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COURSE: **Sperm DNA fragmentation**

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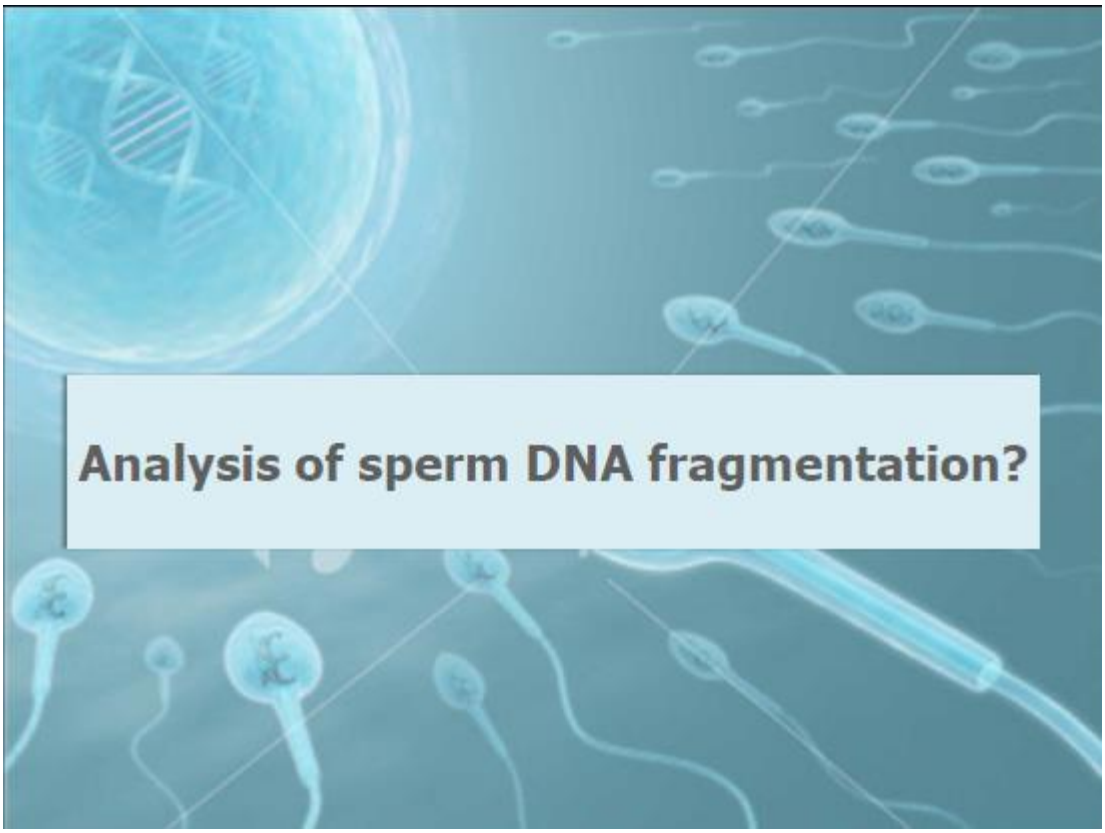


Male infertility etiology

- **Varicocele** (35-40%)
- **Infection** (~10%)
- **Genetics** (~10%)
- **Endocrinology** (<5%)
- **Immunology** (<5%)
- **Obstruction** (<5%)
- ***Cryptorchidism*** (<5%)
- **Idiopathic** (25%)

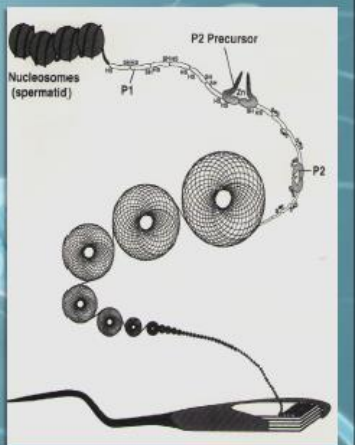
Greenberg et al, J Urology 1978

Potential causes of DNA fragmentation

- **Varicocele** (Saleh, 2003; Fischer, 2003; Zini, 2000)
- **Chemotherapy and radiotherapy** (Chatterjee, 2000; Deane, 2004; Kobayashi, 2001)
- **Smoking** (Mak, 2000; Kunzle, 2003; Potts, 1999, Sun, 1993)
- **Leukocytospermia** (de Lamirande, 1993; Zini, 1995, Twigg, 1998; Iwasaki, 1992; Zini, 1993)
- **Apoptosis** (Baccetti, 1996; Sakkas, 2003)
- **Lack of protamine** (Cho, 2003)
- **Testicular hyperthermia** (Sailer J 1997,Banks Reproduction 2005)

Sperm DNA packaging

- Binding of protamine (P1 i P2) on DNA replaces all other proteines (histones)
- Formation of ring structures (each consists of 50 Kbp DNA)
- Each spermatozoa has approximately 50 000 ring structures



Balhorn, J Cell Biol 1982

Etiology of sperm DNA damage

Intratesticular

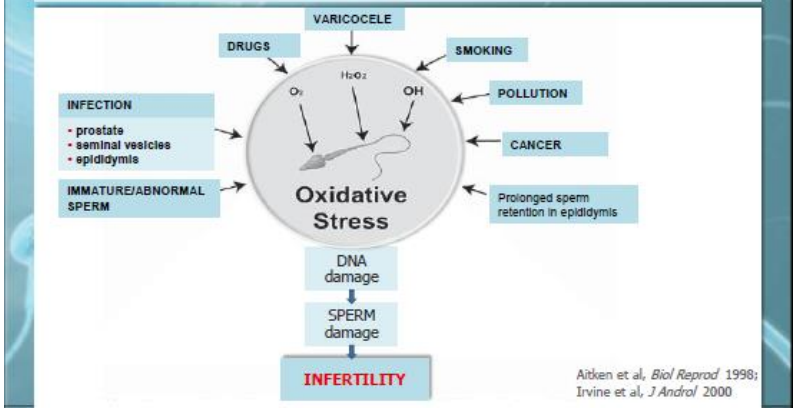
Posttesticular

Intratesticular

- Lack of protamine - a significant cause of sperm DNA damage (found in 5-15% of infertile men)
  De Yebra et al, J Biol Chem 1993; Bianchi et al, Biol Reprod 1993; Kramer et al, Genet Test 1997, Cho, 2003
- Mutations in genes coding for protamine synthesis
- Impaired topoisomeraze II and transition proteins may cause DNA damage
  Balhorn, J Cell Biol 1982; Kierzenbaum, Mol Reprod Dev 2001; Bissoneault, FEBS Lett 2002
- Apoptosis
  Sakkas et al, Exp Cell Res 1999, Sakkas et al. Reprod Biomed Online 2003

Posttesticular

- Reactive oxygen species (ROS) ⇧ in semen of 25% infertile men



Aitken et al, *Biol Reprod* 1998; Irvine et al, *J Androl* 2000

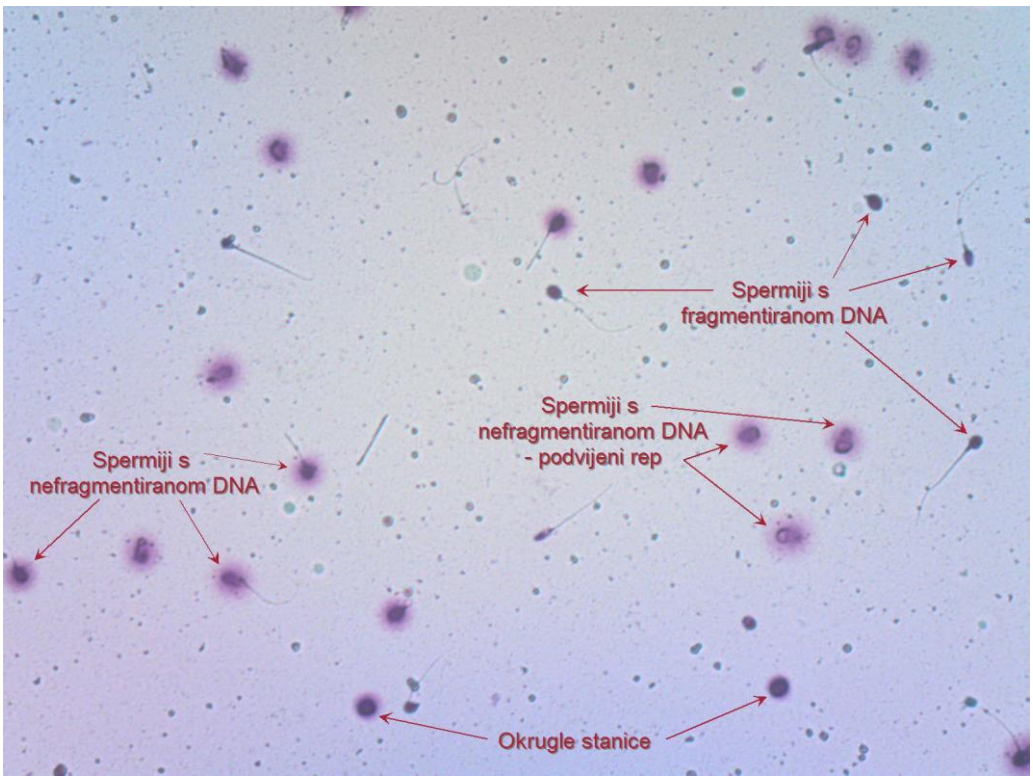
Why investigate sperm DNA integrity?

- Need for better marker of male fertility potential
- Improvement of ART arised issues of sperm quality importance

DNA fragmentation analysis....

....is recommended for

- Patients with more than 3 consecutive unsucessful IVF procedures
- In cases of embryo development arrest
- In cases of reccurent miscarriage
- In cases of idiopathic infertility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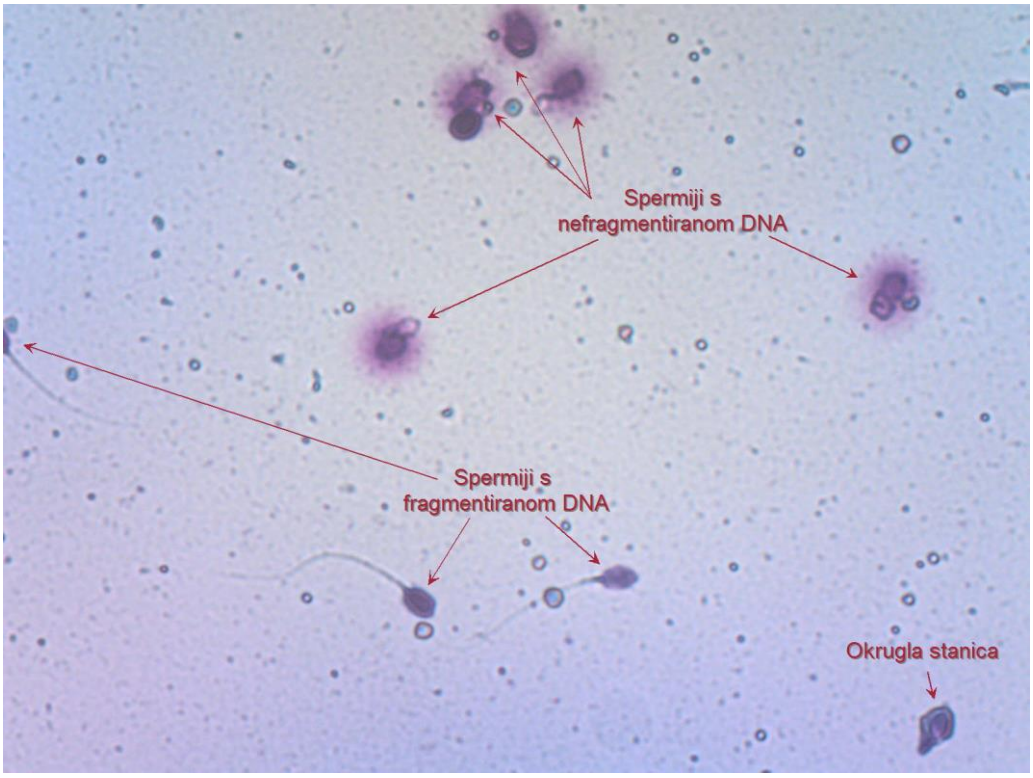
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Alternative techniques for determining the integrity of sperm DNA (SCSA, TUNNEL, ISNT, comet assay) are quite labour-intensive and expensive, which prevents their inclusion in the standard spermiogram used in clinical praxis

PROCEDURE:

Sperm DNA fragmentation can be determined using Halosperm® Sperm DNA Analysis Kit, which utilizes sperm chromatin dispersion (SCD) test (Fernández i sur., 2005) for detection of spermatozoa with poor quality DNA.

1) Prepare adequate sperm concentrations of 5-10 x 10^6/mL using sperm cultivation medium
2) Warm the Lysis Solution (LS) at the room temperature
3) Place the eppendorf tube with agarose (ACS) into the float and incubate in a water bath at 95-100ºC for 5 min
4) Equilibrate the agarose in a water bath for another 5 min at +37ºC
5) Add 25 µL of sample in eppendorf tube with agarose and mix well. Place a drop of 20 µL of sperm suspension on a processed side of the microscope slide (SCS) and cover with coverslip
6) Slides should be kept horizontally during the entire process
7) Place the slide on a surface pre-cooled to +4°C, transfer into the fridge and leave for 5 min to allow for agarose to solidify
8) Prepare the denaturation solution by adding 80 µL of Acid Denaturation (AD) solution to 10 mL of distilled water
9) Remove the coverslips from the slides and place the slides horizontally into the denaturation solution (in 100 mm Petri plates) and incubate 7 min
10) Transfer the slides into next Petri plate and incubate in 10 mL of Lysis solution for 25 min
11) Transfer the slides into next Petri plate and incubate in distilled water for 5 min
12) Incubate in Petri plates with 70%, 90% i 100% ethanol successively for 2 min in each concentration
13) Leave to dry at room temperature
14) Staining: place the slide into Petri dish and incubate in 5 mL of May-Grünwald dye for 6 min, wash with distilled water and transfer in next Petri dish, incubate in 10% Giemsa dye (4.5 mL deH2O + 0.5 mL Giemsa). Wash the dye under tap water and leave vertically to dry at room temperature
15) Examine the slides under microscope and count the spermatozoa
16) Classification:
    - spermatozoa without DNA fragmentation (big halo aura - equal to or bigger than 1/3 of the smaller core diameter, or medium halo aura – sized between the bih and small halo aura)
    - spermatozoa with DNA fragmentation (small halo aura – similar to or smaller than 1/3 of the smaller core diameter, without halo aura, or without halo aura and degraded – irregularly and weekly stained core present, should not be counted)
17) Number of fragmented sperm is reported as % SDF ([number of sperm with fragmented DNA / total sperm count] x 100)