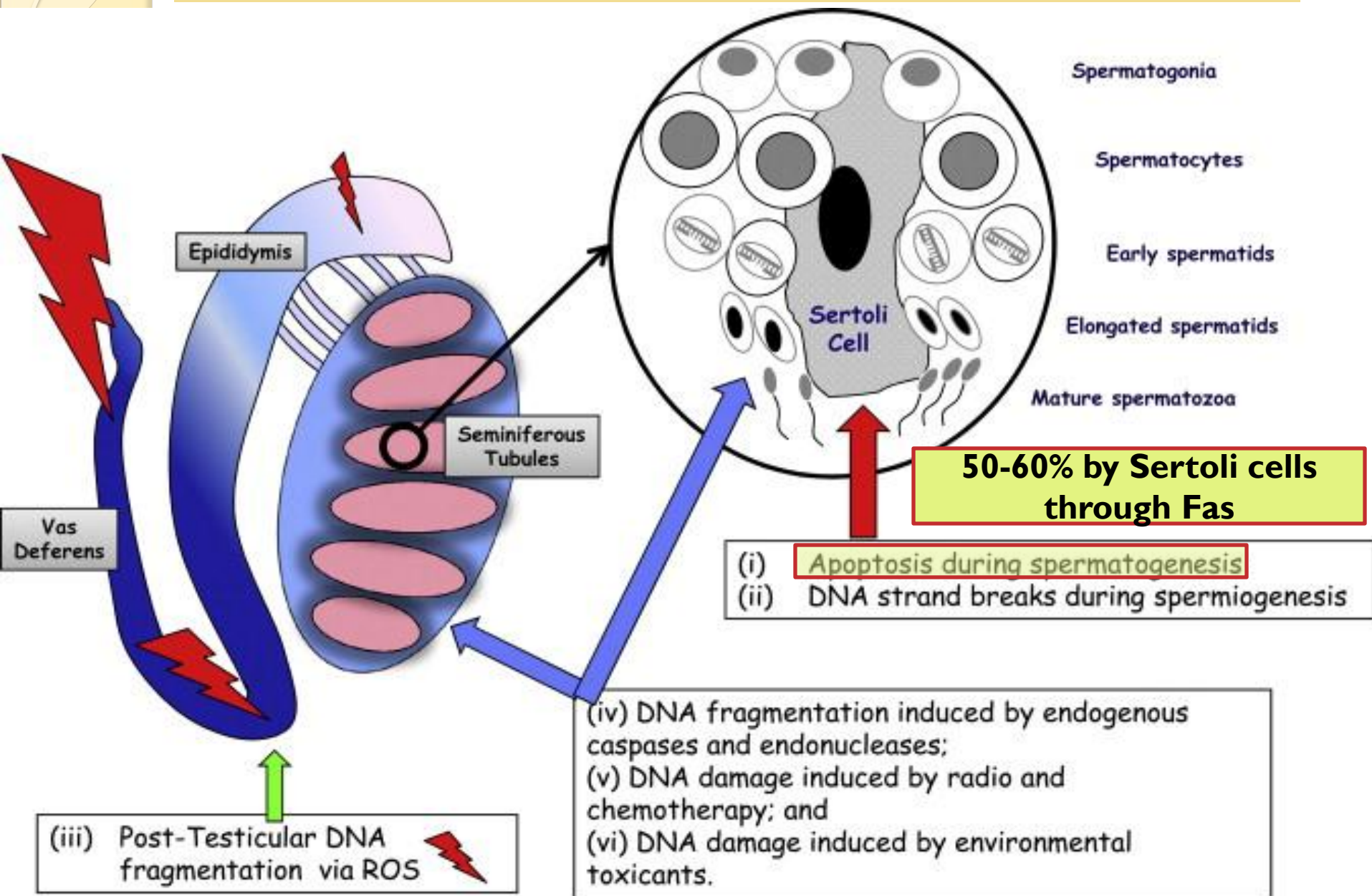
Ο κατακερματισμός του DNA της χρωματίνης ακολουθεί ως αποτέλεσμα της ενεργοποίησης των **ενδοουκλεασών** με **πλήρη απόπτωση** του σπερματικού κυττάρου

Επιπλέον, **επιφανειακοί markers** όπως η phosphatidylserine προσελκύουν **φαγοκύτταρα** που απομακρύνουν τα σημαδεμένα κύτταρα

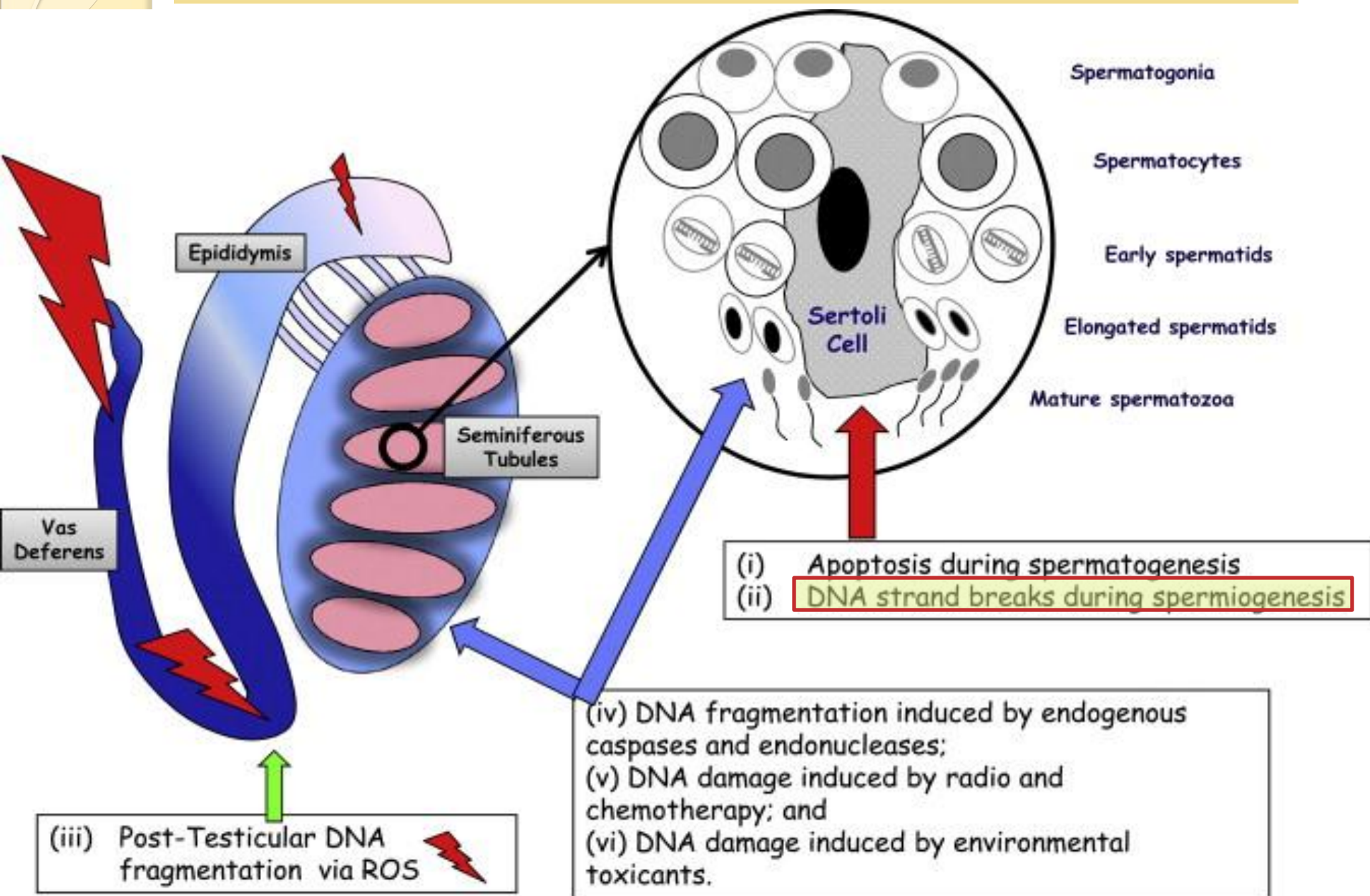
Τέλος, μη διορθωμένα «σπασίματα» στην αλυσίδα του DNA αποτελούν ένα ακόμα μηχανισμό βλάβης του DNA των σπερματοζωαρίων.

Aitken et al *Reproduction* 2011;141:139–50.
Sakkas et al. *Reprod Biomed Online* 2003;7:428–32.
Sotolongo et al. *J Androl* 2005;26:272–80.
Kurosaka et al. *J Immunol* 2003;171:4672–9.
Leduc et al. *Biol Reprod* 2008;78:324–32

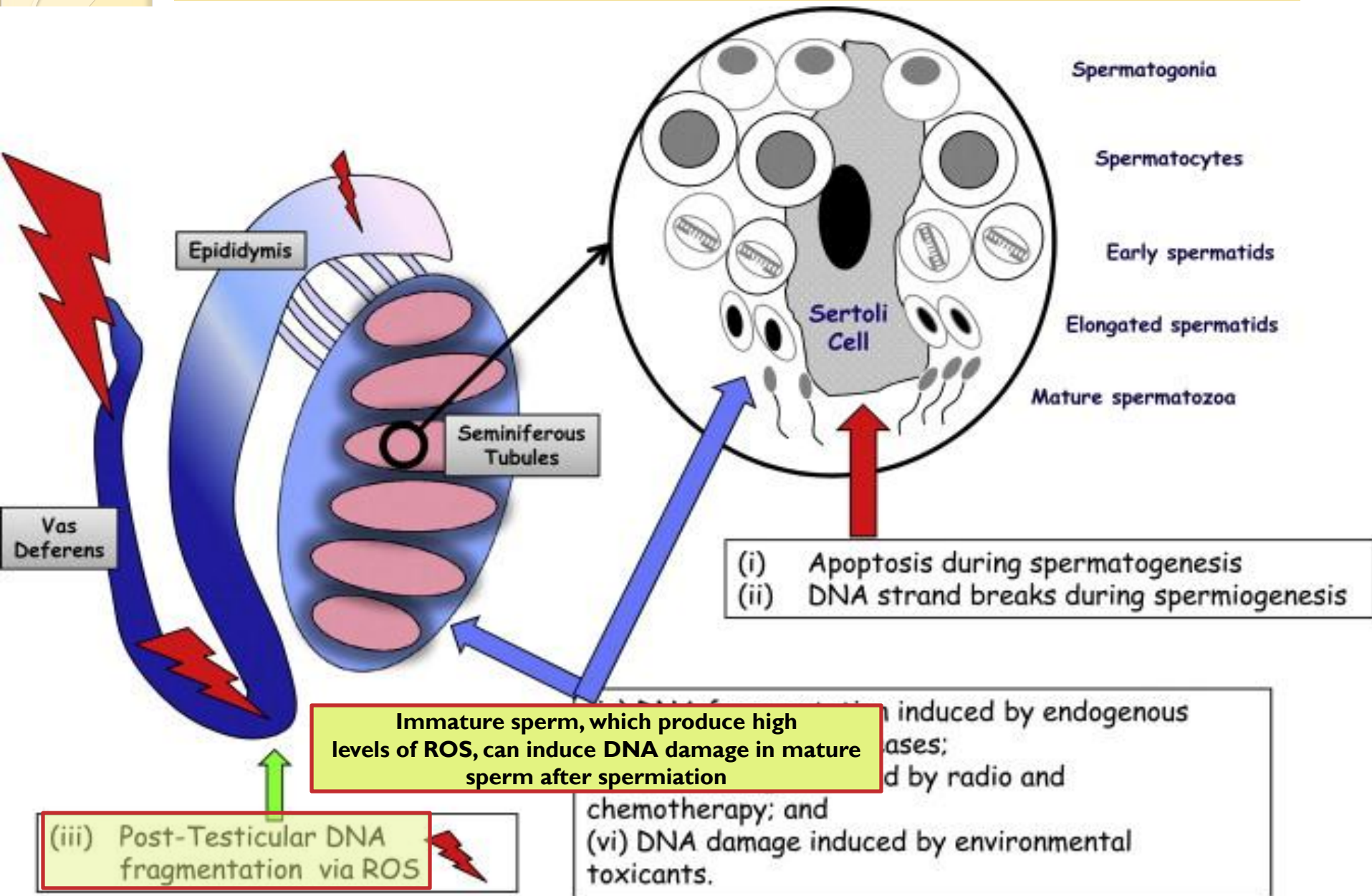
UNDERSTANDING DNA FRAGMENTATION'S ORIGINS



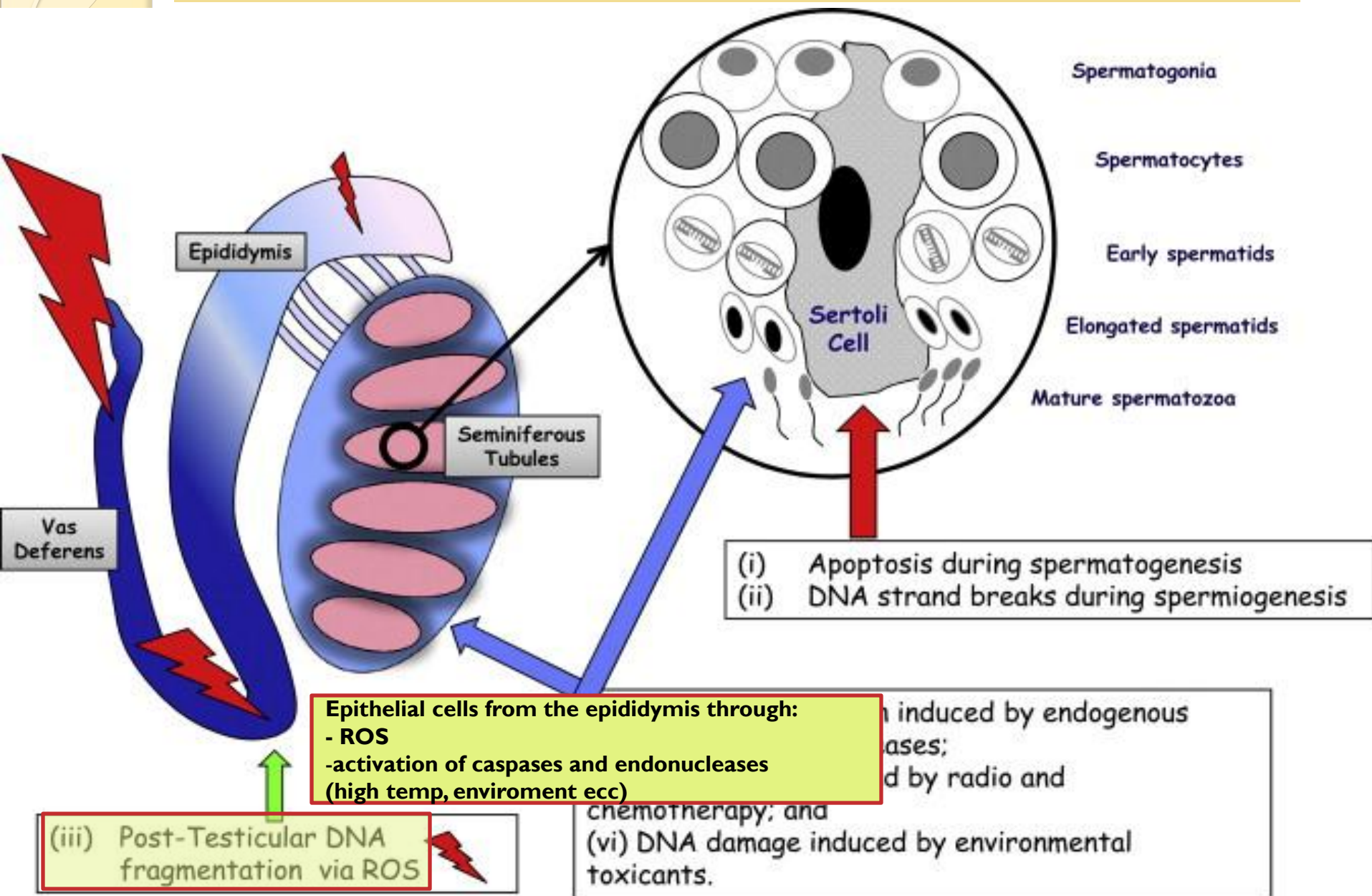
UNDERSTANDING DNA FRAGMENTATION'S ORIGINS



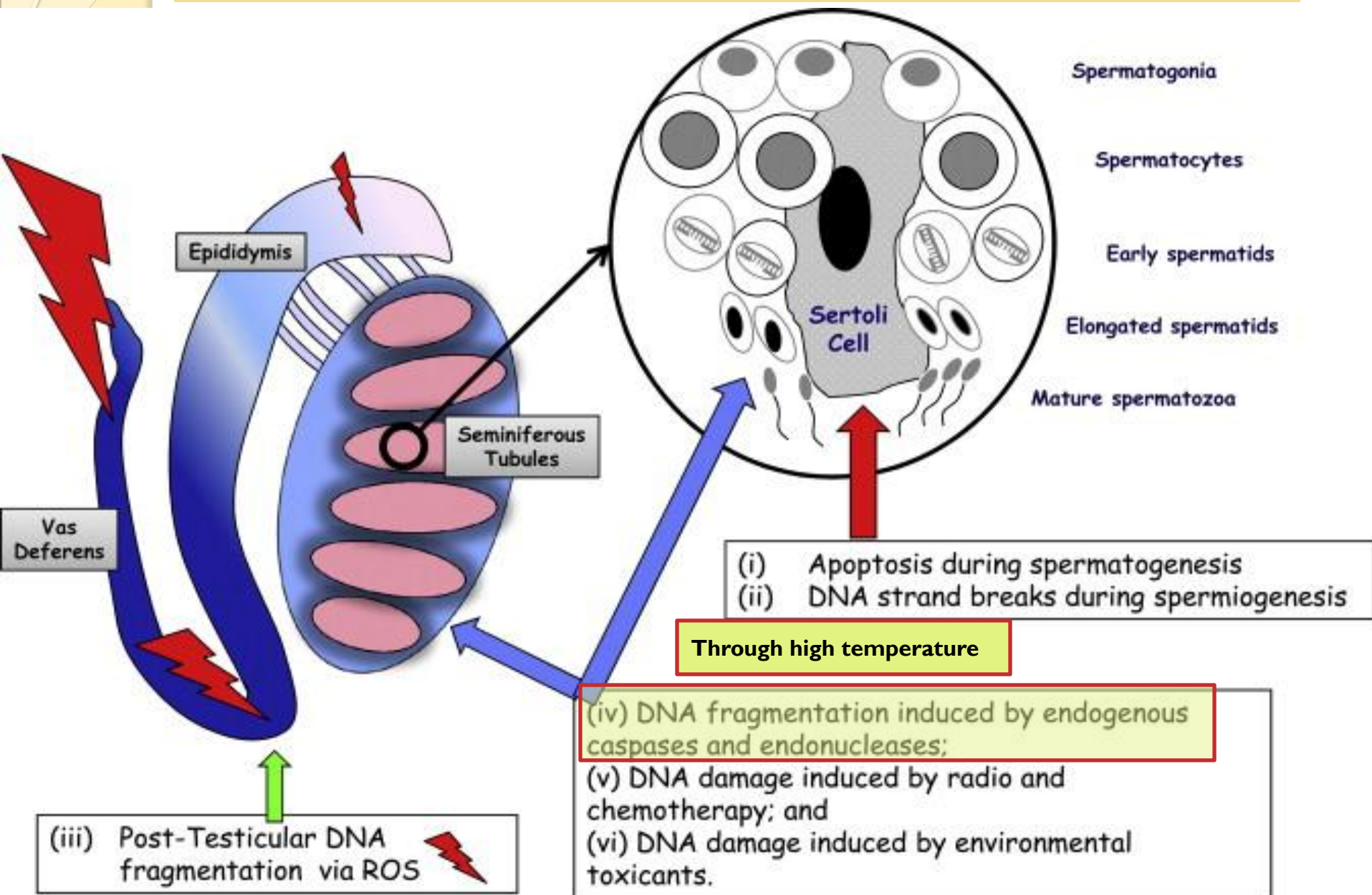
UNDERSTANDING DNA FRAGMENTATION'S ORIGINS



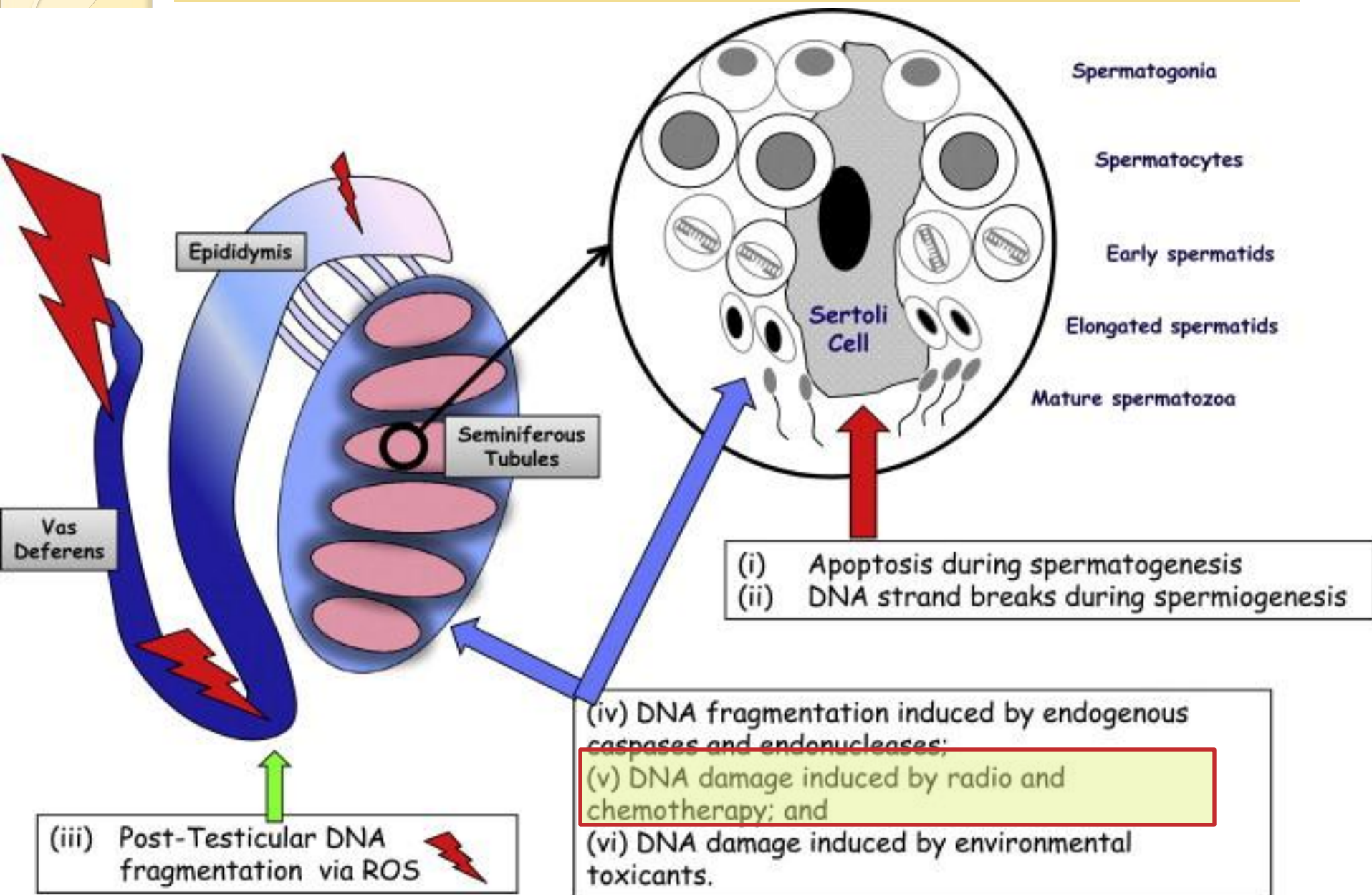
UNDERSTANDING DNA FRAGMENTATION'S ORIGINS



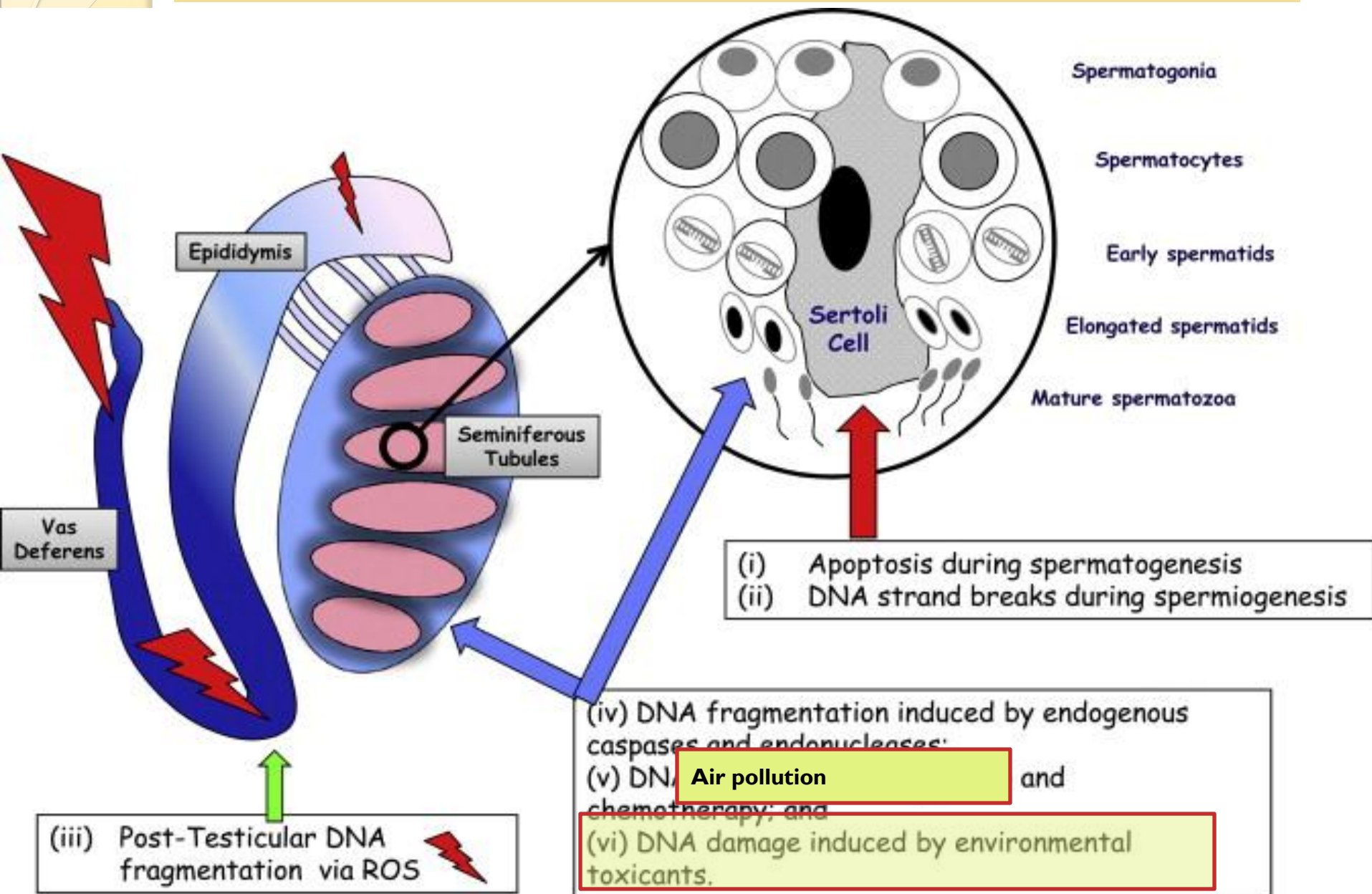
UNDERSTANDING DNA FRAGMENTATION'S ORIGINS



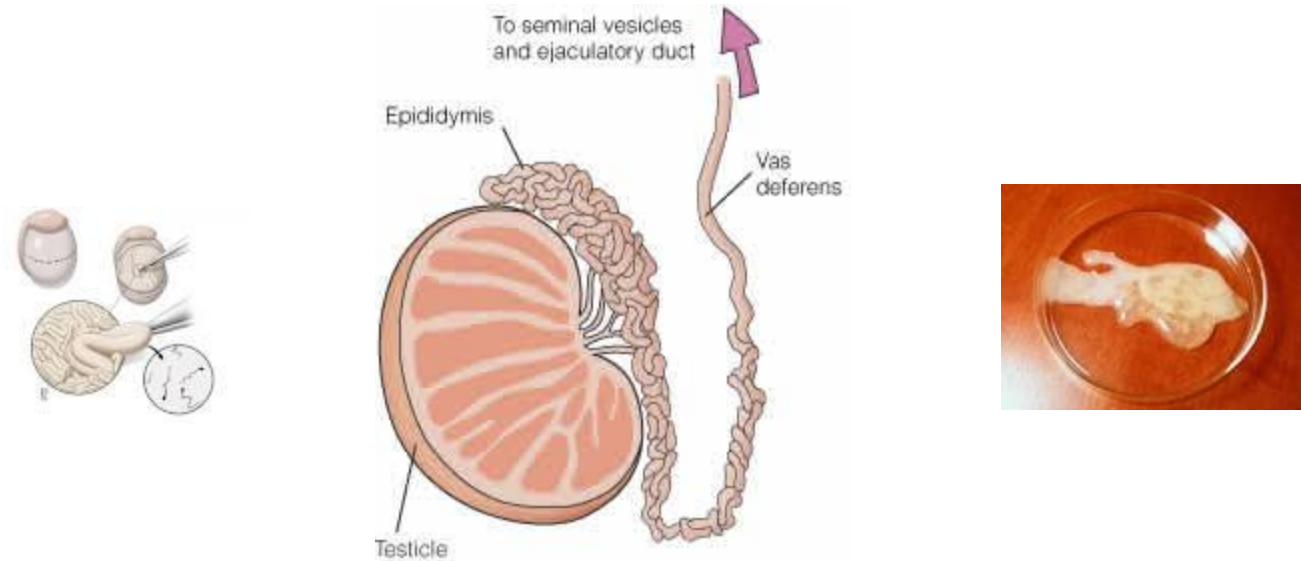
UNDERSTANDING DNA FRAGMENTATION'S ORIGINS



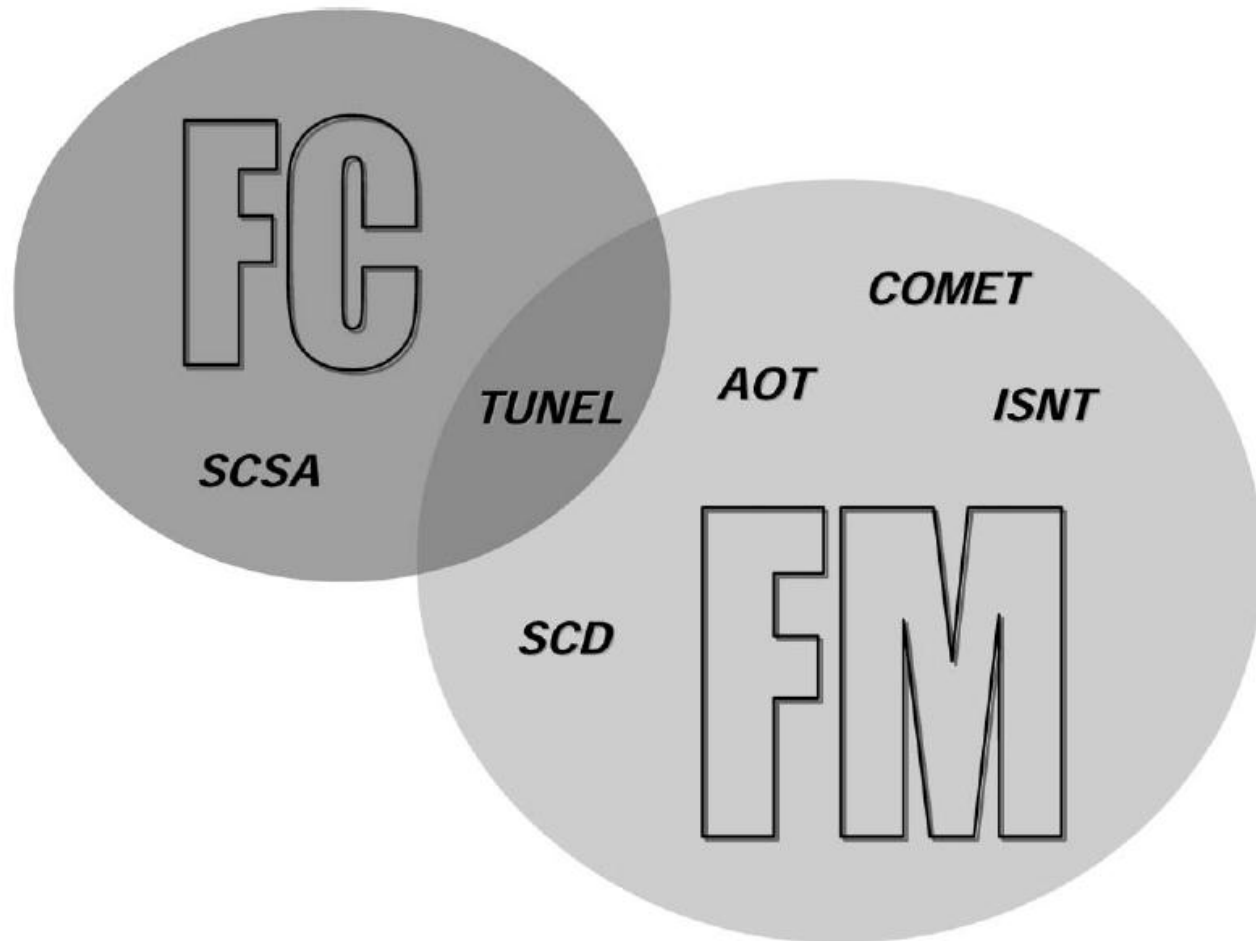
UNDERSTANDING DNA FRAGMENTATION'S ORIGINS



Degree of DNA fragmentation



The paramount importance of the test



AOT, acridine orange test; **COMET**, single-cell gel electrophoresis assay; **ISNT**, in situ nick translation; **SCD**, sperm chromatin dispersion test; **SCSA**, sperm chromatin structure assay; **TUNEL**, terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick-end labelling.

The paramount importance of the test, the sample and the method

Flow cytometry

vs

Fluorescence microscopy

Direct measurement
(TUNEL or comet at
neutral pH)

vs

Indirect measurement (SCSA,
SCD, comet at acid or alkaline
pH)

Standardized methods
(SCSA)

vs

non-standardized methods (all
the others)

Testicular
sperm

vs

Cryopreserved
sperm

vs

Fresh ejaculated
sample

Natural
conception

vs

IUI

vs

IVF

vs

ICSI

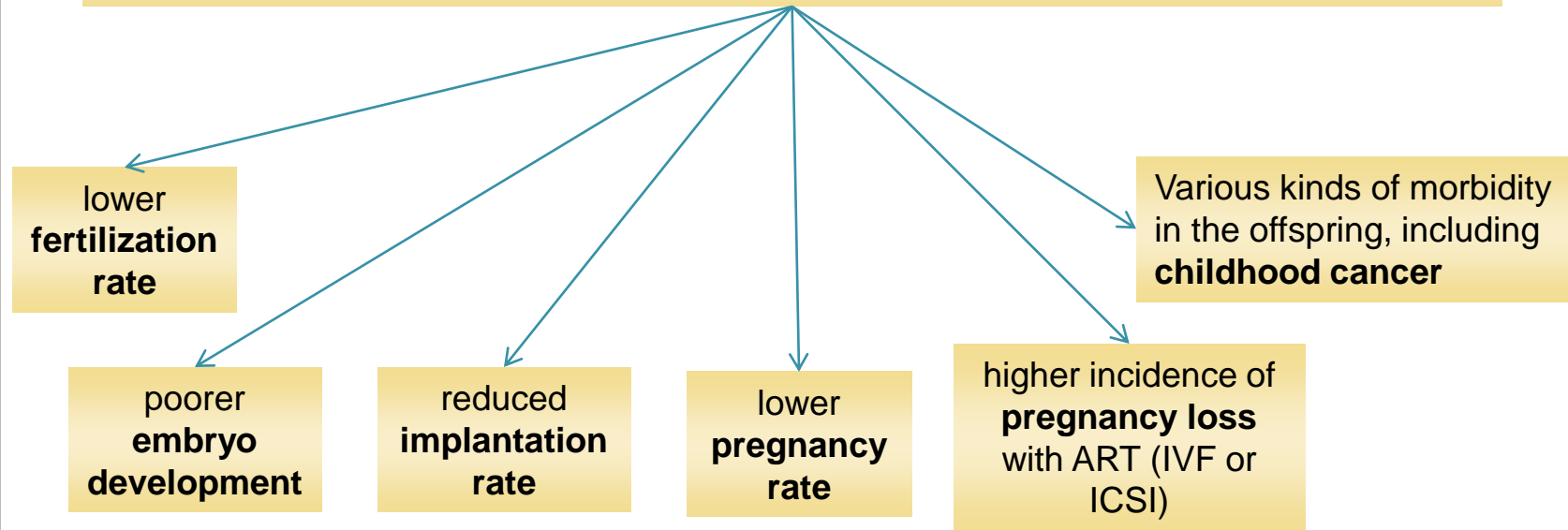
The reason of the debate

Υπάρχει μία **έντονη κλινική ανάγκη** να μπορούμε με ασφάλεια να διαχωρίζουμε τους **γόνιμους** από τους **υπογόνιμους** άνδρες



BUILDING AWARENESS

Semen containing a high proportion of cells with fragmented DNA has been blamed for a:

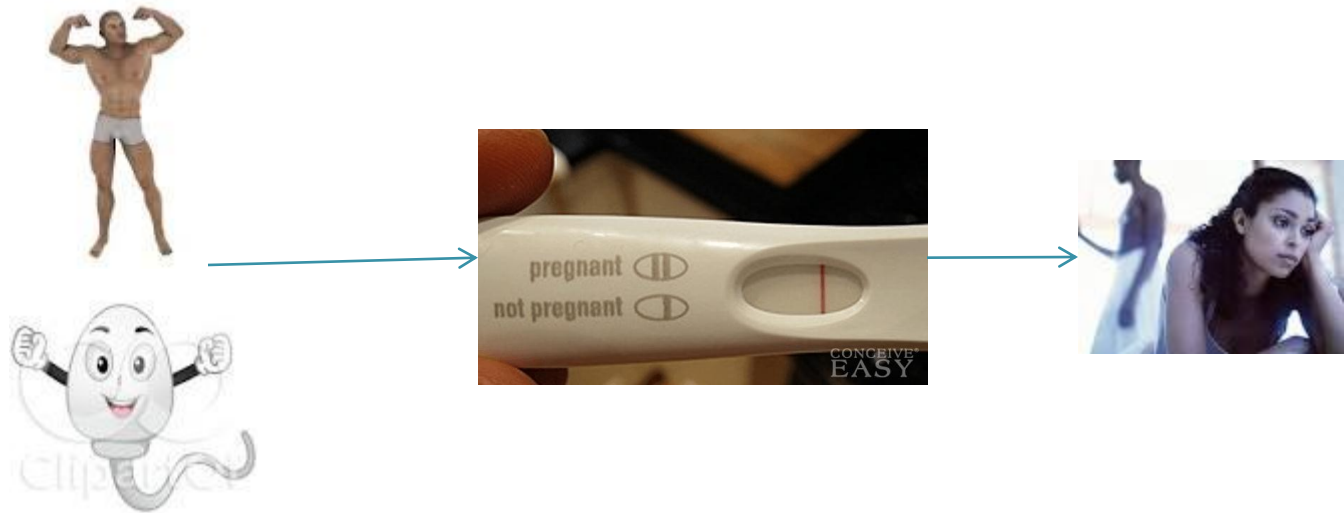


Semen exhibiting a high percentage of damaged spermatozoa with denatured DNA (>30%) has **very low fertility potential**.

Simon et al. et al. *Reprod Biomed Online* 2013;26:68-78
Simon et al. *Syst Biol ReprodMed* 2011;57:133-8.
Aitken et al. *Int J Androl* 2009;32:46-56.
Zini et al. *Hum Reprod* 2008;23:2663-8.
Evenson et al. *Reprod Biomed Online* 2006;12:466-72
Zini et al. *Hum Reprod* 2008;23:2663-8
Zini et al. *Syst Biol Reprod Med* 2011;57:78-85
Aitken and De Iuliis, 2010.

To complicate the issue further...

8% of infertile men have **abnormal DNA integrity** that is **not accompanied** by impairment in semen concentration, motility, or morphology



The interest in assessing the role and effect of DNA fragmentation

European Society of Human Reproduction and Embryology Special Interest Group in Andrology:

- Synthesize the current status of the field
- Provide recommendations for future work

The interest in assessing the role and effect of DNA fragmentation

The European Society of Human Reproduction and Embryology Special Interest Group in Andrology stated that :

- No chromatin assay is definitive
- The DNA fragmentation in unprocessed semen **correlates** with IUI outcome
- Using a selected population of sperm cells with a **normal DFI** originating from raw ejaculate with a >30% DFI **may still have a negative impact** on treatment outcome



Specific Questions

1. Does the DNA integrity test predict male fertility with natural conception?

Studies have looked at:

Time to pregnancy and fertility potential of sperm donors
DNA fragmentation between fertile and infertile men

Overall, **there is an association** with increased DNA fragmentation and reduced fertility in men based on these studies.

In conclusion, there is fair evidence (**Level B**) that increased DNA fragmentation is associated with reduced fertility

Insufficient evidence (**Level C**) to use the test as a predictor of fertility since cut-points have not been clearly established and validated.

Hu J et al. *Andrologia* 2011;43:155–62.

Evenson D et al. *Repro BiomedOnline* 2006;12:466–72.

Giwerzman et al. *Int J Androl* 2010;33:e221–7.

Simon et al. *Syst.Biol.Reprod.Medicine* 2011;57:133–8.

2. Does the DNA integrity test predict pregnancy with intrauterine insemination (IUI)?

A Level II-1 study showed a positive predictive value of the SCSA test with DFI >30% associated with a lower pregnancy and delivery rate.

Other studies did not confirm the cutoff for IUI

In conclusion, there is insufficient evidence (**Level C**) to recommend the **routine use of DNA integrity tests** to predict pregnancy with IUI.

Muriel et al. Hum Reprod 2006;21:738–44.
Bungum et al. Hum Reprod 2004;19:1401–8.
Bungum et al. Human Reprod 2007;22:174–9.
Duran et al. HumReprod 2002;17:3122–8

3. Is DNA fragmentation predictive of pregnancy with in vitro fertilization (IVF)?

One meta-analysis showed that DNA fragmentation was associated with a **modest, but significant**, reduction in IVF pregnancy rates

Increased DNA fragmentation is mildly associated with IVF success overall

In conclusion, there is insufficient evidence (**Level C**) to recommend **routine use of DNA integrity testing** for patients undergoing IVF.

Zini A. Syst.Biol.Repr.Med 2011;57:78–85.
Larson Hum Reprod 2000;15:1717–22.
Larson-Cook Fertil Steril 2003;80:895–902.
Tarozzi Reprod Biomed Online 2009;18:486–95.
Simon Fertil Steril 2011;95:652–7.
Simon Human Reprod 2010;25:1594–608.

4. Is DNA fragmentation predictive of pregnancy with IVF and intracytoplasmic sperm injection (ICSI)?

A meta-analysis concluded that sperm DNA fragmentation was significantly associated with pregnancy in IVF/ICSI cycles.

In conclusion, there is insufficient evidence (**Level C**) to recommend **routine DNA integrity testing** for patients undergoing IVF/ICSI.

Collins et al. Fertil Steril 2008;89:823–31.
Simon et al. Human Reprod 2010;25:1594–608.
Zini A. Syst.Biol.Repr.Med 2011;57:78–85.
Gandini et al. Human Reprod 2004;19:1409–17.
Zini et al. Hum Reprod 2005;20:3476–80.
Micinski et al. Reprod Biol 2009;9:65–70.

5. Is DNA fragmentation predictive of pregnancy loss?

A meta-analysis found a **significant association** between DNA fragmentation and pregnancy loss after IVF or ICSI

However, there is insufficient evidence (**Level C**) to recommend **routine DNA integrity testing** to predict pregnancy loss.

Concluding Remarks

- ❖ DNA fragmentation is the **most frequent DNA alteration** in sperm, and **its clinical relevance is now emerging**
- ❖ **DNA fragmentation has an important impact** independent of the parameters of classic semen analysis, on both natural and assisted reproduction.
- ❖ **Pharmacological treatments** (based on the administration of antioxidants) aimed at decreasing SDF have demonstrate **little beneficial effect**

Concluding Remarks

- ❖ The evaluation of SDF may be useful in those couples who are eligible for **first-level ART**
- ❖ **The impact of SDF on the outcome of second-level ART are inconsistent**, leaving doubts about the clinical utility of SDF in decision-making for couples undergoing these procedures
- ❖ Not only is **standardization needed** for both assays, but also the **definition of threshold values** of SDF in fertile men must be determined.

Concluding Remarks

There are **certain categories of patient** who may benefit from SDF evaluation before undergoing ART or attempting to conceive naturally:

- subjects who have been heavily exposed to **toxicants or radiochemotherapies**
- those affected by **diabetes**
- male partners in couples who have experienced **repetitive, unexplained PL**
- male partners in couples who have experienced **repeated, unexplained failure of fertilization.**
- **cancer patients** may have increased SDF in their semen, even before chemotherapy.