

2ème Journée Ecotoxicologie en Milieux Aquatiques Méditerranéens & Santé Humaine
Biomarqueurs et bioindicateurs en environnement et en santé humaine
29 novembre 2010

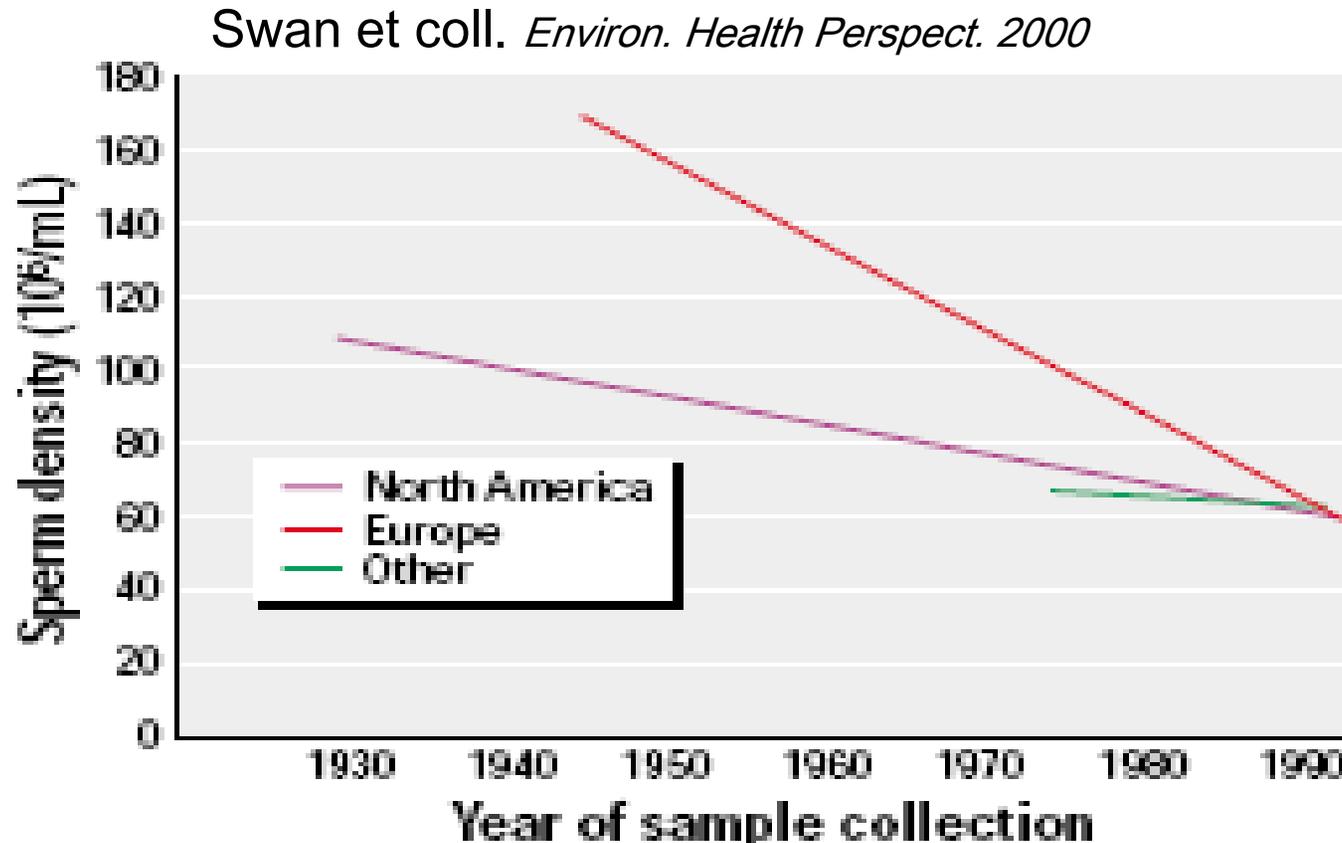
Biomarqueurs de génotoxicité germinale chez l'Homme

Jeanne Perrin

Biogénotoxicologie & Mutagenèse Environnementale
(EA 1784 – FR CNRS ECCOREV 3098)

Reprotoxicologie

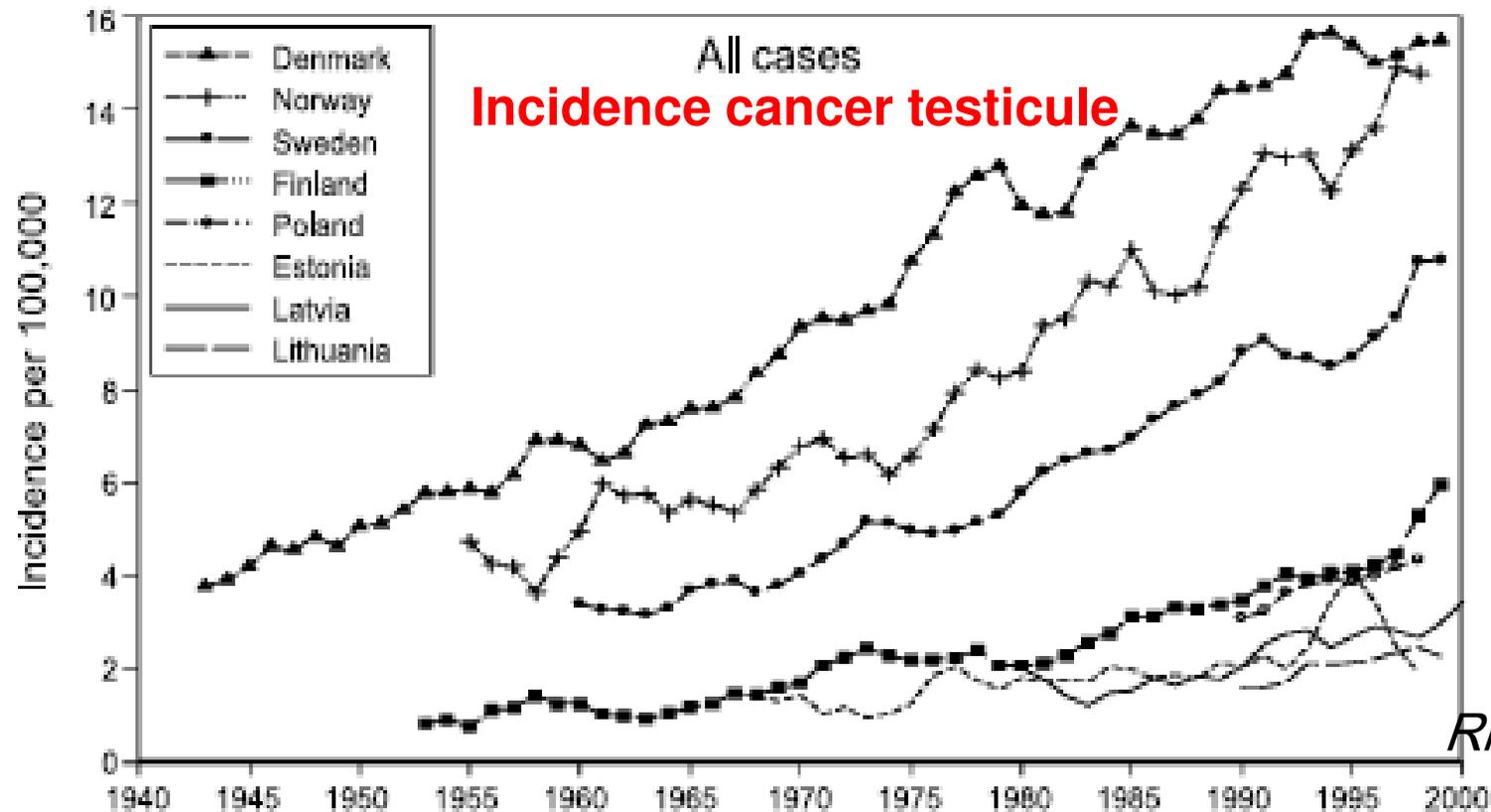
Carlsen et coll. *Br. Med. J.* 1992 : Diminution significative de la concentration du sperme chez les hommes fertiles.



- **15%** des couples consultent pour infertilité (*Juul et coll. 1999*)
- paramètres spermatiques moyens proches de la limite inf. de normalité (*Andersen et coll. 2000 ; Carlsen et coll. 2005*)

Reprotoxicologie

Andersen et coll. 2000 : 21% ♂ jeunes < 20 Millions spz/ml



N. Skakkebaek et coll. 2006 : variations rapides liées à

- mode de vie
- expositions environnementale (perturbateurs endocriniens)

Spermatogenèse : rappels...

Coupe de tube séminifère

Sperma

Prostate

Vésicule
séminal

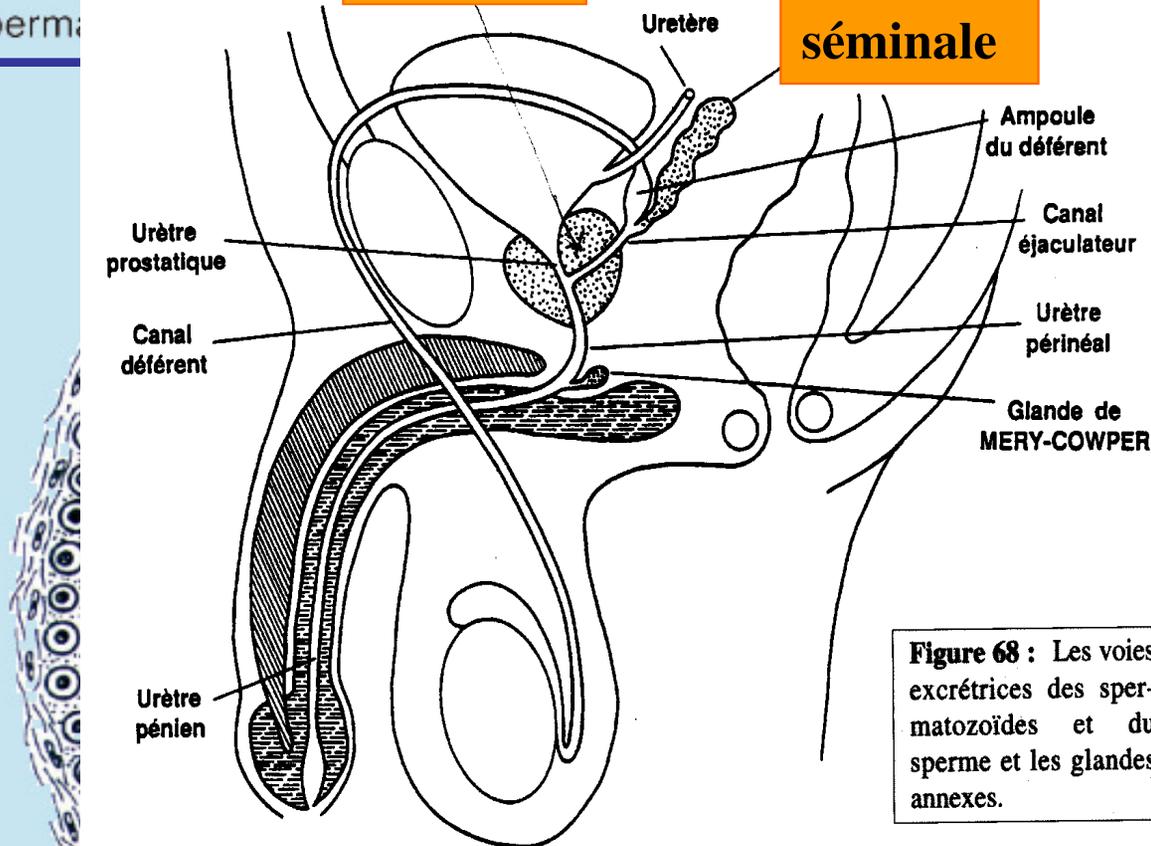


Figure 68 : Les voies excrétoires des spermatozoïdes et du sperme et les glandes annexes.

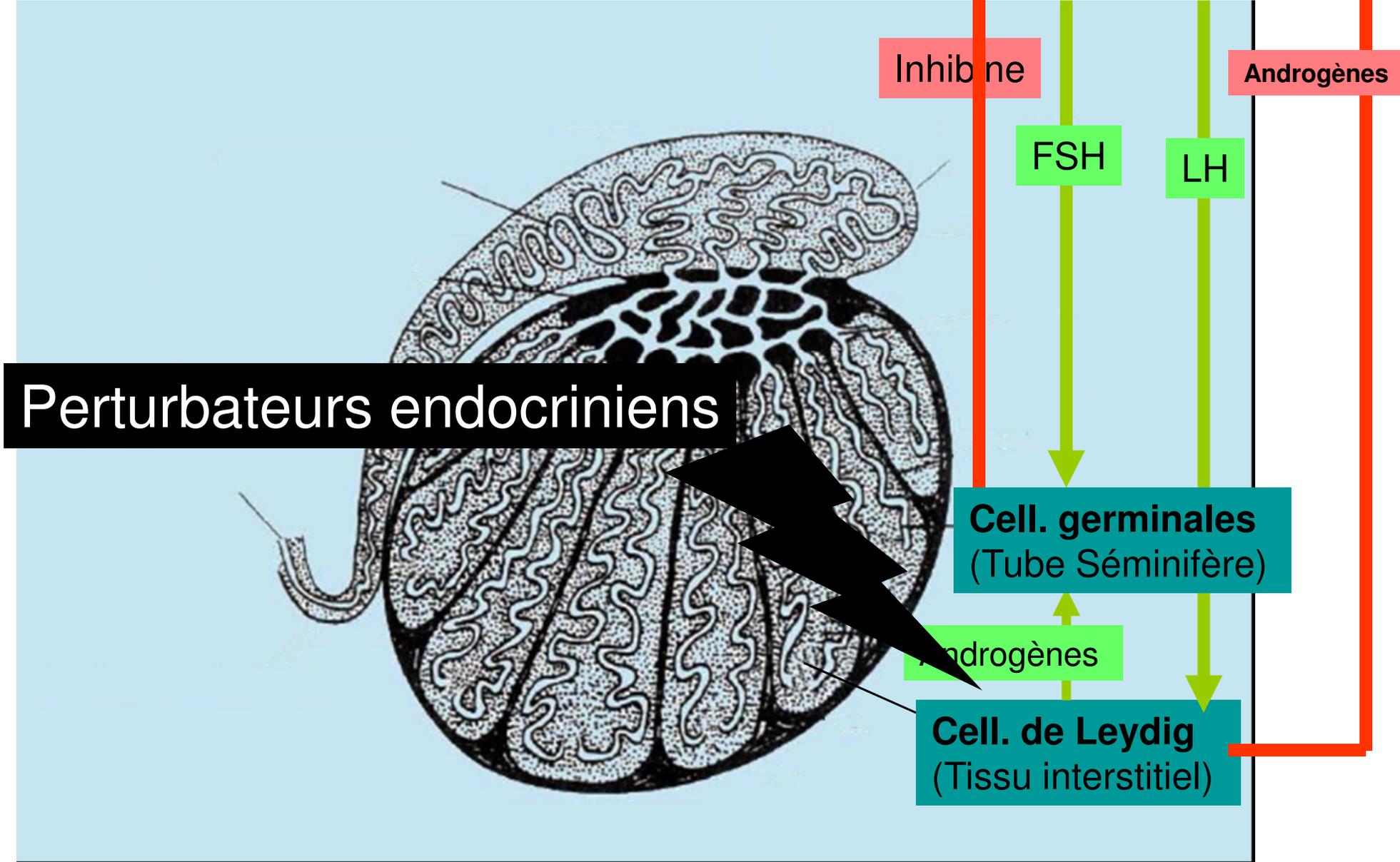
D'après Czyba 1993

ère

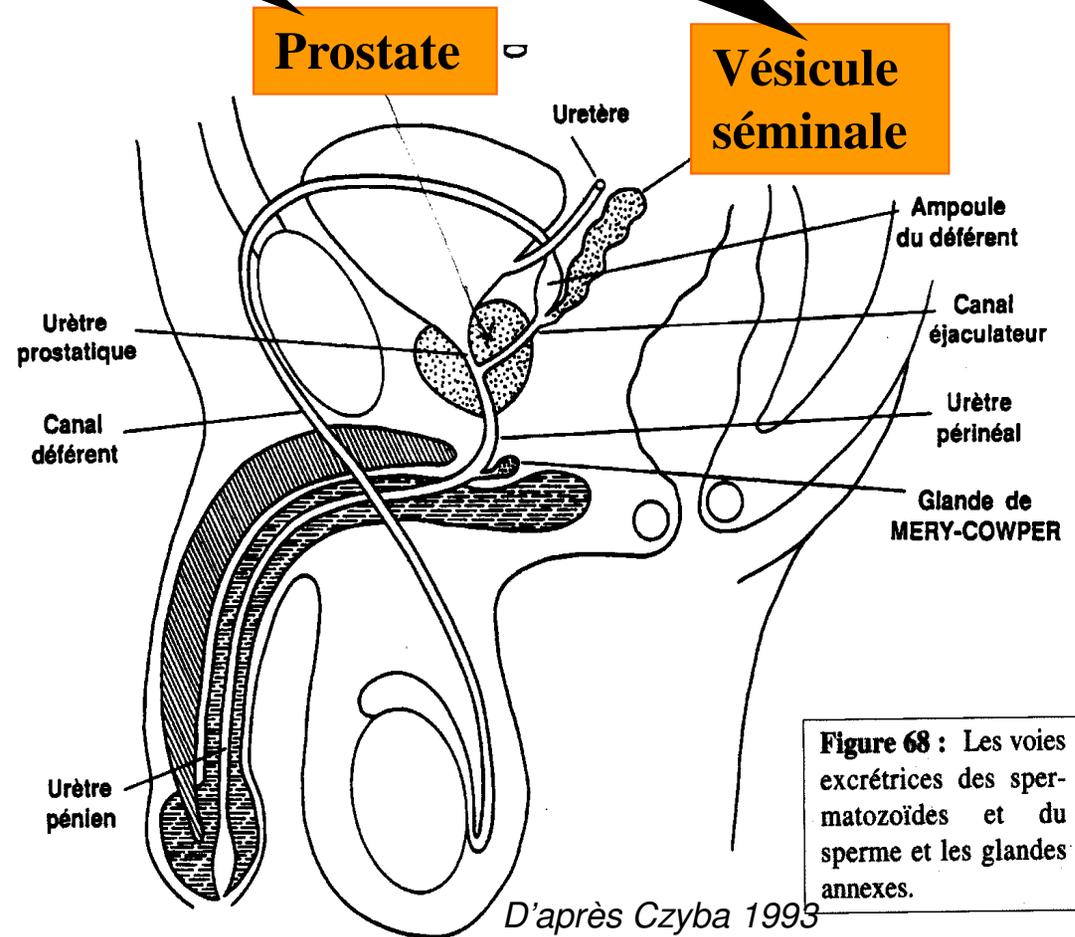
Spermatocytes I

Noyau de cellule
de Sertoli

Contrôle hormonal : axe gonadotrope

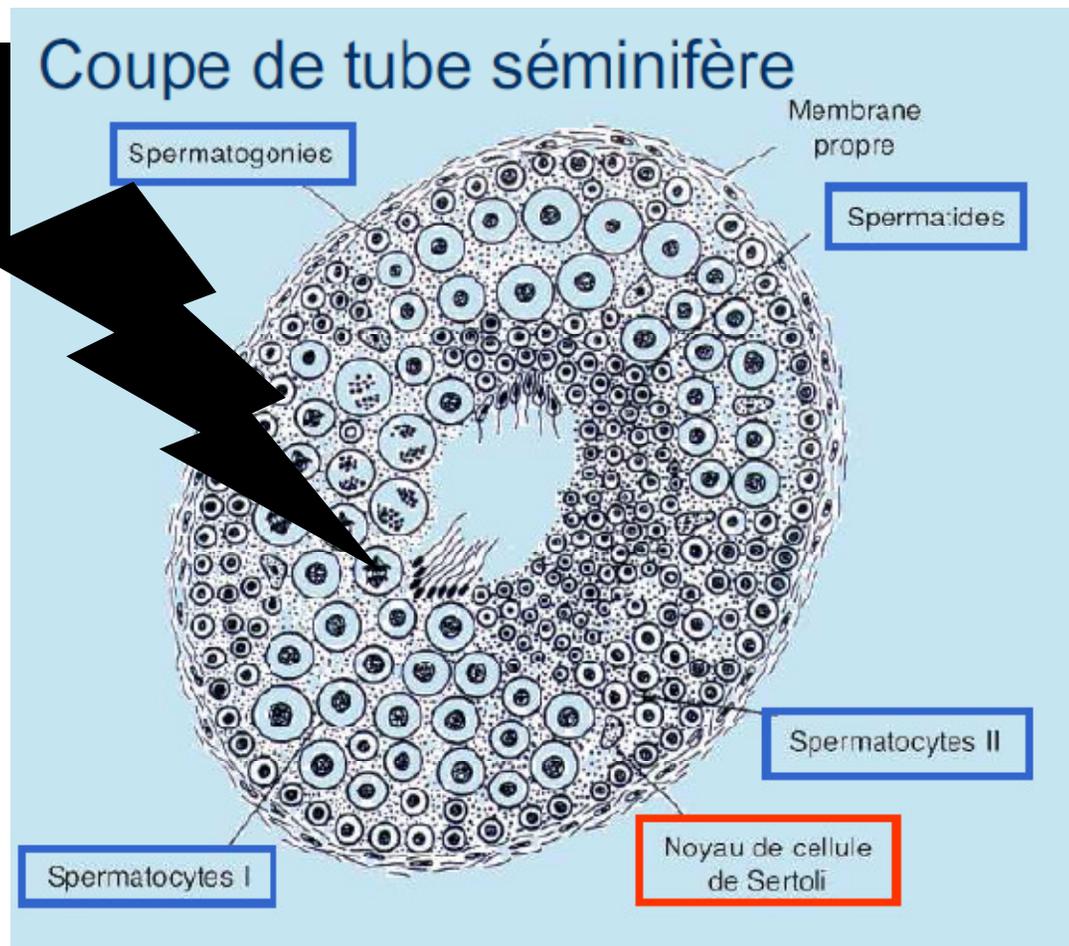


liquide séminal



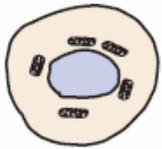
- Stress oxydant
- Toxiques chimiques
- Altération mobilité des spz

Altération de la spermatogenèse :

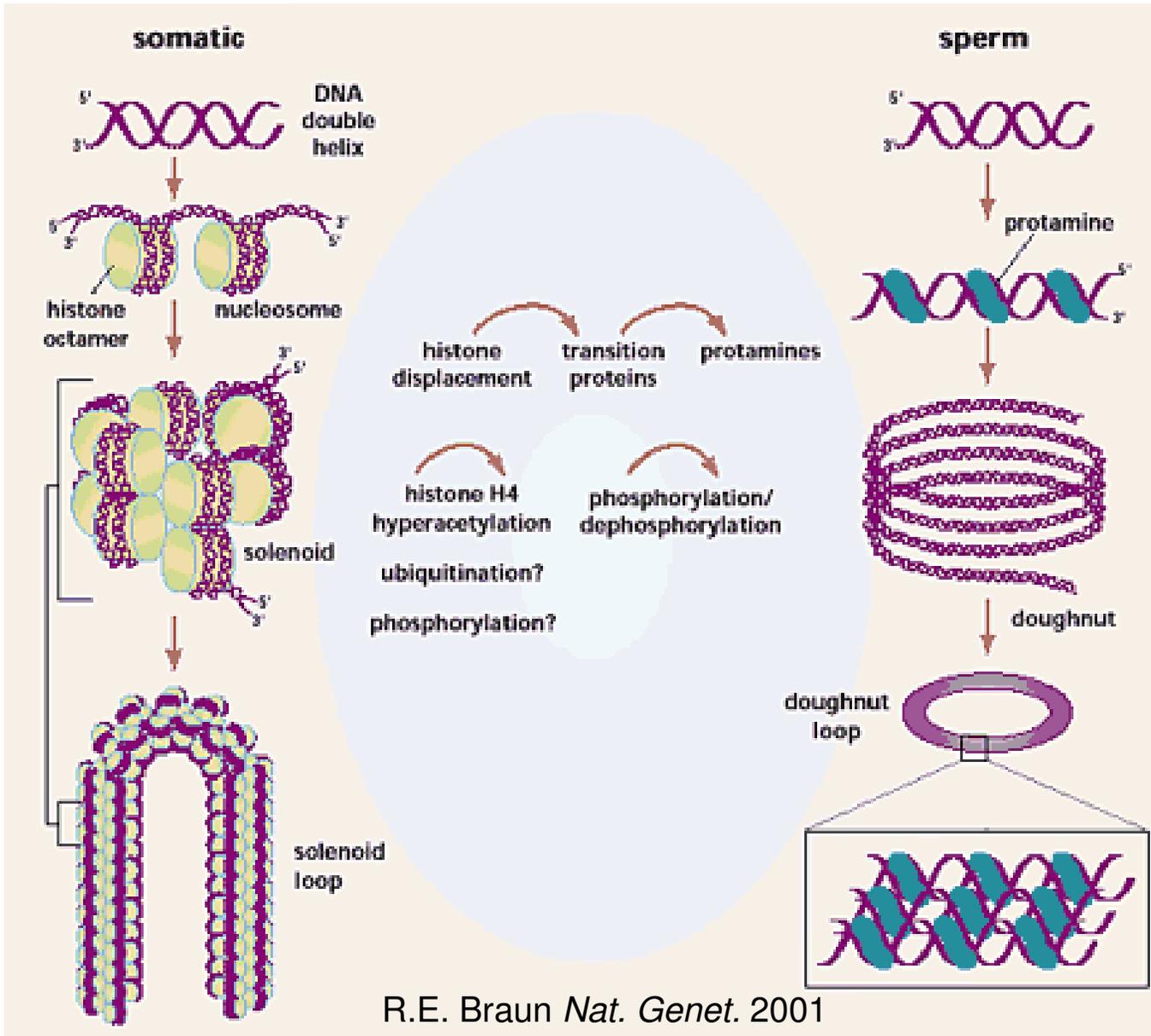


→ Altération des spermatozoïdes :

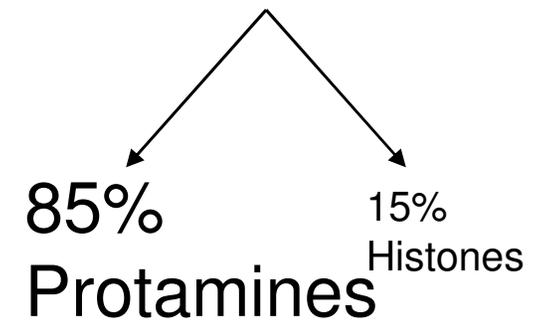
- Baisse concentration
- Altération morphologie
- Altération stabilité de la chromatine
- Dommages à l'ADN



Maturation nucléaire

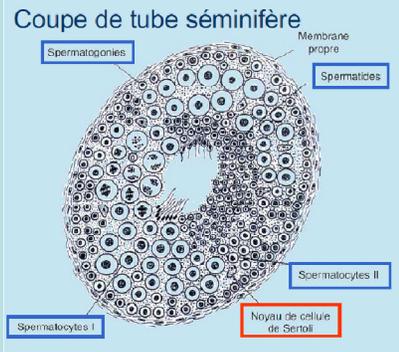


Compaction de la chromatine



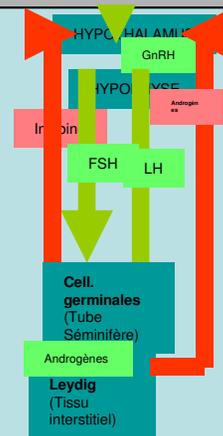
Indicateurs de santé reproductive

- Concentration spz
- Morphologie spz



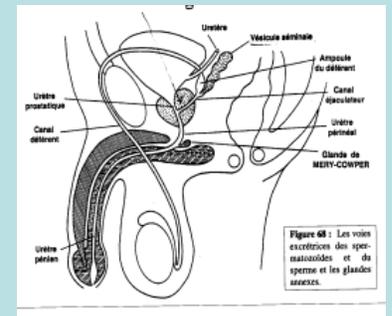
SPERMATOGENESE

Taux hormonaux sériques :
FSH, LH, Inh. B, Sex Ho bind
Prot, Testostérone...



REGULATION HORMONALE

- Volume éjaculât
- Marqueurs biochimiques
- Zinc
- Mobilité spz



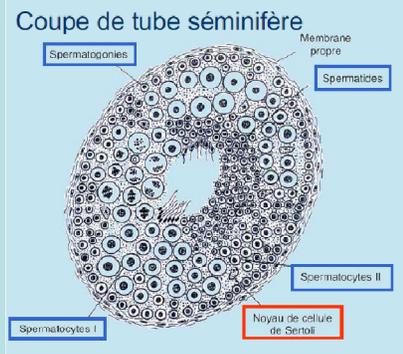
TRACTUS SEMINAL

FERTILITE

- Délai nécessaire à concevoir
- Probabilité de concevoir en un temps donné
- Fécondabilité

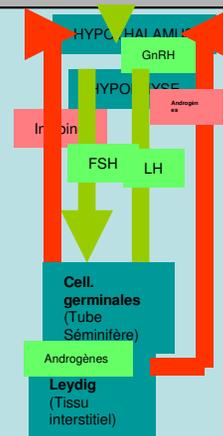
Indicateurs de santé reproductive ♂

- Concentration spz
- Morphologie spz
- **Intégrité ADN**
- **Intégrité chromatine**



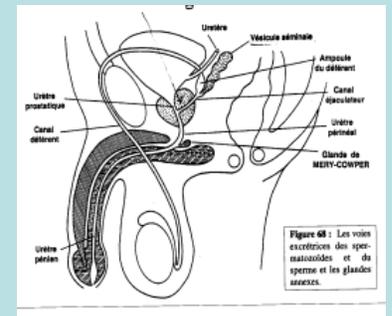
SPERMATOGENESE

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REGULATION HORMONALE

- Volume éjaculât
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- Mobilité spz
- **Intégrité ADN**
- **Intégrité chromatine**



TRACTUS SEMINAL

FERTILITE

- Délai nécessaire à concevoir
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Évaluation des dommages à l'ADN ds la cellule germinale

- **Pourquoi ?**

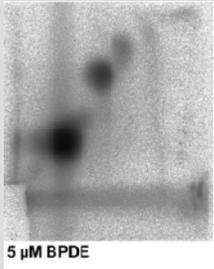
- *Sun et coll. 1997* : augmentation de l'altération de la chromatine des spz quand altération sévère des paramètres spermatiques
- *Spano et coll 2000* ; *Larson et coll. 2000* : impact négatif des dommages à l'ADN des spz sur la fertilité
 - naturelle
 - après Assistance Médicale à la Procréation (AMP)

- **Comment ?**

- Lésions primaires de l'ADN
- Anomalies du contenu chromosomique
- Qualité de la chromatine

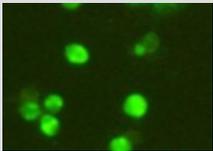
Mesure des dommages à l'ADN du spz

DNA adducts



32P POST LABELLING

TANDEM MASS
SPECTROMETRY/HPLC



IMMUNOLABELLING

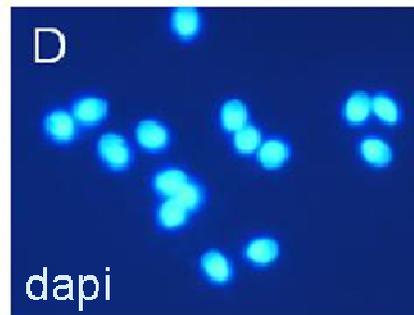
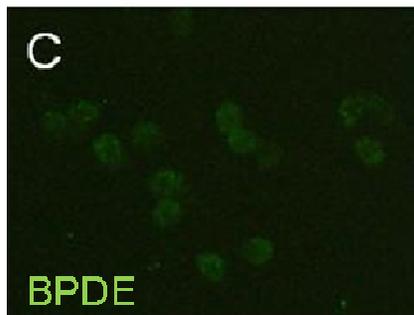
DNA adducts

Sperm DNA

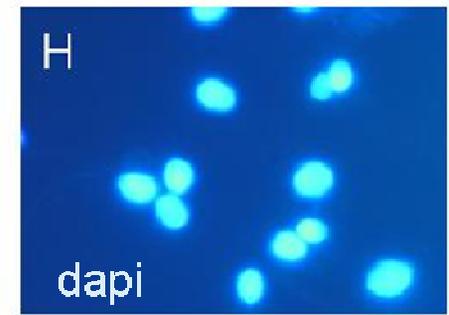
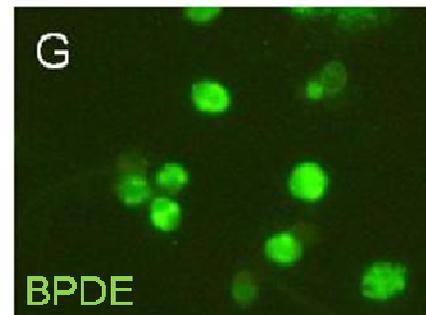


Adduits à l'ADN des spz

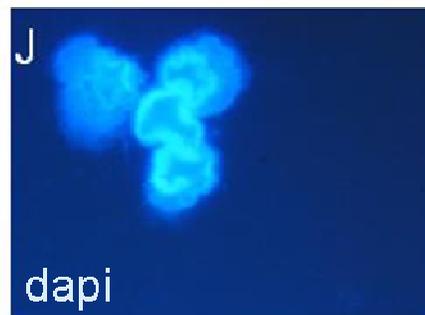
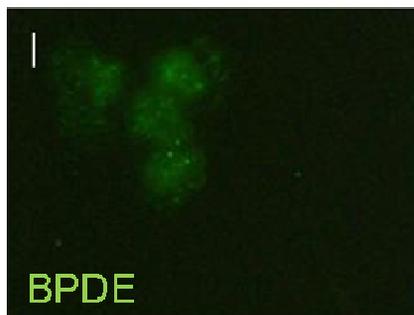
NON FUMEURS



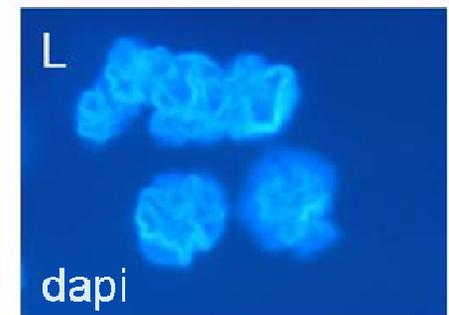
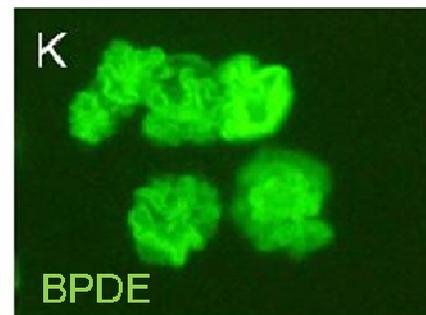
FUMEURS



CONTROLE NEGATIF



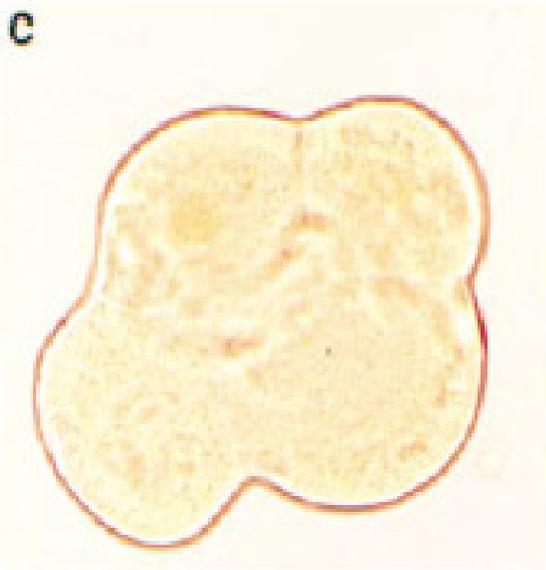
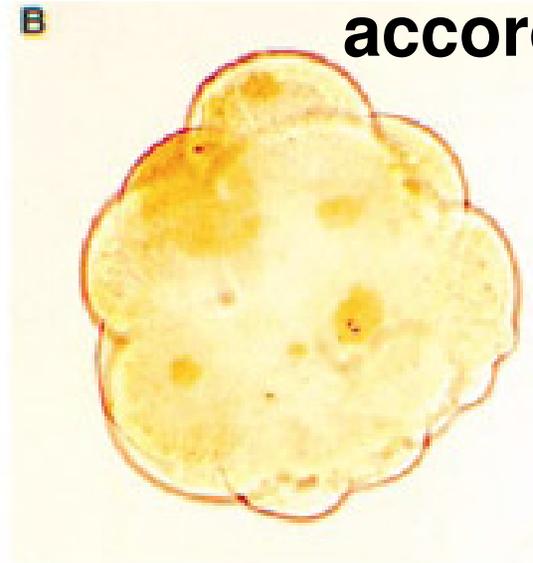
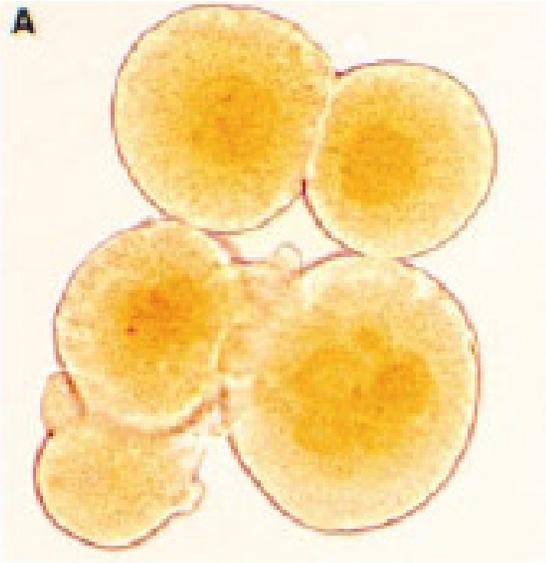
CONTROLE POSITIF



Perrin et coll. unpublished

Adduits à l'ADN des embryons

Immunostaining of BPDE DNA adducts in embryos according to parental smoking.

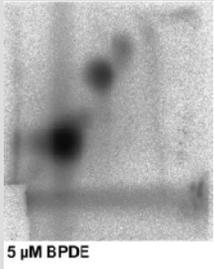


(A) both parents smoke
(B) only father smokes

(C) neither parent smokes
(D) negative control

Mesure des dommages à l'ADN du spz

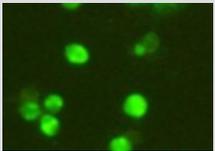
DNA adducts



5 μ M BPDE

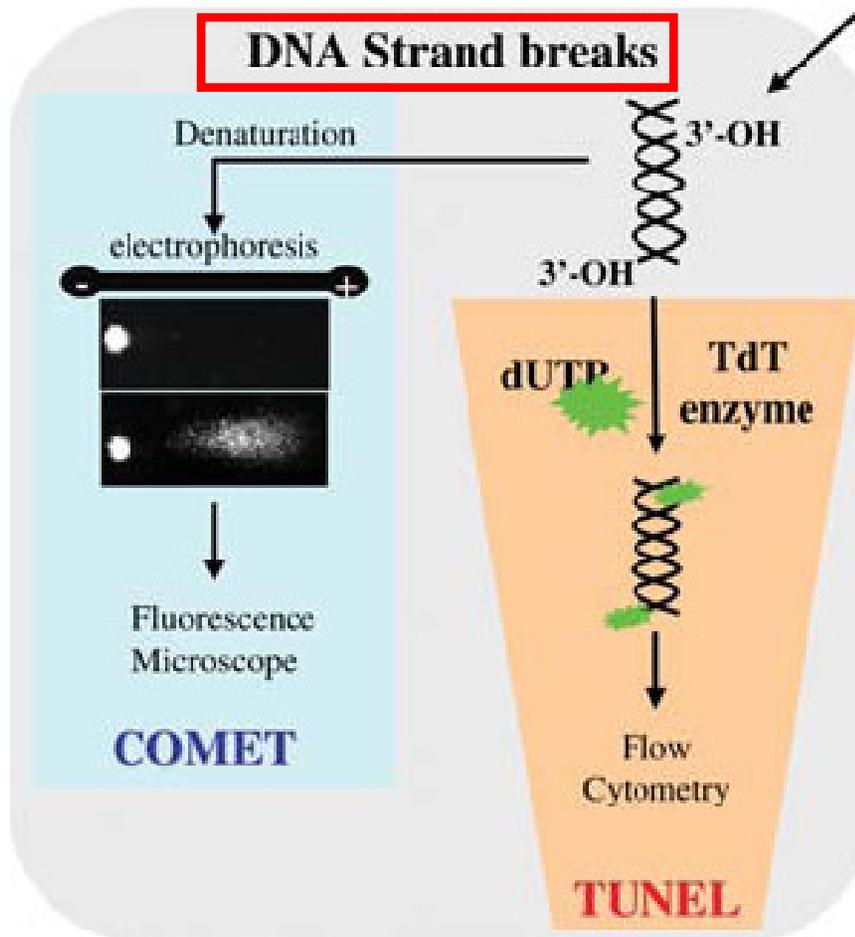
32 P POST LABELLING

TANDEM MASS SPECTROMETRY/HPLC



IMMUNOLABELLING

DNA Strand breaks



DNA adducts

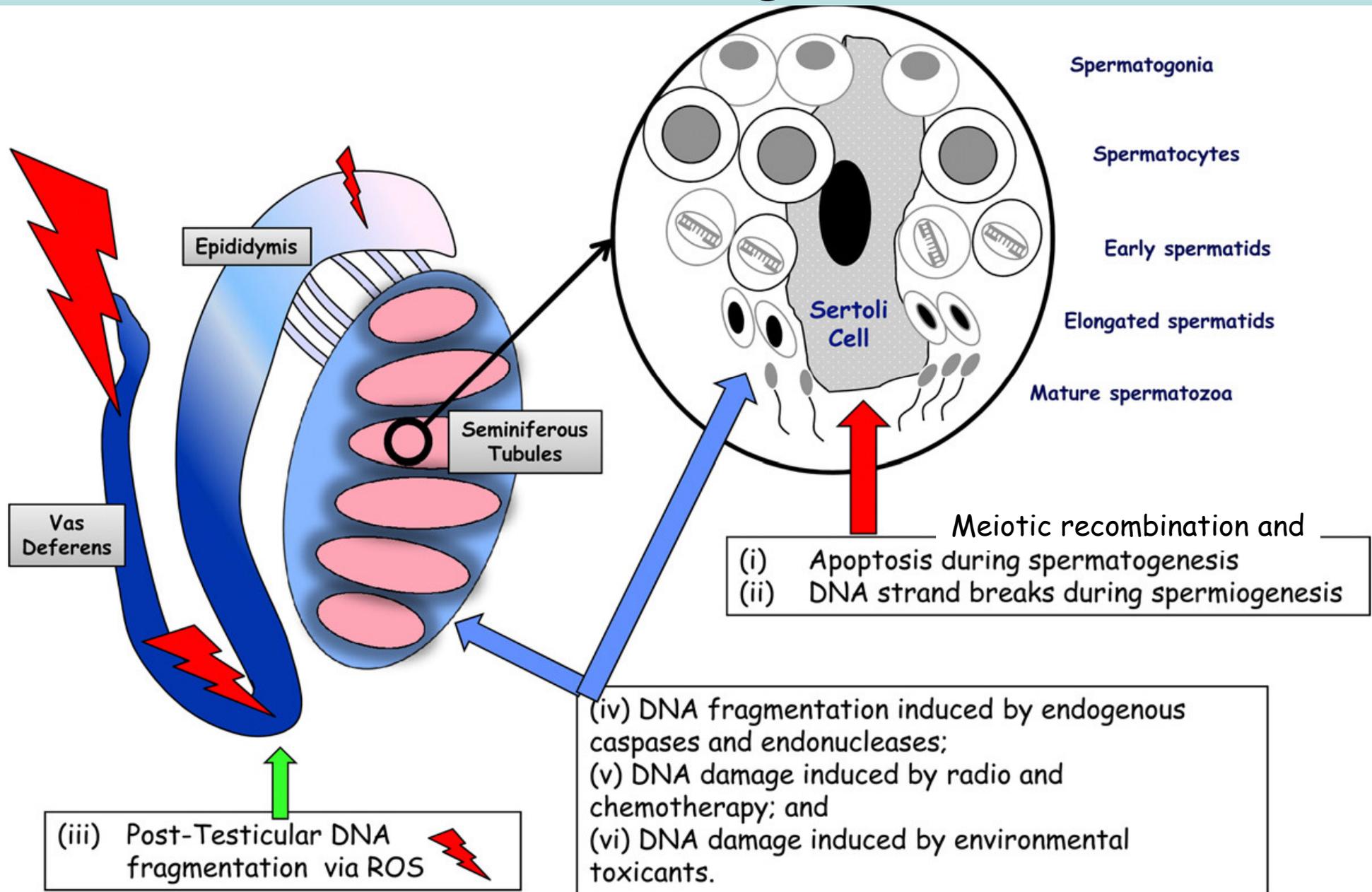
DNA breaks

Sperm DNA



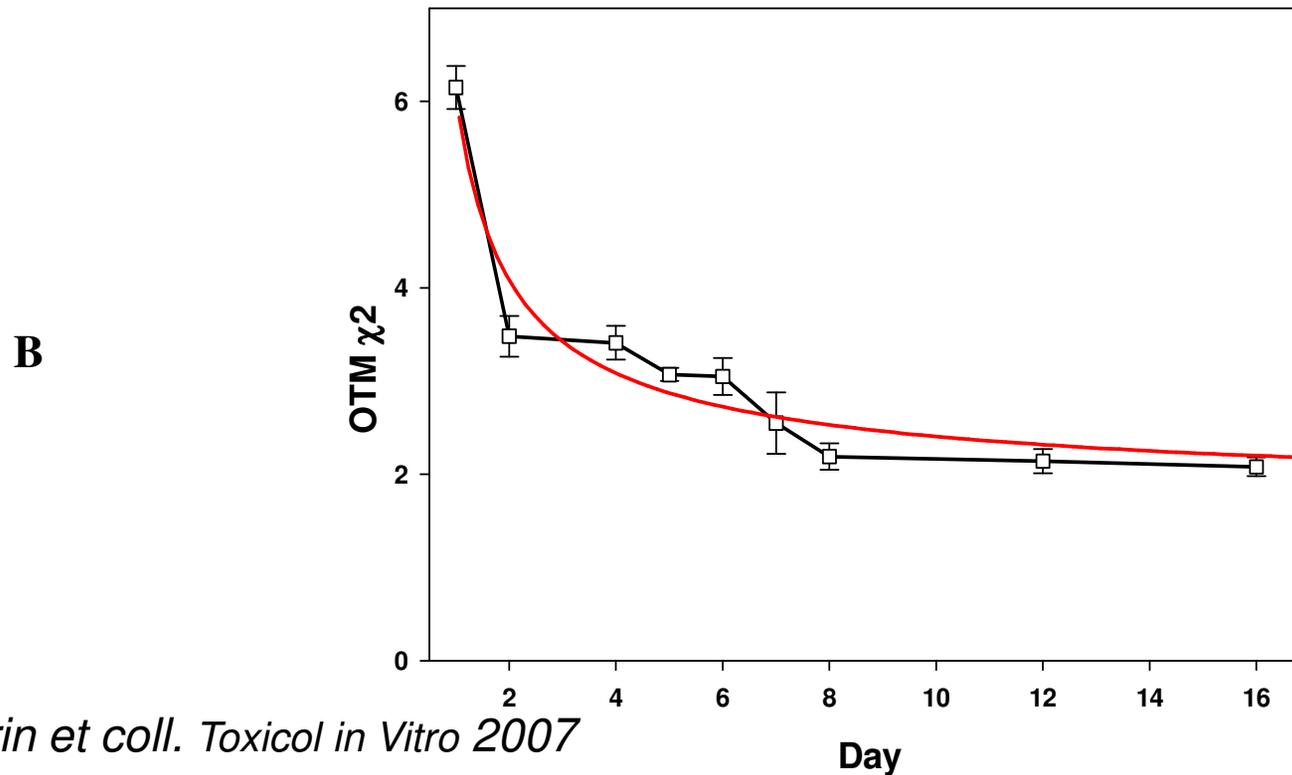
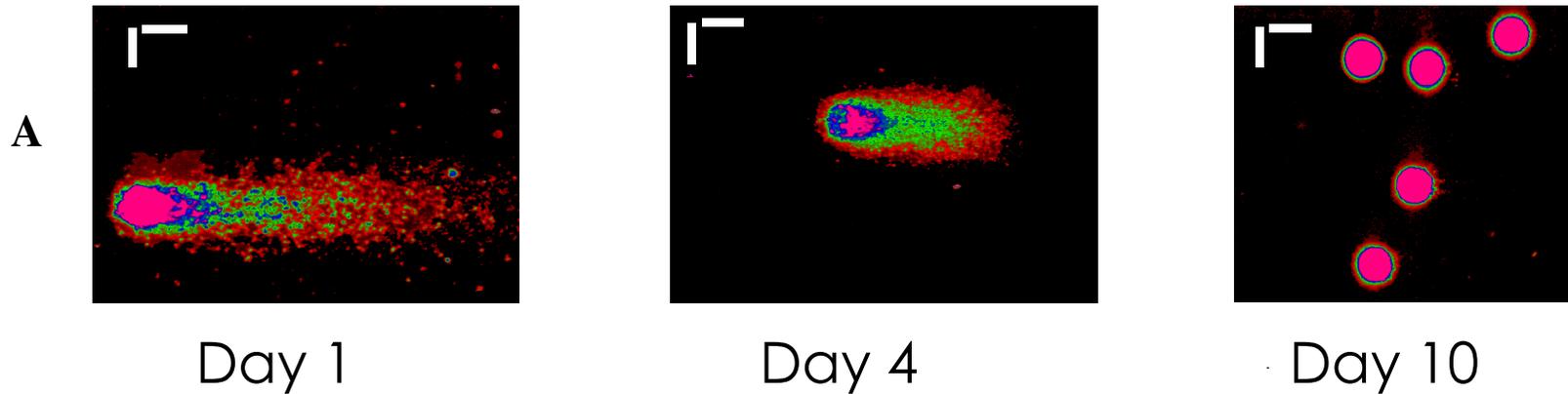
Adapted from Delbes et coll. 2010

Principaux mécanismes de fragmentation de l'ADN

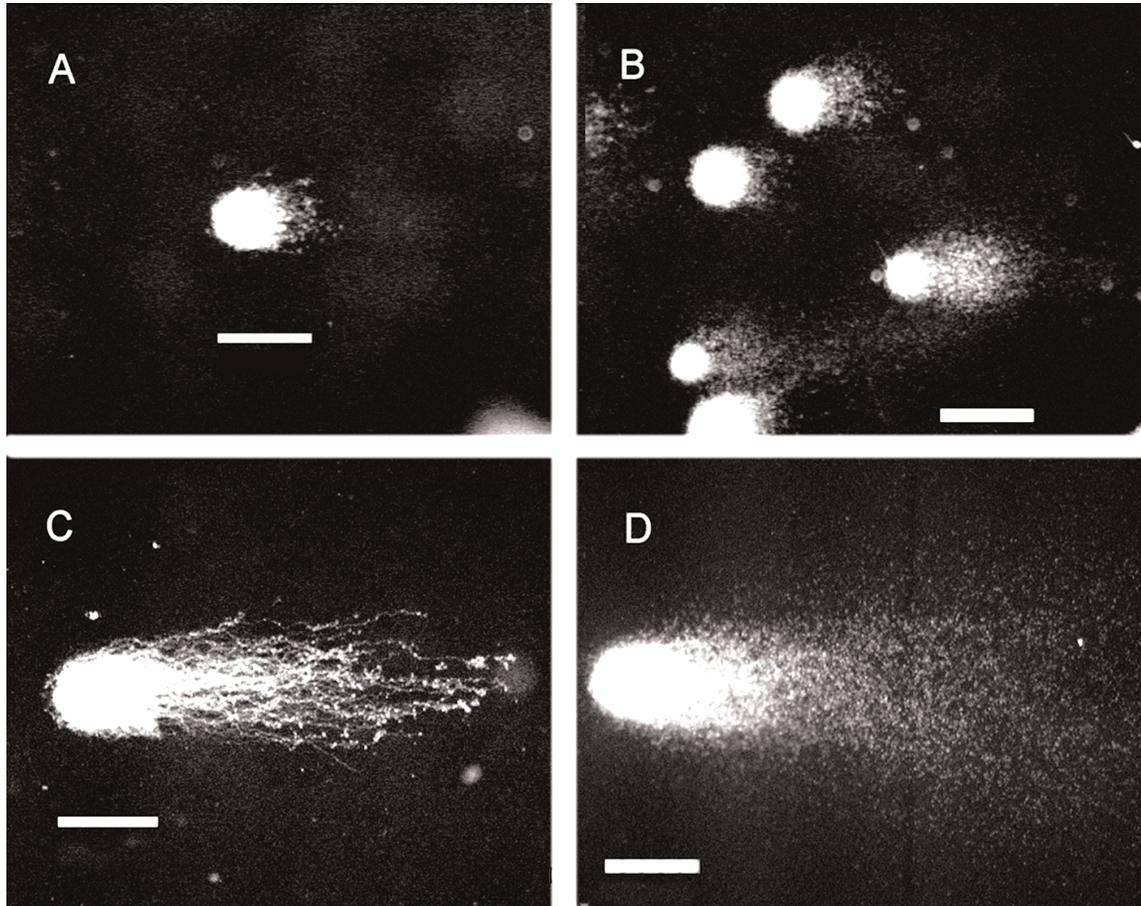


Adapted from Sakkas and Alvarez 2010.

Test des comètes sur cellules germinales en culture in vitro



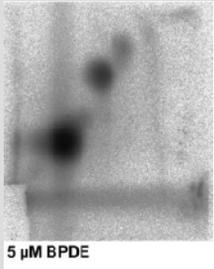
Test des comètes sur spz



Duty et coll. 2003 Phthalates and DNA damage in human sperm

Mesure des dommages à l'ADN du spz

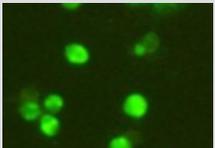
DNA adducts



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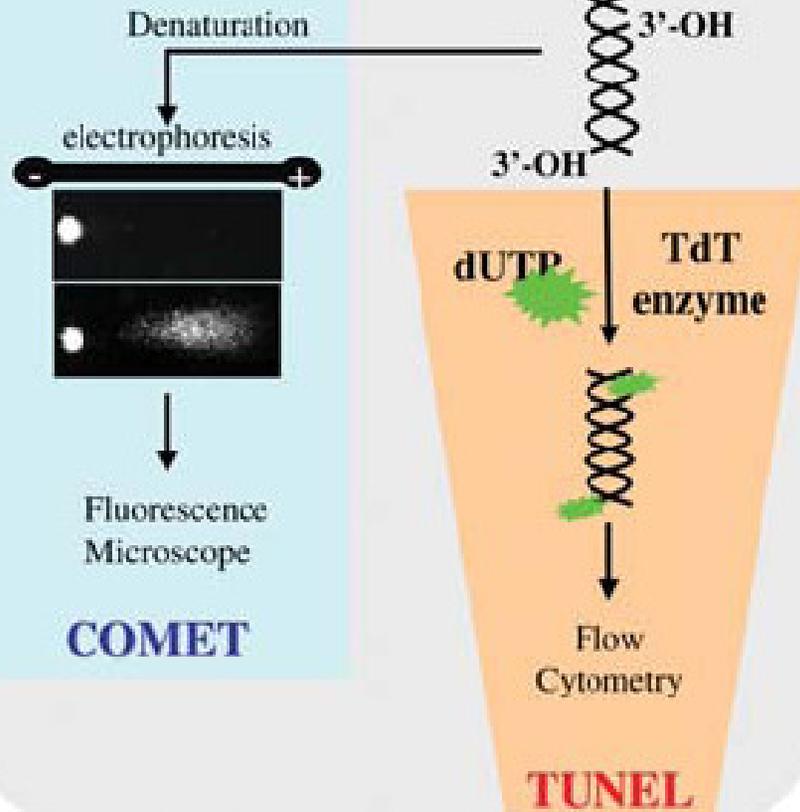
32P POST LABELLING

TANDEM MASS SPECTROMETRY/HPLC



IMMUNOLABELLING

DNA Strand breaks



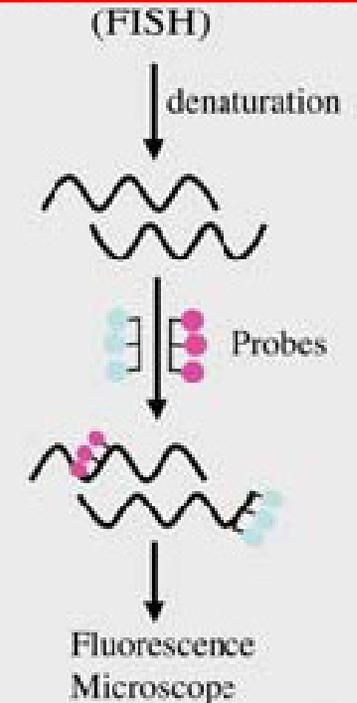
DNA adducts

DNA breaks

Sperm DNA



Chromosome Aberrations (FISH)



Adapted from Delbes et coll. 2010

Anomalies chromosomiques des spz

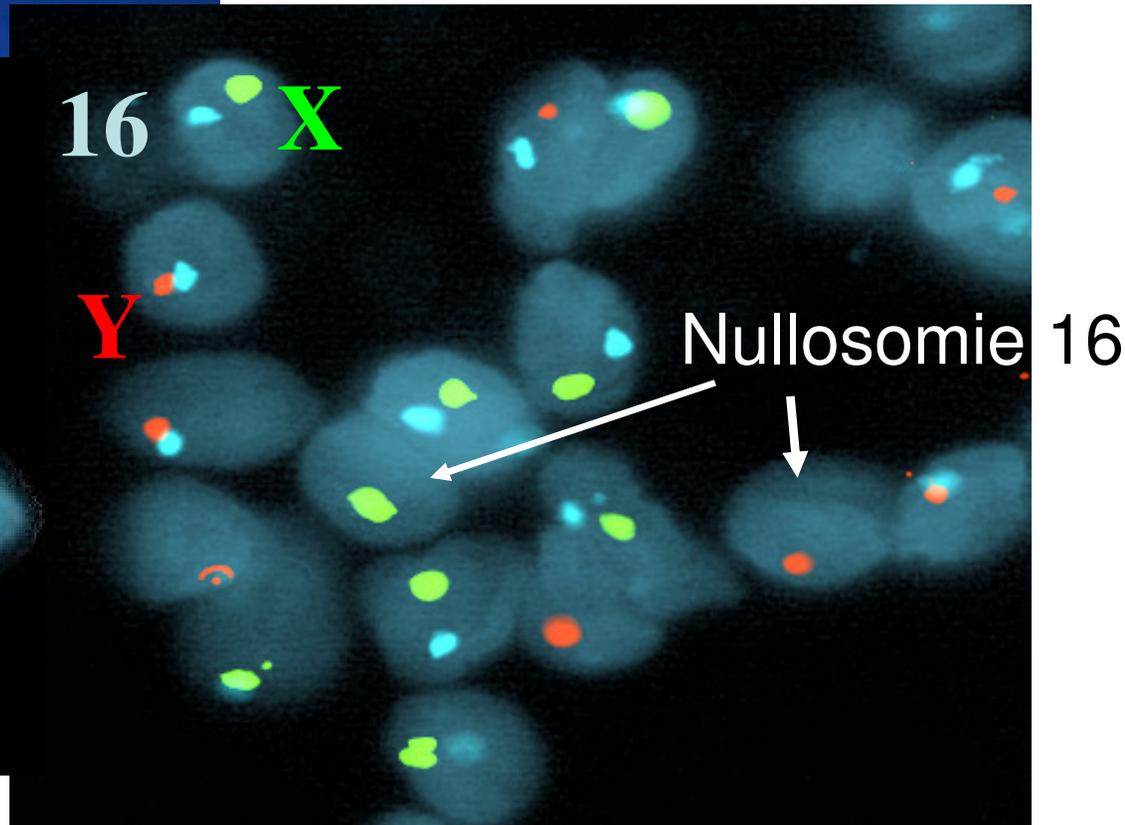
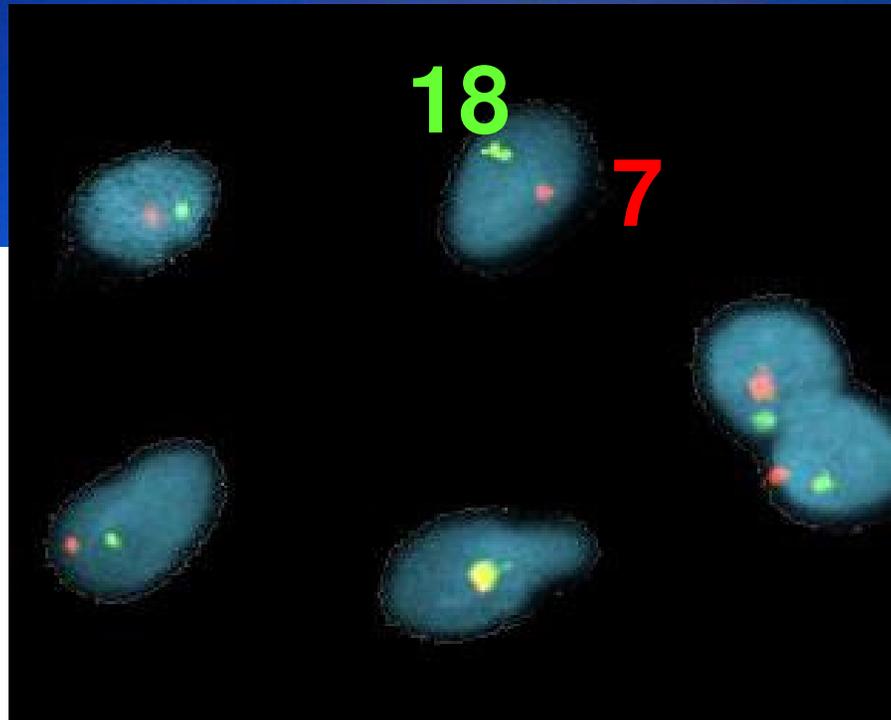
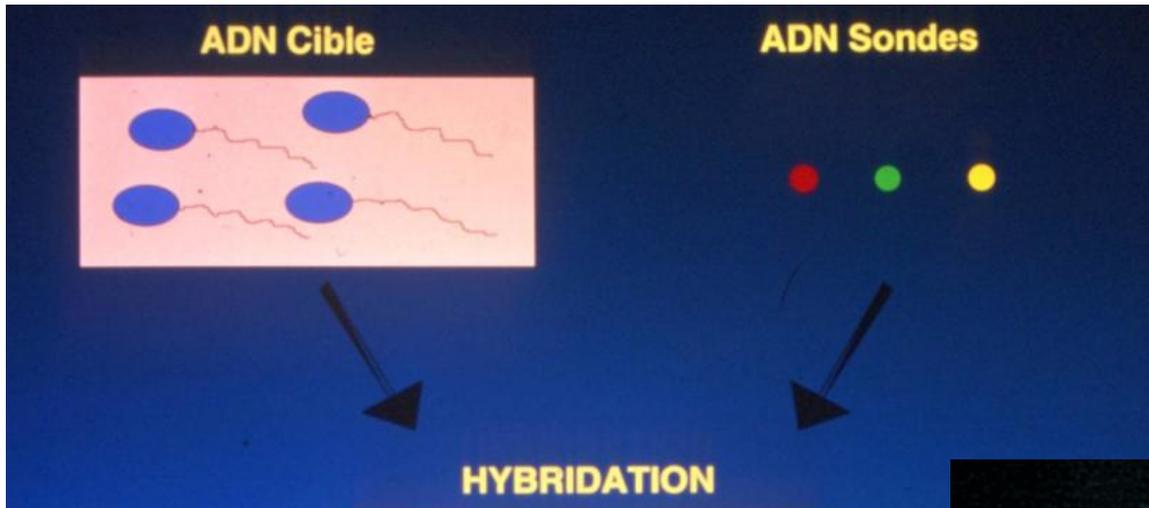


Table II. Disomy and diploidy frequencies in post cisplatin, etoposide and bleomycin chemotherapy (PEB-CT) patients and in controls

%	Post PEB-CT patients						Controls					
	T	P	S	G	B	Mean (%)	Mean (%)	1	2	3	4	5
disomy 7	0.10	0.05	0.04	0.11	0.05	0.07	0.044	0.09	0.03	0.02	0.05	0.03
disomy 16	0.10	0.10	0.08	0.09	0.08	0.09 ^a	0.046	0.04	0.04	0.06	0.03	0.06
disomy 18	0.06	0.04	0.03	0.06	0.03	0.044 ^{**}	0.014	0.01	0.00	0.01	0.02	0.03
disomy X	0.02	0.02	0.03	0.03	0.04	0.028	0.030	0.01	0.05	0.04	0.03	0.02
disomy Y	0.01	0.04	0.02	0.07	0.02	0.032	0.008	0.00	0.00	0.01	0.01	0.02
disomy XY	0.14	0.13	0.15	0.27	0.24	0.186 ^{***}	0.073	0.07	0.08	0.07	0.05	0.09
Diploidy ^a	0.29	0.29	0.16	0.11	0.42	0.254 ^{***}	0.094	0.10	0.06	0.10	0.09	0.12
Diploidy ^b	0.49	0.24	0.16	0.20	0.28	0.274 ^{***}	0.080	0.07	0.04	0.06	0.13	0.10

Values are expressed as percentages.

^aChromosomes 7 and 16 two-colour FISH.

^bChromosomes X, Y and 18 three-colour FISH.

^{*} $P < 0.05$; ^{**} $P < 0.01$; ^{***} $P < 0.001$.

De Mas et coll. 2001

Aneuploid sperm formation in rainbow trout exposed to the environmental estrogen 17 α -ethynylestradiol

Kim H. Brown^{a,1}, Irvin R. Schultz^b, J. G. Cloud^a, and James J. Nagler^{a,2}

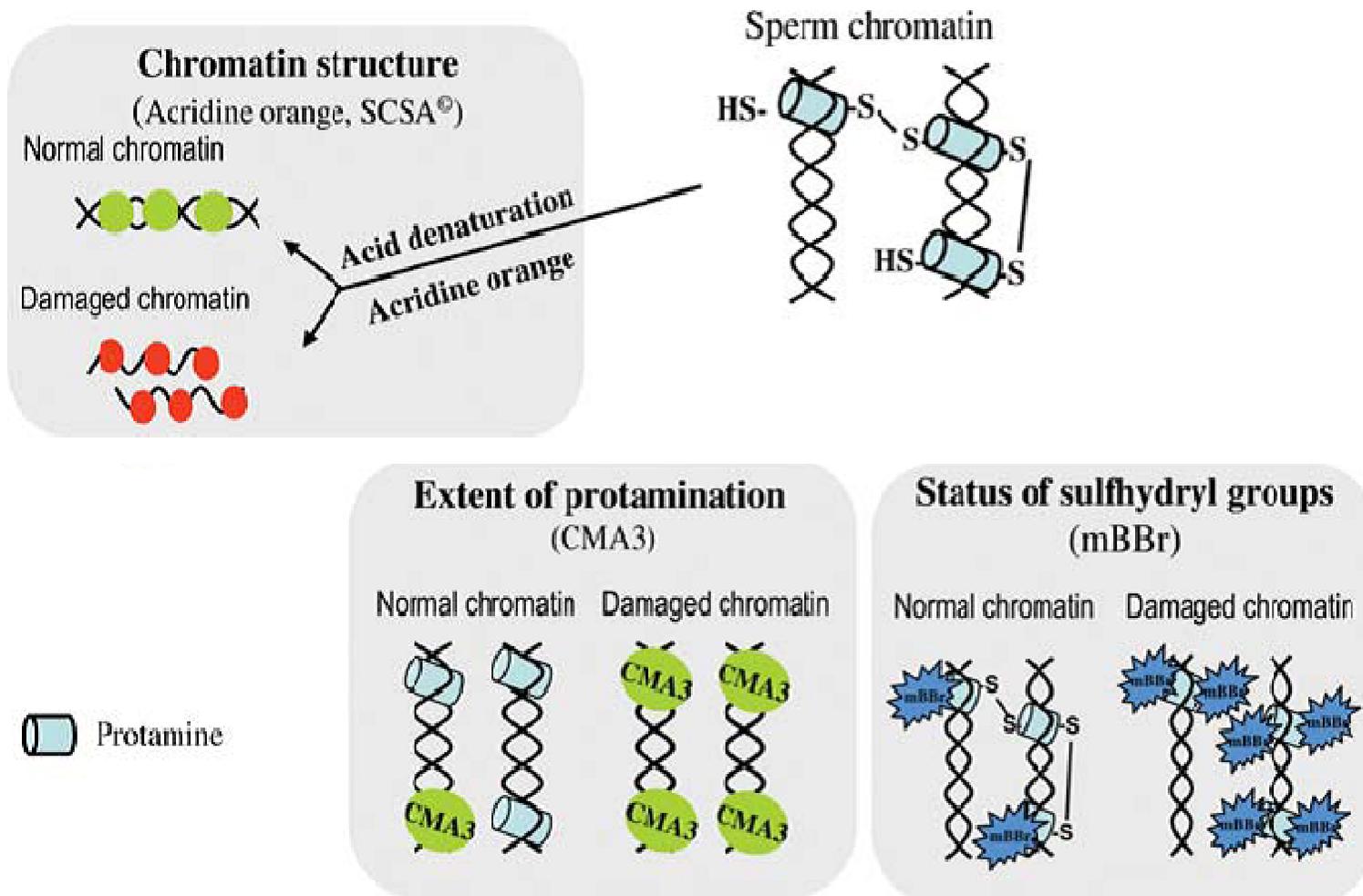
^aDepartment of Biological Sciences and Center for Reproductive Biology, University of Idaho, P.O. Box 443051, Moscow, ID 83844-3051; and ^bBattelle Pacific Northwest National Laboratory-Marine Science Laboratory, 1529 West Sequim Bay Road, Sequim, WA 98382

Edited by Neal L. First, Mississippi State University, Mississippi State, MS, and approved October 27, 2008 (received for review August 22, 2008)

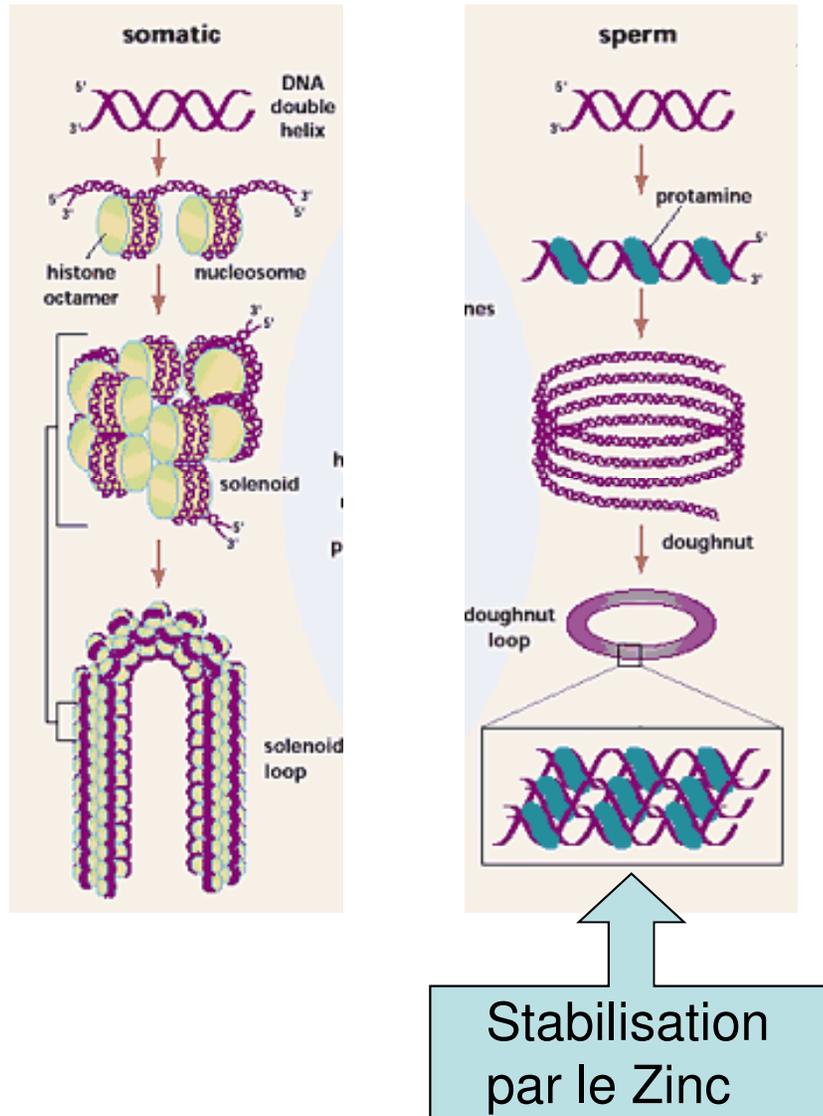
Environmental contaminants that mimic native estrogens (i.e., environmental estrogens) are known to significantly impact a wide range of vertebrate species and have been implicated as a cause

of reproductive failure in many species. Environmental estrogens have a wide variety of chemical structures but are grouped together based on their ability to mimic natural estrogens by interfering with (or binding directly to)

Qualité de la chromatine du spz



Qualité de la chromatine du spz : le Pb



Compétition avec le Zn →
altère la condensation de la
chromatine des spz



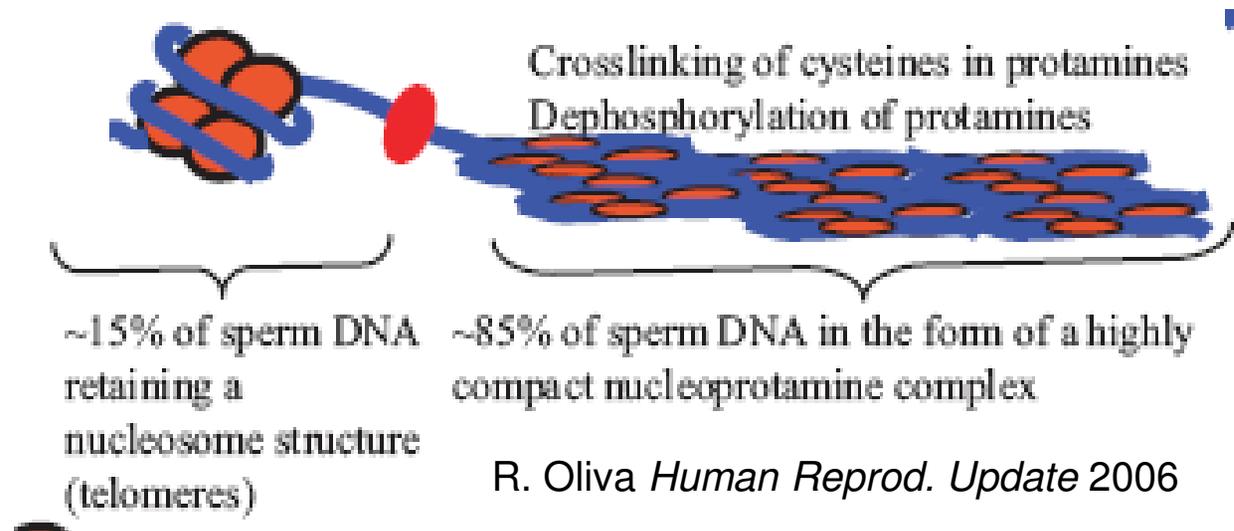
- Diminution de la fécondance
- Dommages à l'ADN des spz (stress oxydant)

Adapted from Braun Nat. Genet. 2001

Information épigénétique de l'ADN du spz

ADN du spz :

- information génétique
- information épigénétique



Structure hétérogène

Information épigénétique de l'ADN du spz

Nature. 2009 Jul 23;460(7254):473-8. Epub 2009 Jun 14.

Distinctive chromatin in human sperm packages genes for embryo development.

Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR.

Howard Hughes Medical Institute, Department of Oncological Sciences,
University of Utah School of Medicine, Salt Lake City, Utah 84112, USA

Au total...

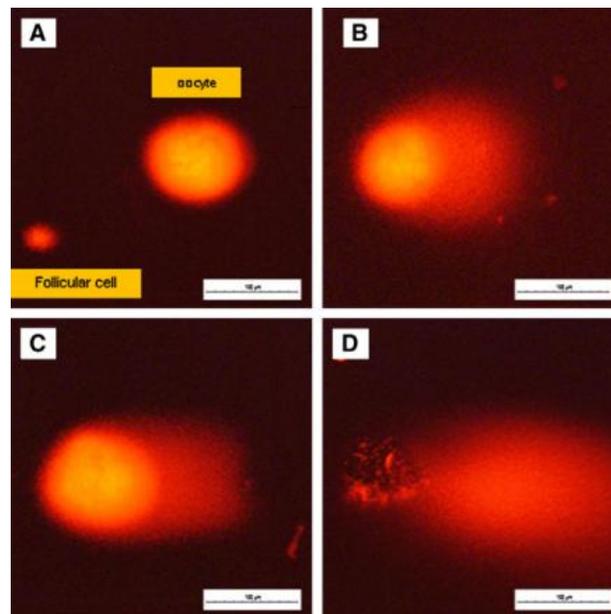
- Paramètres du sperme : spermatogenèse, mais pas qualité de l'ADN
- Nbx toxiques altèrent ADN sans effet visible sur paramètres spermatiques
- FECONDATION POSSIBLE :
 - Déséquilibre chromosomique
 - Altération développement embryonnaire
- Génotoxicité germinale : et la femme ?

Comet assay on mouse oocytes: an improved technique to evaluate genotoxic risk on female germ cells

Anais Berthelot-Ricou, M.D.,^a Jeanne Perrin, M.D., Ph.D.,^{a,b} Carole Di Giorgio, Ph.D.,^a Michel De Meo, Ph.D.,^a Alain Botta, M.D., Ph.D.,^a and Blandine Courbiere, M.D., Ph.D.^{a,c}

^a Laboratoire de Biogénotoxicologie et Mutagenèse Environnementale, Fédération de Recherche CNRS 3098 ECCOREV Université de la Méditerranée Aix-Marseille II, Facultés de Médecine et Pharmacie, Marseille, 27 Bd. Jean Moulin, 13005 Marseille, France; ^b CECOS-Laboratoire de Biologie de la Reproduction, Pr Grillo, AP-HM La Conception, 147 Bd Baille, 13005 Marseille, France; and ^c Service de Gynécologie-Obstétrique, Centre d'Assistance médicale à la Procréation, Pr Gamerre, AP-HM La Conception, 147 Bd Baille, 13005 Marseille, France

Fertility and Sterility® Vol. ■, No. ■, ■ 2010



Examples of different aspects of DNA damages as detected by the comet assay on mouse oocytes. **(A)** Control group: intact oocyte after comet assay process. **(B)** Oocyte exposed in vitro to SSI (12 J/cm²) for 4 minutes: moderate DNA damage. **(C)** Oocyte exposed in vitro for 5 minutes to H₂O₂ (250 µM): major DNA damage. **(D)** Oocyte exposed in vitro for 1 hour to methylmethanesulfonate (100 µM). Maximal DNA damage, with the aspect called *ghost cell*. The direction of electrophoresis was from left to right, and the comet tail containing the DNA fragments was stained by iodide propidium 0.1 mg/mL. Scale bar, 100 µm. Magnification, ×200.