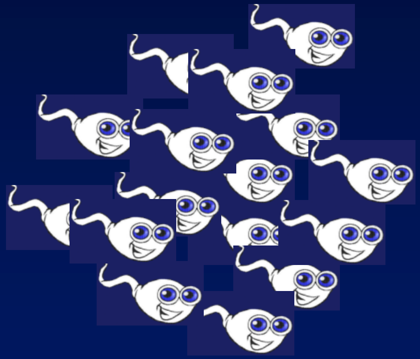
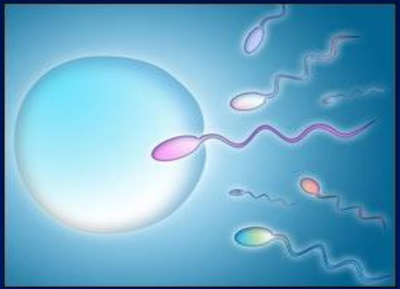
# IMPACT OF NUTRITION ON MALE GAMETE





**Pr R. LEVY and**

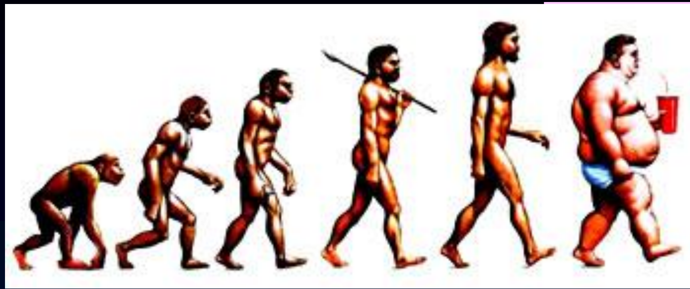
C. FAURE, N. SERMONDADE, C. DUPONT, P. LEVEILLE , I. CEDRIN,

S. HERCBERG and S. CZERNICHOW

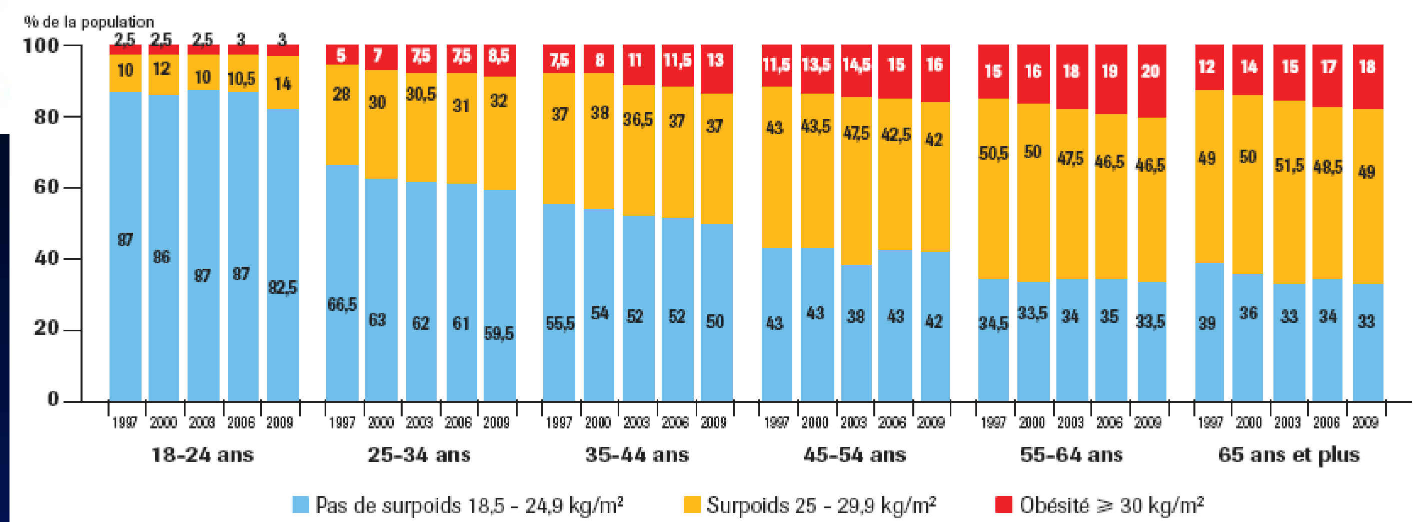
**Histologie Embryologie Cytogénétique CECOS, CHU Jean Verdier, Bondy**
**UMR Inserm U557 / Inra /Cnam /Paris 13, UREN " épidémiologie nutritionnelle " - Pr. S. Hercberg**

# MALE INFERTILITY AND LIFESTYLE FACTORS

- 80 millions infertile (WHO)
- 1/6 couple
- 1/10 ART
- 50%  :  a « male » factor

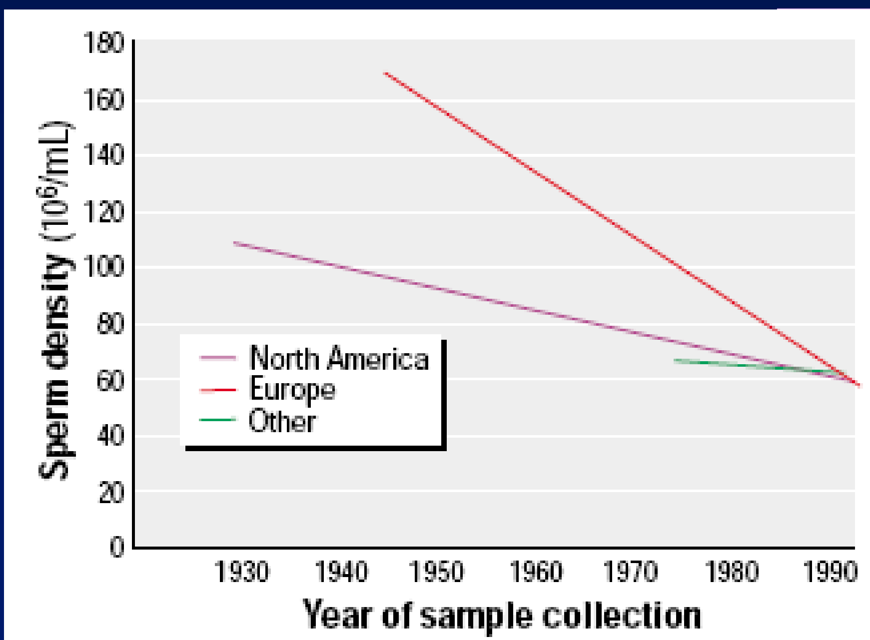


# MALE OBESITY




**Overweight 30 - 50%**

**Obesity > 15%**




# MALE INFERTILITY

**Declining semen quality**

**1% / year USA**

**3% / year Europe/Australia**

# QUANTITY AND QUALITY

## Dietary intake, physical activity and nutritional status in adults: the French nutrition and health survey (ENNS, 2006–2007)

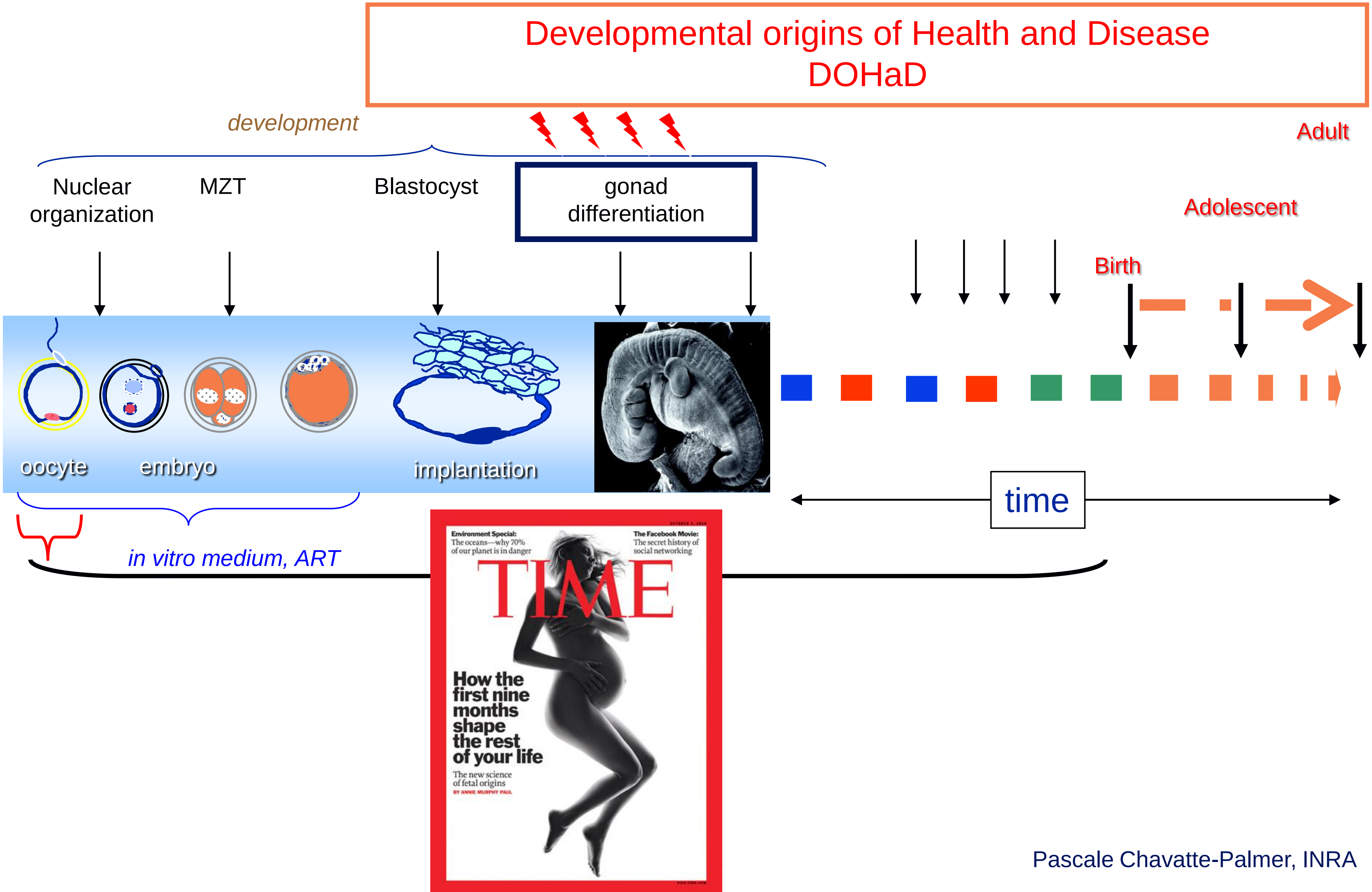
Programme National Nutrition Santé

| | Men | | Women | |
|---|---|---|---|---|
| | Mean or % | SE | Mean or % | SE |
| Iron status | | | | |
| Hb | | | | |
| Mean (g/l) | 153 | 0·5 | 137 | 0·4 |
| % M: <130 g/l; W: <120 g/l | 1·9 | 0·6 | 4·5 | 0·7 |
| Serum ferritin | | | | |
| Mean (μg/l) | 291 | 93 | 81 | 3 |
| % <15·0 μg/l | 1·3 | 0·5 | 8·7 | 1·2 |
| % 15·0–30·0 μg/l | 2·6 | 1·0 | 18·0 | 1·6 |
| Iron-deficiency status (%) | | | | |
| W: Hb <120 g/l and ferritin <15·0 μg/l | | | 1·9 | 0·4 |
| M: Hb <130 g/l and ferritin <15·0 μg/l | 0·5 | 0·3 | | |
| Serum 25-hydroxyvitamin D | | | | |
| Mean (nmol/l) | 59·8 | 1·2 | 56·4 | 1·1 |
| % <25 nmol/l | 3·3 | 0·7 | 5·5 | 0·9 |
| Plasma folate | | | | |
| Mean (nmol/l) | 14·2 | 0·3 | 16·4 | 0·3 |
| % <7 nmol/l | 6·7 | 1·3 | 5·8 | 1·1 |
| Plasma vitamin $B_{12}$ | | | | |
| Mean (pmol/l) | 289 | 4 | 304 | 6 |
| % <150 pmol/l | 2·9 | 0·8 | 3·7 | 0·8 |

**OVERALL POOR NUTRITIONAL STATUS**

Castetbon et al. 2009

# Maternal nutritional imprinting




Pascale Chavatte-Palmer, INRA

# LOW MATERNAL NUTRITION

## SERTOLI CELLS

- Reduces the number of Sertoli cells in the newborn lamb *Alejandro et al. 2002*

- Impacts on the hypothalamic-pituitary-adrenal axis responsiveness in sheep at different ages postnatal *Chadio et al. 2007*

- Reduces Sertoli cell number and altered pituitary responsiveness in lambs / smaller seminiferous tubules diameter in adulthood *Kotsampasi et al. 2009*

- Lower testicular weight and lower Sertoli cell number in adult life,

For review, Dupont et al. , 2011

*Genovese et al. 2010*

# MATERNAL OBESITY

**SERTOLI CELLS**

Human Reproduction Vol.22, No.10 pp. 2758–2762, 2007
Advance Access publication on August 17, 2007
doi:10.1093/humrep/dem219

**Is maternal obesity related to semen quality in the male offspring? A pilot study**

C.H. Ramlau-Hansen[1,2,4], E.A. Nohr[3], A.M. Thulstrup[1], J.P. Bonde[1], L. Storgaard[1] and J. Olsen[2]

**Cohort Health Habits for Two**

**1984-87 :**

**5109 male offsprings**

**Inhibin B in the male offspring decreased**

**with increasing maternal BMI**

Ramlau-Hansen et al. 2007


# MATERNAL VITAMIN B12 DEFICIENCY

**Maternal vitamin B12 deficiency affects spermatogenesis at the embryonic and immature stages in rat**

Watanabe et al., 2003; 2007

- ❑ **Affects spermatogenesis (developing embryo)**
- ❑ **Results in <u>reversible</u> OAT (adult)**

# LOW BIRTH WEIGHT

**Low birth weight for gestational age and**

**subsequent male gonadal function**

Smaller testicular size

Lower testosterone level

Higher LH value

Low Inhibin B value

Cicognani et al. 2002

Lower fertility

in adult males ?

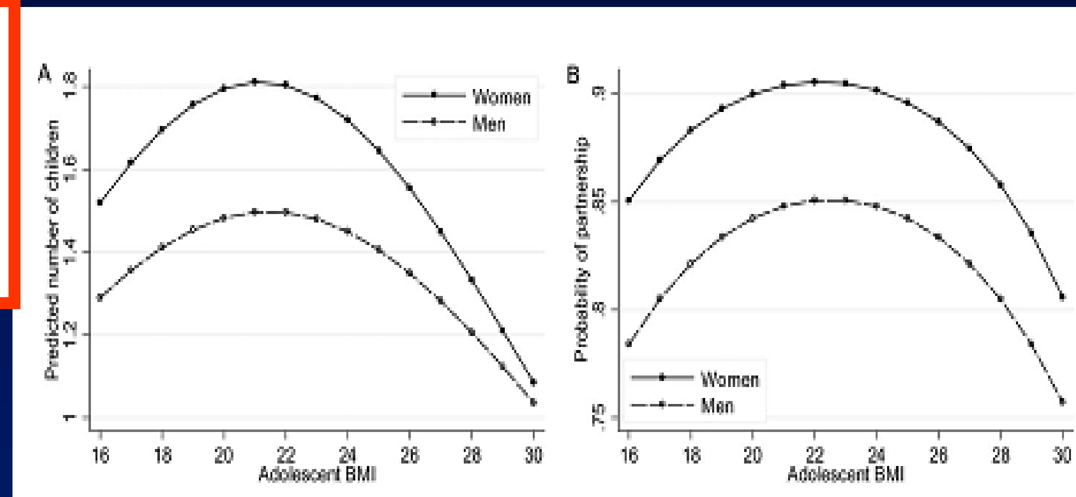
# BMI IN ADOLESCENCE

Body Mass Index in Adolescence and Number of Children in Adulthood

*Markus Jokela,* Mika Kivimäki,*† Marko Elovainio,*†‡ Jorma Viikari,§ Olli T. Raitakari,¶ and Liisa Keltikangas-Järvinen**

Lower fertility associated with obesity and underweight: the US National Longitudinal Survey of Youth[1–3]

*Markus Jokela, Marko Elovainio, and Mika Kivimäki*


**Obese adolescent / men are 22% less likely to have fathered a child than their NW counterparts by the time they had reached their late 40s**

# MALE BMI AND INFERTILITY

- **USA : 1 329 couples**
  - ✓ OR for infertility OR = 1.12
  - ✓ BMI>32

Sallmen et al. 2006

- **Denmark : 47 835 couples**
  - ✓ OR for infertility : overweight OR=1.15, obesity OR=1.49

Ramlau-Hansen et al. 2007

- **Norwegian Mother and Child cohort study : 26 303 couples**
  - ✓ OR for infertility : overweight OR=1.19, obesity OR=1.36
  - ✓ BMI>35

Nguyen et al. 2007

Human Reproduction Update Advance Access published November 4, 2009
Human Reproduction Update, Vol.00, No.0 pp. 1–19, 2009
doi:10.1093/humupd/dmp047

human reproduction update

## The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis

A.A. MacDonald[1], G.P. Herbison[2], M. Showell[3], and C.M. Farquhar[4,5]

[1]School of Medicine, Faculty of Medical and Health Sciences, University of Auckland, Auckland 1142, New Zealand [2]Preventive and Social Medicine, Dunedin School of Medicine, University of Otago, Dunedin 9054, New Zealand [3]Cochrane Menstrual Disorders & Subfertility Group, Faculty of Medical and Health Sciences, University of Auckland, Auckland 1142, New Zealand [4]Department of Obstetrics and Gynaecology, Faculty of Medical and Health Sciences, University of Auckland, Auckland 1142, New Zealand

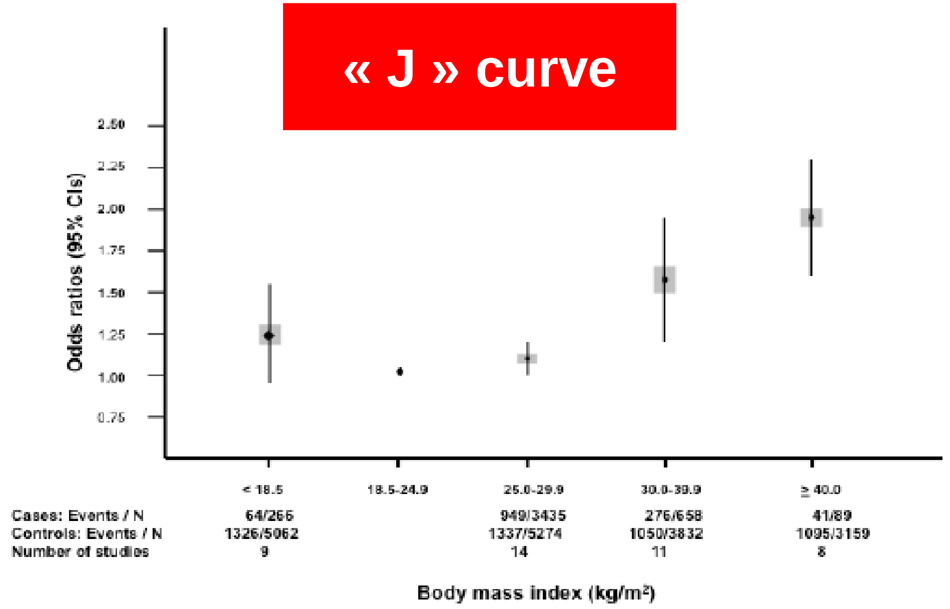
**No evidence of association between increased BMI and semen parameters**

**Body Mass Index in relation to semen parameters : an updated systematic review and meta-analysis**

**13 studies, 9 779 men**

**Overweight and obesity are associated with an increased prevalence of azoospermia or oligozoospermia**

Figure 2




Sermondade et al. , 2011

DNA Damage in Human Spermatozoa Is Highly Correlated with the Efficiency of Chromatin Remodeling and the Formation of 8-Hydroxy-2′-Deoxyguanosine, a Marker of Oxidative Stress[1]

Geoffry N. De Iuliis,[3] Laura K. Thomson,[5] Lisa A. Mitchell,[3,4] Jane M. Finnie,[3,
Andrew Hedges,[6] Brett Nixon,[3] and R. John Aitken[2,3]

**BMI ?**

| | Population | N | Results |
|---|---|---|---|
| Kort et al. 2006 | Partner of infertile couple | 520 | ↗ **DNA fragmentation** |
| Chavarro et al., 2009 | Partner of infertile couple | 483 | ↗ **DNA fragmentation** |
| La Vignera et al., 2011 | General population | 150 | ↗ **DNA fragmentation** |
| Rybar et al., 2011 | Partner of infertile couple | 153 | **No effect on chromatin integrity nor condensation** |
| Tunc et al., 2010 | Partner of infertile couple | 81 | **No effect on DNA fragmentation** |

First International Journal of Andrology
ANDROLOGIA

ORIGINAL ARTICLE

# Impact of body mass index on seminal oxidative stress

O. Tunc[1,2], H. W. Bakos[1,2] & K. Tremellen[1,2]

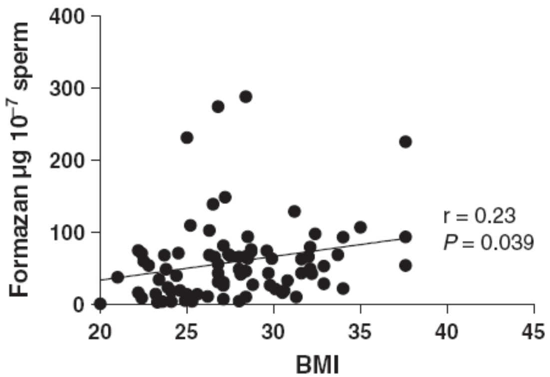



**Fig. 1** Correlation between reactive oxygen species production ($\mu$g formazan per $10^7$ sperm) and body mass index.

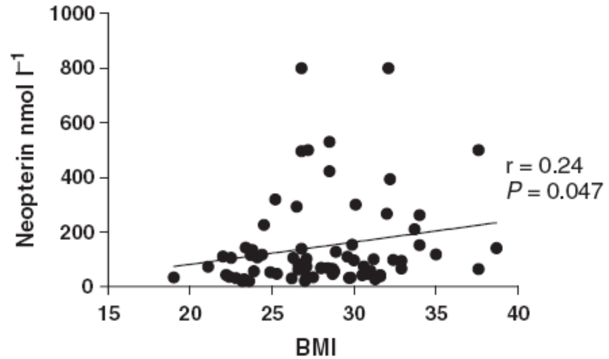



**Fig. 2** Correlation between seminal plasma neopterin concentration (nmol $l^{-1}$) and body mass index.

**A positive correlation between BMI and oxidative stress**

**A positive correlation between BMI and macrophage activity**

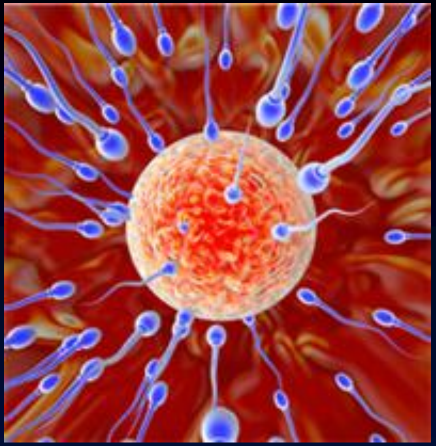
The **systemic immune activation** seen in obese individuals extends to the male reproductive tract, resulting in **an enhanced state of macrophage activity and seminal oxidative stress**

**Tunc et al. 2010**

# Hypercholesterolemia Impaired Sperm Functionality in Rabbits

Tania E. Saez Lancellotti[1,2,9], Paola V. Boarelli[1,9], Maria A. Monclus[1], Maria E. Cabrillana[1], Marisa A. Clementi[1], Leandro S. Espínola[2], Jose L. Cid Barría[2], Amanda E. Vincenti[1], Analia G. Santi[3], Miguel W. Fornés[1,2*]

Lancellotti et al 2010



# The effect of paternal diet-induced obesity on sperm function and fertilization in a mouse model

H. W. Bakos,*† M. Mitchell,* B. P. Setchell‡ and M. Lane*†

*Discipline of Obstetrics & Gynaecology, School of Paediatrics & Reproductive Health, Research Centre for Reproductive Health, Robinson Institute, University of Adelaide, Adelaide, SA, Australia, †Repromed, Dulwich, SA, Australia, and ‡Discipline of Anatomy and Physiology, School of Medical Sciences, University of Adelaide, SA, Australia

| | Control | HFD | *p*-Value |
|---|---|---|---|
| Capacitation at insemination (%) | 21.1 ± 5.1 | 12.3 ± 2.5 | <0.01 |
| Capacitation 4 h post-insemination (%) | 36.8 ± 3.3 | 34.6 ± 2.1 | NS |
| Acrosome reaction at insemination (%) | 12.9 ± 1.9 | 12.1 ± 1.3 | NS |
| Acrosome reaction 4 h post-insemination (%) | 45.1 ± 2.8 | 49.6 ± 4.1 | NS |
| Sperm binding numbers 4 h post-insemination | 58.4 ± 2.4 | 41.1 ± 2.5 | <0.01 |
| Fertilization rate (%) | 43.9 ± 4.2 | 25.9 ± 4.7 | <0.01 |

Bakos et al. 2010

# Paternal diet-induced obesity impairs embryo development and implantation in the mouse

Megan Mitchell, Ph.D.,[a] Hassan W. Bakos, B.Hlth.Sc.(Hons.).,[a,b] and Michelle Lane, Ph.D.[a,b]

**TABLE 3**

**Effect of paternal obesity on embryo development on day 5 of culture, average total, TE, and ICM cell number, and proportion of apoptotic cells in blastocyst-stage embryos.**

| Parameter | Lean | Obese | *P* value |
|---|---|---|---|
| Day 5 ≤morula (%) | 5.6 | 25.5 | <.001 |
| Day 5 EB, B, and ExpB (%) | 45.2 | 49.1 | NS |
| Day 5 ≥HB (%) | 46.0 | 25.5 | <.001 |
| Total cell number | 47.9 ± 2.0 | 34.7 ± 3.8 | <.001 |
| TE cell number | 32.1 ± 1.7 | 24.6 ± 3.1 | <.05 |
| ICM cell number | 15.8 ± 0.8 | 10.1 ± 1.0 | <.001 |
| Proportion ICM (%) | 33.3 | 30.3 | NS |
| Proportion apoptosis (%) | 14.1 | 21.3 | <.05 |

**TABLE 4**

**Effect of paternal obesity on blastocyst viability and fetal and placental characteristics after transfer into pseudopregnant females.**

| Parameter | Lean | Obese | *P* value |
|---|---|---|---|
| Implantation/ET (%) | 86.7 | 73.3 | <.05 |
| Fetal development/ ET (%) | 38.7 | 21.3 | <.05 |
| Fetal development/ implantation (%) | 44.6 | 29.3 | <.075 |
| Fetal weight (mg) | 872.8 ± 21.1 | 870.5 ± 28.4 | NS |
| Placental weight (mg) | 131.6 ± 5.8 | 133.5 ± 7.8 | NS |
| Fetal/placental ratio | 6.9 ± 0.3 | 6.8 ± 0.4 | NS |
| Fetal length (mm) | 19.6 ± 0.3 | 19.4 ± 0.4 | NS |

Mitchell et al. 2010

# Paternal body mass index is associated with decreased blastocyst development and reduced live birth rates following assisted reproductive technology

Hassan W. Bakos, Ph.D.,[a,b] Richard C. Henshaw, M.D.,[b] Megan Mitchell, Ph.D.,[a] and Michelle Lane, Ph.D.[a,b]

**TABLE 3**

**Embryo development outcom**

| Embryo development outcom | Obese (n = 62) | Morbidly obese (n = 32) |
|---|---|---|
| IVF fertilization rate (%) | 60.2 | ND |
| ICSI fertilization rate (%) | 78.7 | 65.4 |
| Day 3 grade 1 and grade 2 emb | 61.3 ± 4.6 | 42.1 ± 7.4 |
| Day 5 on-time blastocyst develo | 20.3 ± 3.9 | 18.7 ± 5.7 |
| Day 5 expanded blastocyst, 2PI | 10.7 ± 2.9 | 8.5 ± 4.2 |

*Note:* Results are expressed as ±SE

[a] Linear decrease in blastocyst deve ... statistically significantly different.

*Bakos. Paternal obesity and ART pregna*

**TABLE 4**

**Pregnancy and live birth outcomes according to paternal BMI.**

| Outcome | Normal (n = 63) | Overweight (n = 148) | Obese (n = 62) | Morbidly obese (n = 32) |
|---|---|---|---|---|
| β-hCG/OPU (%) | 46.03[a] | 36.49[b] | 35.48[b] | 15.15[c] |
| β-hCG/ET (%) | 50.88[a] | 41.54[a,b] | 38.60[b] | 20.83[c] |
| Sac/OPU (%) | 44.44[a] | 31.76[b] | 32.26[b] | 12.12[c] |
| Sac/ET (%) | 49.12[a] | 36.15[b] | 35.09[b] | 16.67[c] |
| Heart/OPU (%) | 42.86[a] | 29.73[b] | 25.81[b] | 12.12[c] |
| Heart/ET (%) | 47.37[a] | 33.85[b] | 28.07[b] | 16.67[c] |
| Pregnancy loss (%) | 10.3[a] | 38.5[b] | 36.4[b] | 20.0[a,b] |
| Live birth/OPU (%) | 41.3[a] | 26.4[b] | 22.6[b] | 12.12[c] |

*Note:* Data are expressed both per oocyte pickup (OPU) and also per embryo transfer (ET). Linear decrease with increasing BMI, $P < .05$, for all values except pregnancy loss. Different superscripts are significantly different within each outcome, $P < .05$.

*Bakos. Paternal obesity and ART pregnancy. Fertil Steril 2011.*

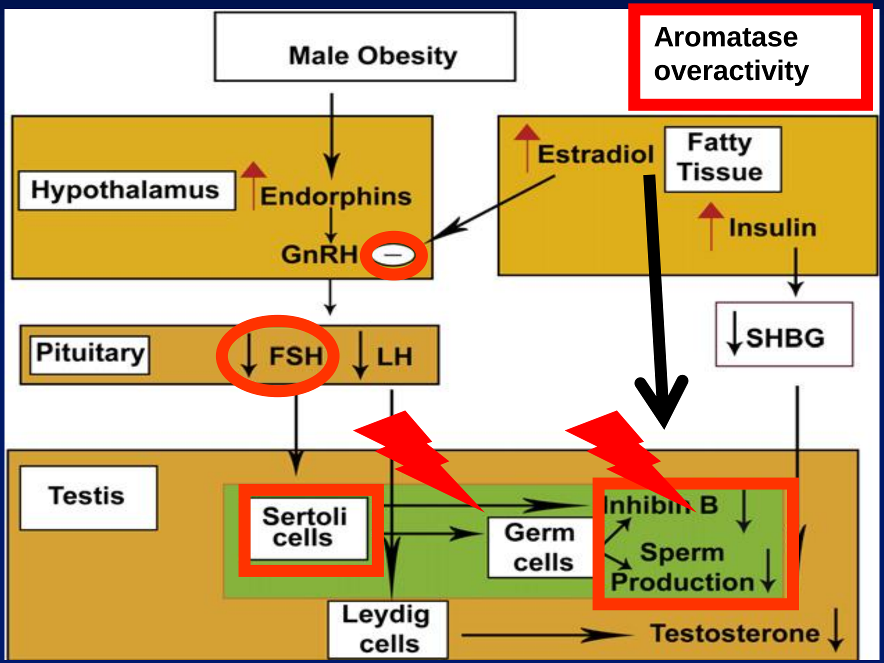
Lane et al. 2011

# MECHANISMS

- Relative hypogonadotrophic state (debated)
- Peripheral conversion of androgens to oestrogens
- Direct damage : spermatogenesis and spermatozoa
- Toxic : endocrine disruptors /pollutants accumulate in adipose tissue
- Scrotal temperature
  - fat tissue
  - sedentary lifestyle

**INSULIN**

**LEPTIN**



# NORMAL WEIGHT OBESE SYNDROME


## Continuum between NWO and overweight

- **The proinflammatory cytokines :**

**significant prognostic indicators of the risk of obesity, CVD, metabolic syndrome <u>and infertility</u>**

- **Systemic oxidative stress :**

**Extends to <u>testicular micro-environment</u>**

**ADIPOSOPATHY**

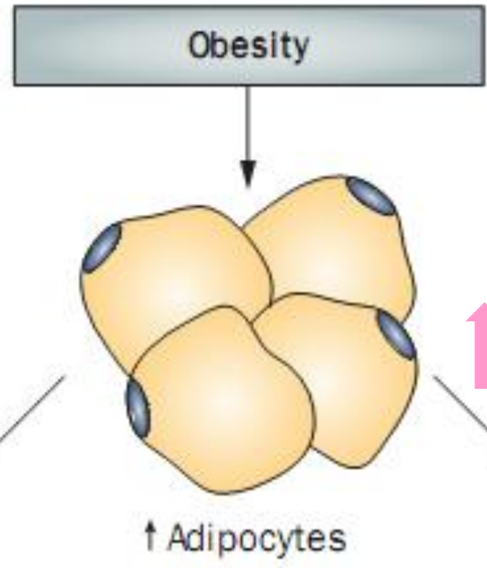
Table. Laboratory values in women, by weight and presence of

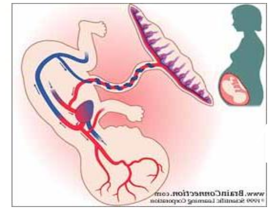
| Variable | Normal weight | NWO | Overweight |
|---|---|---|---|
| BMI, $kg/m^2$ | 19.2 | 22.6 | 27.9 |
| Fat mass, % | 23.3 | 34.9 | 42.9 |
| *Lipids, mg/dL* | | | |
| LDL-C | 107.2 | 103.8 | 116.0 |
| HDL-C | 69.1 | 68.2 | 70.2 |
| Triglycerides | 66.3 | 86.1 | 111.5 |
| Total cholesterol | 178.4 | 187.9 | 218.1 |
| *Cytokines, pg/mL* | | | |
| TNF-α | 20.1 | 42.8 | 56.4 |
| IL-6 | 5.9 | 11.4 | 13.7 |
| IL-1α | 14.8 | 26.9 | 29.8 |
| IL-1β | 5.0 | 15.0 | 19.0 |

*Note:* All values are means.
LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.
*Source:* De Lorenzo A, et al. Normal-weight obese syndrome: early inflammation. *Am J Clin Nutr.* 2007;85:40-45.

**Polygenic obesity**

- **Heredity of dietary behavior**
- **Genic environnement**
- **FTO, PTER, MC4R, MAF, NPC1**



**Environment**

**Toxic and**

**Endocrine disruptors**

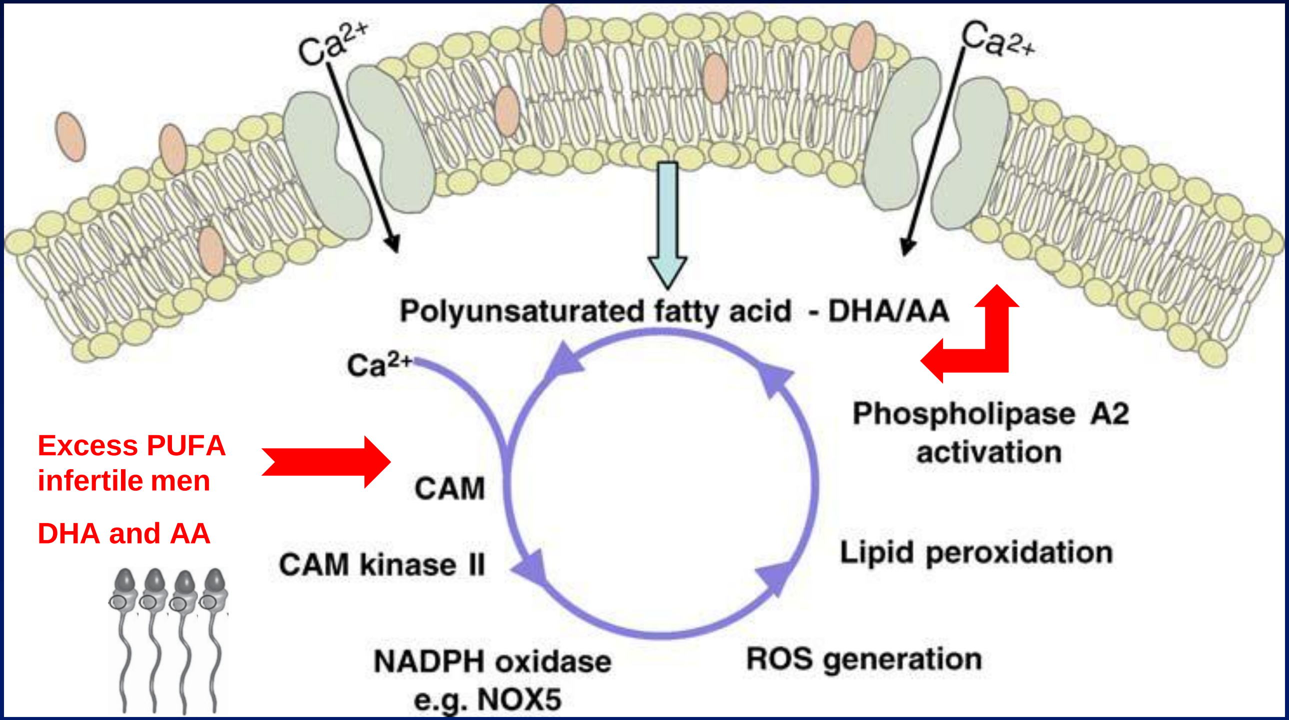
# OXIDATIVE STRESS

lessis 2010

# POLYINSATURATED FATTY ACIDS (PUFA)

**Membrane fluidity (fertilization)**

**Oxidative stress (Lipid peroxidation)**




- Morphologically abnormal sperm (RC)
- Excessive seminal leukocytes
- Antioxidant deficiency

Wathes and Aitken 2007; Chavarro et al., 2011

# GENE POLYMORPHISMS

❑ ***GST*** (GSTM1, GSTT1, GSTP1 Ile105Val)

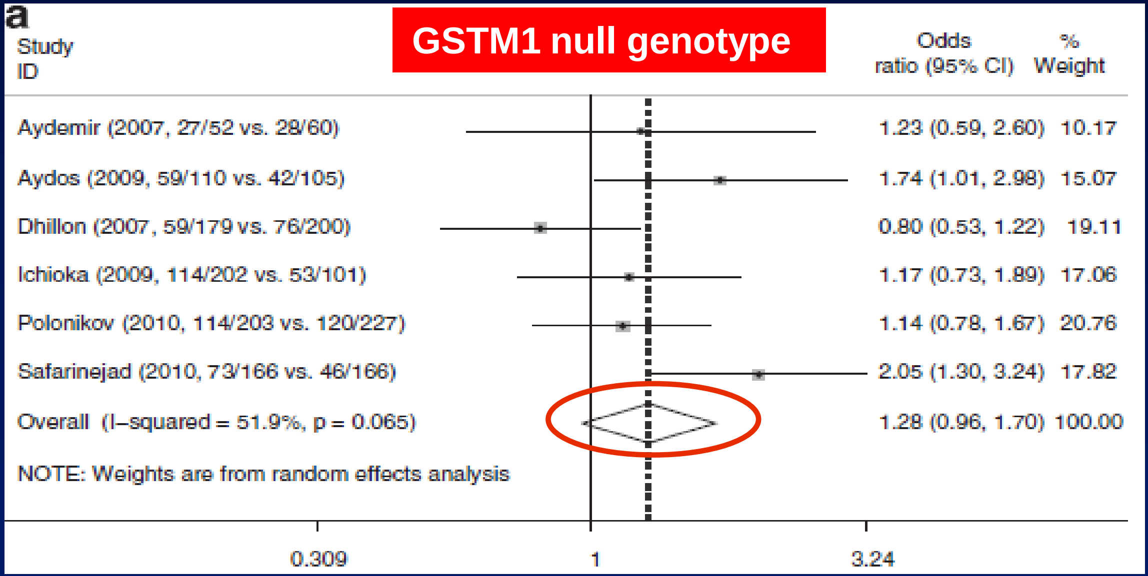
- A positive association between male infertility and GSTM1null, GSTT1null, GSTP1 Ile/Ile Which mutually potentiate the effects of each other **(Safarinejad et al, 2010)**

**Promising,**

**but results not reproducible**

**at the meta analytical level !**

**(Economopoulos et al, 2010)**




❑***eNOS*** (T-786C, G894T, and 4a/b)

**-*eNOS* 786CC (p=0.001), 894TT (p=0.001) and 4aa (p=0.004) more frequent in infertile subjects**

**-Correlation genotype/phenotype (Safarinejad 2010)**

# ANTIOXIDANT INTAKE AND MALE INFERTILITY

**Table IV** Semen parameters according to the tertiles of the two major dietary patterns

| | 'Health Conscious' | | | | 'Traditional Dutch' | | | |
|---|---|---|---|---|---|---|---|---|
| | Low ($n$ = 40) | Intermediate ($n$ = 44) | High ($n$ = 42) | P-value[1] | Low ($n$ = 44) | Intermediate ($n$ = 43) | High ($n$ = 39) | P-value[1] |
| *Semen parameters* | | | | | | | | |
| DFI (%) | 25.2 (21.1–29.2) | 25.0 (20.2–29.8) | 20.6 (17.5–23.6) | 0.05 | 23.5 (20.1–26.9) | 25.0 (20.0–30.1) | 22.0 (18.5–25.6) | 0.53 |
| Volume (ml) | 3.4 (2.9–3.8) | 2.8 (2.4–3.2) | 3.1 (2.6–3.5) | 0.41 | 3.1 (2.6–3.5) | 3.1 (2.7–3.5) | 3.0 (2.6–3.5) | 0.89 |
| Concentration ($\times 10^6$ cells/ml) | 46.2 (30.8–61.5) | 39 (27.6–50.4) | 48.2 (33.1–63.2) | 0.89 | 36.7 (26.8–46.6) | 36.4 (23.1–49.8) | 61.7 (44.5–78.9) | 0.01 |
| Motility (%) | 35 (30–40) | 33 (28–39) | 37 (32–42) | 0.06 | 35 (31–39) | 34 (29–40) | 37 (31–43) | 0.98 |
| Morphology (%) | 5 (4–6) | 5 (4–6) | 5 (4–6) | 0.74 | 5 (4–6) | 5 (4–6) | 6 (5–7) | 0.34 |

Data are presented as median with range.
[1] P-values are adjusted for age, BMI, smoking, vitamin supplement use and presence of varicocele.

Vujkovic et al., 2009


OAT

BMI 23,2


Normal semen

BMI 23,5

**DIET CAN INFLUENCE SEMEN QUALITY, DFI**

A low intake of antioxidant nutrients is associated with poor semen quality in patients attending fertility clinics

Mendiola 2009 et 2010

# ANTIOXIDANT INTAKE AND MALE INFERTILITY




**REVERSIBILITY**

**SPECIFICITY**

**HEALTHY**

**WELL-BALANCED**

**DIET**



- ❑**Endocrine effect**
- ❑**Exocrine (semen quality) effect**
- ❑**Fertilization/embryo development/implantation**


# FOLATE

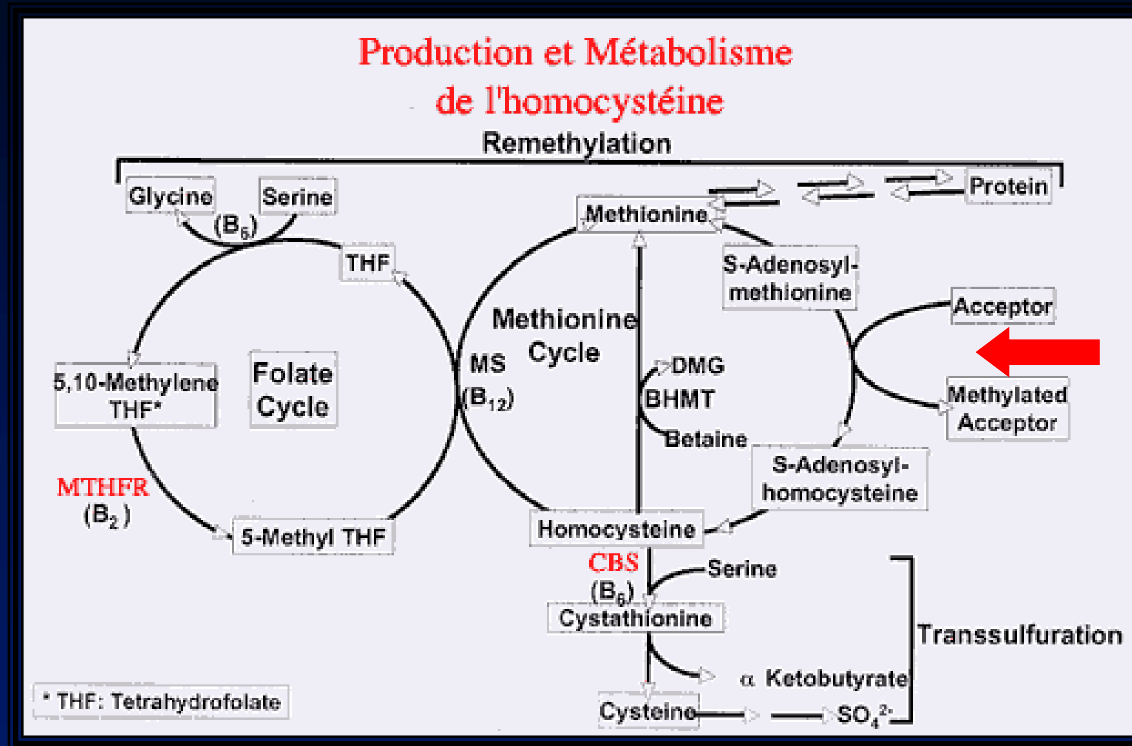
Low folate in seminal plasma is associated with increased sperm DNA damage

Jolanda C. Boxmeer, M.D., ... Utomo, M.D.,[a] Johannes C. Romijn, Ph.D.,[b] Marinus J. C. Eijkemans, Ph.D.,[c] Jan Lindemans, Ph.D.,[d] Joop S. E. Laven, Ph.D.,[a] Nick S. Macklon, Ph.D.,[a,e] Eric A. P. Steegers, Ph.D.,[a] and Regine P. M. Steegers-Theunissen, Ph.D.[a,f,g,h]

Human Reproduction pp. 1–9, 2008 Hum. Reprod. Advance Access published March 19, 2008 doi:10.1093/hum

The association of folate, zinc and antioxidant intake with sperm aneuploidy in healthy non-smoking men

S.S. Young[1], B. Eskenazi[1,4], F.M. Marchetti[2,3], G. Block[1] and A.J. Wyrobek[2,3]




**GENETIC QUALITY**

**EPIGENETIC QUALITY**

European Journal of Human Genetics (2010) 18, 73–80
www.nature.com/ejhg


ARTICLE

## Specific epigenetic alterations of *IGF2-H19* locus in spermatozoa from infertile men

Céline Chalas Boissonnas[1,2,3], Hafida El Abdalaoui[4], Virginie Haelewyn[1], Patricia Fauque[1,2,3], Jean Michel Dupont[2,3,5], Ivo Gut[4], Daniel Vaiman[2,3], Pierre Jouannet[1], Jörg Tost[4] and Hélène Jammes*[,2,3,6]

European Journal of Human Genetics (2009) 17, 1582–1591
www.nature.com/ejhg


EJHG Open

ARTICLE

## DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm

Marques et al, 2004 et 2008; Kobayashi et al., 2007 et 2009; Boissonnas et al. 2010

Cell

# Paternally Induced Transgenerational Environmental Reprogramming of Metabolic Gene Expression in Mammals

LETTER

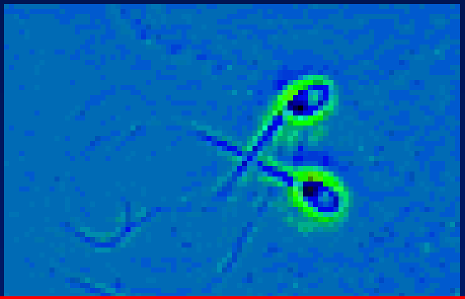
doi:10.1038/nature09491

## Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring

Sheau-Fang Ng[1], Ruby C. Y. Lin[2], D. Ross Laybutt[3], Romain Barres[4], Julie A. Owens[5] & Margaret J. Morris[1]

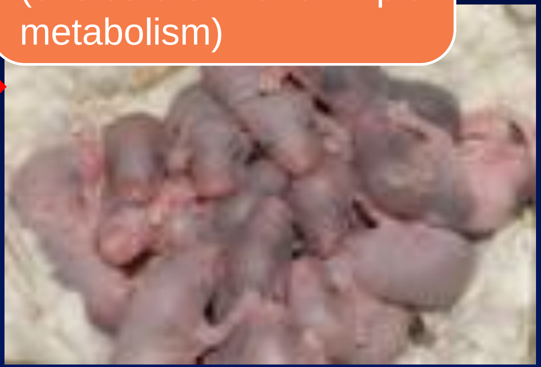
LOW PROTEIN DIET

**Non genetic intergenerational paternal transmission of metabolic phenotype**



**to offspring via epigenetic heritable marks on male germ cells**

Upregulation of the biosynthesis of genes (cholesterol and lipid metabolism)



# Paternal nutritional imprinting

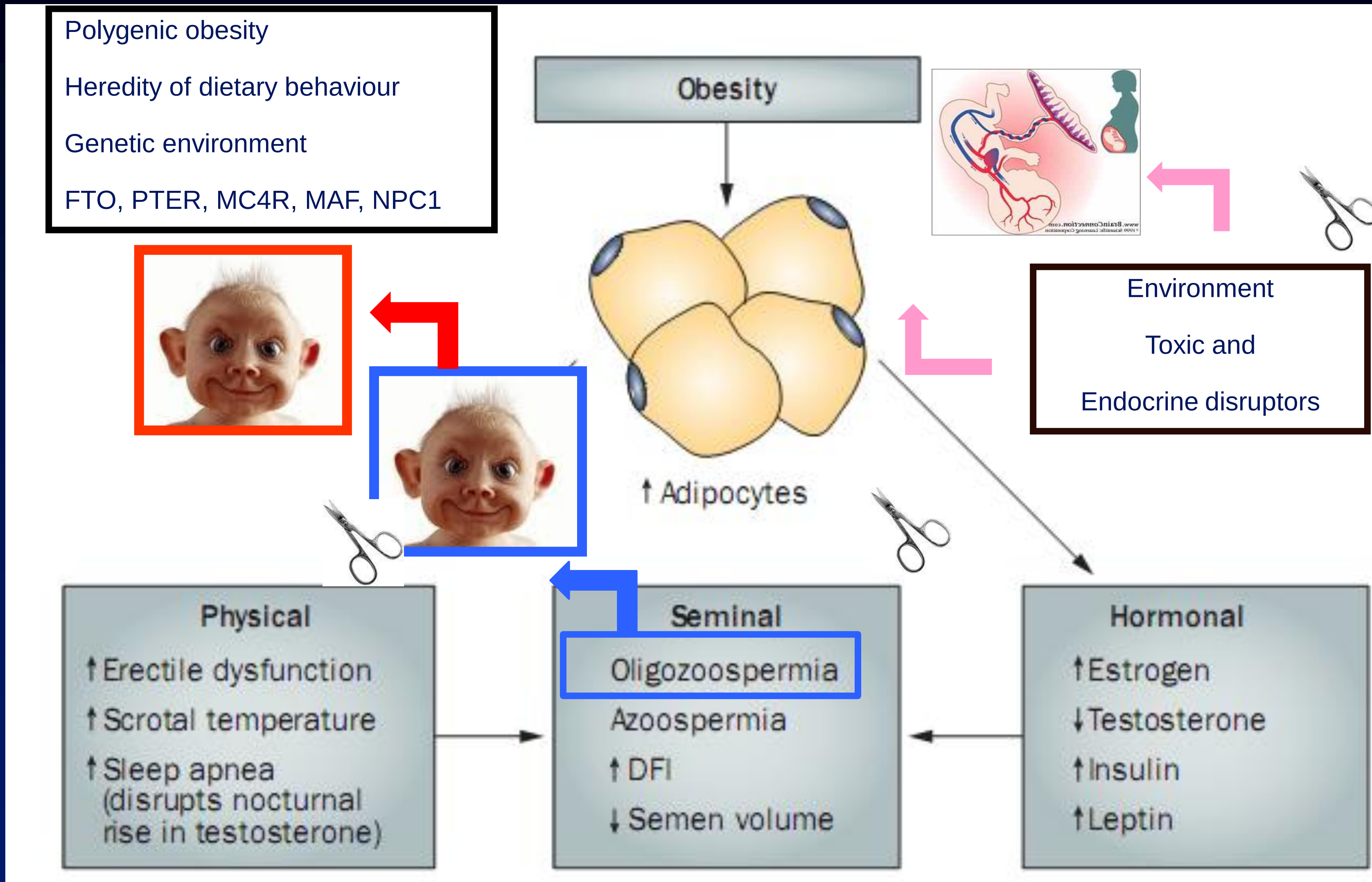
Ng et al., 2010; Carone et al., 2010

Cell Metabolism
Previews

# You Are What Your Dad Ate

Anne C. Ferguson-Smith[1,2,*] and Mary-Elizabeth Patti[3,*]

**the father's nutritional and metabolic status certainly merits our attention, too, if we are to optimize health of <u>his children and grandchildren</u>**


Polygenic obesity
Heredity of dietary behaviour
Genetic environment
FTO, PTER, MC4R, MAF, NPC1
Obesity
↑ Adipocytes
Environment
Toxic and
Endocrine disruptors
Physical
↑ Erectile dysfunction
↑ Scrotal temperature
↑ Sleep apnea (disrupts nocturnal rise in testosterone)
Seminal
Oligozoospermia
Azoospermia
↑ DFI
↓ Semen volume
Hormonal
↑ Estrogen
↓ Testosterone
↑ Insulin
↑ Leptin

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