Lebanese International Fertility Summit
2 – 3 October 2015
Hilton Beirut Habtoor Grand
Germinal cell testicular tumors, microlithiasis, Cis & infertility

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Testicular microlithiasis™
Testicular microlithiasis ™

- 1.5-5.6% of healthy men
- Calculi in seminiferous tubules.
- Incidentally diagnosed/US.
  - multiple bright foci, 1-2 mm limited to the testis.
  - Classical TM: > 5
  - Limited TM: <5
- No palpable, not visible on MRI
- TM associated
  - Male pseudohermaphroditisme
  - Cryptorchidism,
  - Subfertility infertility
  - Hypogonadism, varicocele, testicular torsion,
  - Klinefelter syndrome and Down syndrome.

Decastro et al J Urol 2008
TM and Testicular Tumors

- Increased incidence of testicular cancer
  - RR = 12.70, 95% CI: 8.18-19.71, \( P < .001 \)

- 98.6%: No TGCT in the subsequent five years,
  - Surveillance of these patients is of little benefit.

- TM in the contralateral testis of men with unilateral TGCT: x 28.6 increased risk of ITGCN

- Infertile men:
  - 2-20% TM on US,
  - Increased risk of ITGCN (bilateral, sonographic heterogeneity or atrophy)

Priebe and Garret Pediatrics 1970
Wang et al J Urol 2015
US in subfertile patients

- 16204 chinese patients,
  - TM in 1.39% on US: 60% CTM and 40% LTM
  - Testis Volume and sperm count and motility: CTM < LTM and non-TM
  - Azoo in 20%
    - 60% CTM (FSH: 20) et 40 LTM (FSH: 8)
- TM is negatively correlated with semen parameters
- Extent of ML correlates inversely with semen parameters

Table 2. The comparison of semen parameters of CTM, LTM, and non-TM group

<table>
<thead>
<tr>
<th>Contents</th>
<th>CTM (N = 97)</th>
<th>LTM (N = 62)</th>
<th>Non-TM (N = 120)</th>
<th>F Value</th>
<th>P Value *</th>
<th>P Value †</th>
<th>P Value ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (mL)</td>
<td>3.77 ± 1.48</td>
<td>3.83 ± 1.48</td>
<td>3.41 ± 1.16</td>
<td>3.670</td>
<td>.056</td>
<td>.056</td>
<td>.051</td>
</tr>
<tr>
<td>Sperm concentration (million/mL)</td>
<td>38.01 ± 31.58</td>
<td>52.31 ± 33.26</td>
<td>67.16 ± 36.94</td>
<td>38.755</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.006</td>
</tr>
<tr>
<td>Total sperm number</td>
<td>135.13 ± 111.85</td>
<td>187.26 ± 123.90</td>
<td>224.82 ± 150.69</td>
<td>24.481</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.071</td>
</tr>
<tr>
<td>Total motility, PR+ NP (%)</td>
<td>46.03 ± 23.69</td>
<td>55.37 ± 24.16</td>
<td>62.08 ± 20.45</td>
<td>27.227</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.057</td>
</tr>
<tr>
<td>PR, (%)</td>
<td>35.88±20.17</td>
<td>43.15 ± 21.08</td>
<td>47.10 ± 17.84</td>
<td>17.701</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.195</td>
</tr>
</tbody>
</table>

Xu et al J urol 2014
TM and infertility

- Seminiferous tubule calculi of hydroxyapatite ($\text{Ca}_5\text{(PO}_4\text{)}_3\text{(OH)}$)
  - inflammation
  - degeneration of seminiferous tubular cells

- Obstruction of seminiferous tubules /sloughing degenerative tubular epithelium

- 30%-60% of seminiferous tubules obstructed / concretions.

- Reduction in sperm motility

- **Bilateral TM in infertile men : 20% ITCGN**

deJong et al. J Urol 2014
Follow-up guidelines for TM

• All TM patients must be well informed and educated to practice **monthly self-examination** of testicles.

• The presence of TML alone in the absence of other risk factors is not an indication for regular scrotal US.

• If TM is associated with other risk factors of cancer, annual US follow-up examination is recommended until 55yo. **A biopsy should be offered**.
  - Infertility
  - bilateral TM,
  - atrophic testes,
  - undescended testes,
  - a history of TGCT, and contralateral TM

Richenberg et al Eur Radiol 2015, EUA recommendations
Patient leaflet for testicular microlithiasis

• What is testicular microlithiasis?
  - Small lumps of calcium lie in the small tubes within the testicle. There must be at least 5 such calcifications in one (or both) testicles before the label testicular microlithiasis (TML) is applied. TML is seen in about 2 or 3 men in every hundred.

• How is TML detected?
  - The calcium lumps cannot be felt and they do NOT cause discomfort. They can only be seen on ultrasound. In other words, TML was discovered incidentally during your ultrasound scan of the testes.

• Why is TML important?
  - At the end of the 1990s, there was some concern that TML might lead to cancer of the testicle. Since then, many studies across the world have looked at TML. They have NOT confirmed the initial worries. There is no evidence that TML on its own leads to cancer.

• What should I do?
  - Like every man, including men who do not have TML, you should practice monthly self-examination of the testicles. If you are uncertain about how to do this, please ask your doctor. Nothing else is required. You do not need regular ultrasound scans. The calcium in the testicles is not related to your diet or to any sexual or other activity.

• What should I do if I feel a new lump during self-examination?
  - Please contact your family doctor or specialist. Your family doctor or specialist will examine you and if thought appropriate will refer you on for a specialist opinion. It is likely that you will be referred also for an urgent ultrasound scan. This will be usually at the hospital where the initial scan was performed.
IntraTubular Germ Cell Neoplasia (ITGCN)
Intratubular germ cell neoplasia (ITGCN)

• Initial description by Skakkebaek in 1972
  ▪ pre-invasive lesion for TGCTs in the testis biopsies of two infertile patients who went on to develop TGCTs.

• Precursor lesion for invasive testicular germ cell tumors (TGCTs) of adolescents and young adults

• Bilateral testicular cancer is closely linked with ITGCN, as patients with unilateral testicular cancer are at the highest risk for a future malignancy in the contralateral testicle.

Risk and Matterson Indian J Androl 2010
Intratubular germ cell neoplasia (ITGCN)

- **Incidence:** 0.4 to 0.8 %
  - Controlat testis of TCGT: 5%
  - Testicular atrophy: x 2.5-4.3
  - **Infertile men:** 0.6 to 2.2 %
    - + Bilat TM: 20%
  - Cryptorchidism: x 4.5-6.6

- **Evolution:** 50% of patients with ITGCN develop TGCTs at 6 years, probably almost all at 10 years f.u.

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>L</th>
<th>Diagnosis</th>
<th>Year</th>
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<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>L</td>
<td>Azoospermia</td>
<td>1977</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>L</td>
<td>U.D.T.*</td>
<td>1973</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>L</td>
<td>U.D.T.</td>
<td>1976</td>
</tr>
</tbody>
</table>

* U.D.T. = undescended testes.

Skakkebaek et al., 1978
Bettochi et al J Androl 1994
Linke et al J Urol 2005
Olesen et al Int J androl 2007

LIFE 2015
Intratubular germ cell neoplasia (ITGCN)

- **Testicular biopsies** should be placed in Bouin’s or Stieve's solution, as formalin can make morphologic diagnosis more difficult.

- Markers can be used to identify ITGCN / IHC
  - PLAP
  - Oct 3/4 (dominant regulatory factors of pluripotency)
  - ...

  J. E. Nielsen et al. Andrologia 2011

- Positive carcinoma in situ (CIS)-like cells detected in the ejaculate of men with ITGCN
  - sensitivity of 0.67 and a specificity of 0.98.

Testicular biopsy for ITGCN

• **Rationale:**
  - to detect ITGCN in the contralateral testicle of patients with unilateral TGCT. (Germany and Denmark)
  - In patients with TGCT in association with other risk factors, such as testicular atrophy, US heterogenicity, TM age or UDT.
  - In infertile men with bilateral TM

• **Consequences:**
  - Positive: Radiation therapy, preservation of endocrine function(?), avoid complications of delay diagnosis of TGCT.
  - negative biopsies will be reassured, and potentially can undergo less intense surveillance.
Management of ITGCN:
Fertility expectation, uni or bilateral, associated condition.

- **Orchiectomy**: standard
  - unilateral ITGCN and a contralateral normal testicle
  - Atrophic, poorly functioning testis
  - oligospermia + ITGCN in pts on ART

- **Irradiation**: 18-20 Gy (2x10)
  - Preservation of endocrine function (75%)

- **Surveillance**: Solitary testicle
  - infertility and dependence on exogenous testosterone following orchiectomy.

Risk and Matterson Indian J Androl 2010
EAU recommandations 2015
Testicular Germ Cell Tumors (TGCT)
Epidemiology of testicular cancers

**INCIDENCE.**
- Rare tumors. 2 to 10 new cases /y/100 000
- Most common solid tumor in young men
  - 15-35 yo (median 29) NSGT
  - 25-55 yo (median 39) seminoma.

**Risk factors: GENVIRONMENTAL MODEL**

**Testicular Dysgenesis Syndrome**
- Cryptorchidie (x2,75-8), Hypospadias, Infertilité (x20)

- Bilateral: 3-4% (x 12)
- Genetic: 20% (X, 12p), Familial: 3%
  - Father with TGCT: x 3,8
  - Brother with TGCT: x 6,6 to 10,8

- Infertilité: x2 (1%) (increase risk of melanoma, PC, BC, HL, NHL and leukemia)

- ITGCN (Cis): ≈ 100%

Address for correspondence: Dr Niels E. Skakkebæk, Depa of Growth and Reproduction, Rigshospitalet, Copenhagen

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Testicular dysgenesis syndrome

Genetic defects, epigenetic factors polymorphisms

Environmental factors e.g. endocrine disruptors

toli cell proliferation (12), and possibly function (10), consistent with changes predicted in the TDS hypothesis (1). The focal dysgenesis induced by fetal DBP exposure in rats (10, 11), comprising malformed seminiferous cords, Sertoli cell-only tubules with immature-appearing Sertoli cells, and the abnormal occurrence of intratubular Leydig cells, are all features also reported in the testes of men with TGCC (13–15). The one TDS feature notably lacking in DBP-exposed rats is the occurrence of TGCC or its precursor, carcinoma in situ (CIS) cells. However, recent studies have shown that DBP exposure results in a transient delay of fetal germ cell differentiation in rats (16), which may have some analogy to the formation of CIS cells, which are thought to result from failure of normal germ cell differentiation in fetal life (17). Overall, the studies in rats exposed to DBP have provided strong support at every level for the TDS hypothesis in human males. Therefore, this potentially provides a model system in which to identify and dissect the mechanisms via which TDS and its disorders may arise in fetal life, which can then be only provides a model for human will have limitations.

REFINEMENT AND ELABORATION OF THE CENTRAL ROLE OF ANDROGENS

As a result of the clinical and animal studies outlined above, there has been widespread acceptance of the concept of a testicular dysgenesis syndrome being added gradually to the hypothesis of the syndrome. A particularly important recent development has been the demonstration that inhibition of androgen production/action in rodents by transgenesis (18), DBP exposure (12), or flutamide treatment (19) reduces Sertoli cell number in the perinatal period. This implies that androgens play a determining role during the most important period of Sertoli cell proliferation (20) (Fig. 1). This would fit with data for the human showing that Sertoli cell number increases during fetal life (21). This would fit with data for the human showing that Sertoli cell number increases during fetal life (21).
Increased incidence of TT

Testicular Cancer (C62): 1975-2011
European Age-Standardised Incidence Rates per 100,000 Population, by Age, Males, Great Britain
Prevalence of Testicular cancer

(a)

Central and South America
- Ecuador, Quito
- Costa Rica
- Colombia, Cali
- Brazil, Goiana

Northern America
- USA (SEER 9 White)
- Canada (except Quebec)
- USA (SEER 9 Black)
- Israel
- Jordan
- Japan (4 registries)
- China (2 registries)
- Singapore
- Philippines (2 registries)
- India, Chennai
- Republic of Korea
- Saudi Arabia: Saudi
- Thailand (2 registries)

Asia
- Slovakia
- Czech Republic
- Bulgaria
- Poland (3 registries)
- Belarus
- Russian Federation
- Norway
- Denmark
- UK, Scotland
- UK, England
- Ireland
- Sweden
- Iceland
- Finland
- Estonia
- Latvia
- Lithuania

Central and Eastern Europe
- Slovenia
- Croatia
- Italy (6 registries)
- Spain (3 registries)
- Germany (2 registries)
- Switzerland (2 registries)
- Austria
- The Netherlands
- France (6 registries)

Western Europe
- Portugal
- France (6 registries)
- New Zealand
- Australia

Oceania

Age-standardised incidence rate (W) per 100,000
Testicular cancer and infertility

• 35 to 45% of men had conceived before their cancer diagnosis

• At the time of TC diagnosis sperm parameters are frequently altered / fertile control men.

• After orchiectomy concentration decrease but motility remains unchanged.

• **Azoospermic patients** (145):
  - 7.5% testicular nodules (2/11 palpable)
  - SCOS: 26% nodules (Leydig cell T or hyperplasia) and 10% cancers and ITGSN
  - No SCOS: 1.7%

Maccini et al Human Reprod 2007
Cancers and sperm parameters

### Table 2: Age, semen parameters and patients' histories according to histological tumour types. Values are median (range)

<table>
<thead>
<tr>
<th></th>
<th>S (n = 53)</th>
<th>NS (n = 70)</th>
<th>HL (n = 41)</th>
<th>NHL (n = 23)</th>
<th>OC (n = 40)</th>
<th>F (n = 102)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>33 (20–41)</td>
<td>29 (16–48)</td>
<td>25 (16–54)</td>
<td>28 (20–47)</td>
<td>37 (20–67)</td>
<td>39 (28–54)</td>
</tr>
<tr>
<td><strong>Ejaculate volume (ml)</strong></td>
<td>3.5 (0.9–6.0)</td>
<td>3.0 (0.7–6.0)</td>
<td>2.5 (0.5–6.0)</td>
<td>3.5 (1.0–11.0)</td>
<td>3.0 (1.5–6.0)</td>
<td>3.0 (0.7–11.0)</td>
</tr>
<tr>
<td><strong>Sperm concentration (10^6 ml⁻¹)</strong></td>
<td>12 (0.1–130)</td>
<td>19 (0.01–70)</td>
<td>15 (0.3–150)</td>
<td>50 (3–130)</td>
<td>71 (0.1–180)</td>
<td>46.5 (4–190)</td>
</tr>
<tr>
<td><strong>Total sperm count (10^6 per sample)</strong></td>
<td>48 (0.1–360)</td>
<td>63.8 (0.025–300)</td>
<td>37.8 (0.9–300)</td>
<td>190 (4–770)</td>
<td>198.7 (0.3–720)</td>
<td>135 (4.5–807.5)</td>
</tr>
<tr>
<td><strong>Progressive motility (%)</strong></td>
<td>35 (0–60)</td>
<td>40 (0–55)</td>
<td>45 (0–70)</td>
<td>45 (10–55)</td>
<td>45 (0–65)</td>
<td>45 (10–60)</td>
</tr>
<tr>
<td><strong>Normal morphology (%)</strong></td>
<td>22 (0–32)</td>
<td>22 (0–33)</td>
<td>20 (0–27)</td>
<td>22 (12–32)</td>
<td>24.5 (0–36)</td>
<td>23 (5–32)</td>
</tr>
<tr>
<td><strong>Leucocytes (10^6)</strong></td>
<td>0.4 (0.2–1.1)</td>
<td>0.6 (0.2–2.8)</td>
<td>1.0 (0.3–8.5)</td>
<td>0.9 (0.4–2.0)</td>
<td>0.75 (0.4–3.8)</td>
<td>0.5 (0.2–2.8)</td>
</tr>
</tbody>
</table>
Fertility preservation in men with cancer

Tournaye et al Lancet 2014

Few patients use their samples (often <10%). 45

Up to now, follow-up information applies to children born after spontaneous conception, and might be disquieting for children born after artificial reproductive techniques. Unfortunately, follow-up data for large cohorts of children born after assisted reproductive treatment by use of frozen-thawed sperm from men with cancer are currently not available in the scientific literature.

Peripubertal options for prevention of germ cell loss

The onset of production of spermatozoa (spermatozoa) starts at puberty, but it is not exactly known in what stage of pubertal development spermatozoa are produced. Some data from urine examination and electro-ejaculation done in pubertal boys suggest that clinical parameters of puberty (eg, testicular size), Tanner stage, and a rise in reproductive hormones do not always coincide with spermatogenesis. 31,47 Spermatozoa can even be found in the urine of boys who have no clinical signs of puberty. In a cohort of 80 pubertal boys, van Casteren and colleagues 9 could not do semen cryopreservation.
Fertility preservation

- Sperm Banking: Preferentially before orchiectomy
- Onco-TESE: sperm extraction from the affected testis immediately after orchiectomy

<table>
<thead>
<tr>
<th></th>
<th>Preorchiectomy</th>
<th>Postorchiectomy</th>
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<tbody>
<tr>
<td>Prefreeze progressive motility (WHO a + b combined), %</td>
<td>34.70 ± 19.82</td>
<td>36.90 ± 20.24</td>
</tr>
<tr>
<td>Postthaw progressive motility (WHO a + b combined), %</td>
<td>14.70 ± 13.18</td>
<td>18.90 ± 13.287</td>
</tr>
</tbody>
</table>

Abbreviation: WHO, World Health Organization.
Bilateral Testicular Cancer

- 1-4% of TGCTs
- 62-88% are metachronous, 50-76 months after the first cancer.
- Stage 1 in 43-90%
- 93% 10-year OS in the metachronous group compared with 85% in the synchronous group

Fossa et al. J Natl Cancer Inst 2005
Bilateral Testicular Cancer

- 25 cases of bilateral synchrone and metachrone testicular tumours treated TSS
- Age was 31.9 (±1.04) years, tumour size was 11.66 (±1.49) mm.
- 11 seminoma, 9 non-seminomatous or mixed germ cell tumours, 4 Leydig T. and 1 hamartoma.
- OS :100%, 12% (3 pts) local recurrence after a mean follow-up of 42.7 months.
- Radical orchiectomy was performed for six patients.
- No patient with a preserved testicle required androgen therapy;
- The mean postoperative total testosterone level was 4.0 ng/mL.

Ferretti et al BJUInt 2014
Testicular sparing surgery

- Mass <2 cm (30%)
- Simultaneous bilateral tumours,
- in solitary testicle
- normal serum testosterone,
- Biopsy on adjacent parenchyma (Cis: 80%),
- 20 Gy radiotherapy if Cis.

- Radiotherapy can be delayed / fertility preservation.
- Simultaneous TESE is possible

Reco EAU and AUA / Russo et al EMJ 2015
Testicular Sparing surgery / Testicular Tumor and infertility

• Series of 22 TT discovered in infertile men
  ▪ Mean diameter: 8.1 mm

• Peroperative frozen sections:
  ▪ Good diagnosis 17/21

• Final pathology:
  ▪ 19 Leydiig cell tumors
  ▪ 1 seminoma
  ▪ 1 teratoma
  ▪ 1 burned-out

• Surveillance/ US must be offered in nodules < 5mm

• Testicular sparing surgery must be offered when feasible

• ONCO-TESE should be performed when needed
Fertility after TC treatment

• 1/3 try to conceive

• 2/3 achieve to conceive: 20% /ART (10% using cryopreserved Spz)

Fig. 1. Number (n) of patients attempting to conceive and post-treatment outcome, without the use of cryopreserved semen, in relation to pretreatment fatherhood. The median age (range) in years (y) at orchietomy is given.

Fig. 2. Actuarial post-treatment paternity rates in each treatment group for patients who attempted conception without the use of cryopreserved semen. P<.001 from two-sided log-rank test. RPLND = retroperitoneal lymph node dissection; RT = radiotherapy; cis = cisplatin. Vertical bars indicate 95% confidence intervals.

Bridoy et al J Natl Cancer Inst 2005
Conclusions

• Patients with isolated TM should be reassured and educated to practice self-examination.

• Patients with TM associated with TC risk factor must be follow and should be offered a biopsy.
  □ Bilateral TM in infertile men : 20% ITCGN

• In patients with Cis Surveillance, Radiotherapy or Orchiectomy must be discussed according to fertility expectation, number of testicles, associated condition.

• Infertility: increased risk of Testicular cancer x2 (increase risk of melanoma, PC, BC, HL, NHL and leukemia)

• In infertile men with testicular tumor
  □ Surveillance must be proposed if <5mm
  □ Partial orchiectomy+biopsies if tumor <30% of testicular volume
    □ Bilateral TT
    □ Single testicle
    □ (Small testicles)
Cas clinique
Mr L. 23 ans

- 1993: Tumeur du testicule droit: orchidectomie dte
- Carcinome embryonnaire pT1 - stade I
  - Pas de conservation de sperme pré-opératoire
  - Surveillance simple
- 2001: Consulte pour infertilité
  - Échographie testiculaire G: Formation hypo-échogène sous capsulaire de 14 mm de grand axe
  - Marqueurs normaux
  - Spermogramme : OATS
    - Cryopréservation: 0% typiques
- Pas de partenaire mais désir de paternité
Conduite à tenir ?

1. Surveillance
2. Orchidectomie gauche
3. Tumorectomie + biopsies à distance
4. Irradiation 18-20gy
Tumorectomie

- **Tumorectomie testicule gauche, biopsie de pulpe testiculaire à distance.**
- **Histologie** : séminome pur associé à de la néoplasie germinale intratubulaire
- **TESE** : cryoconservation de spermatozoïdes
Conduite à tenir ?

1. Irradiation 18gy
2. Carboplatine AUC 7
3. Orchidectomie gauche
4. Surveillance
Mr L. 31 ans

• 2001-2005

• Surveillance :
  - plage intra parenchymateuse hypo-échogène ovale de 5 mm de grand axe au pôle supérieur du testicule
  - Testostérone normale
  - PET scan et scanner normaux
  - Marqueurs normaux

• Rencontre M. 30 ans
  - FIV-ICSI 10/2005 avec paillettes (Azoo)
    - Maxime
    - 2 embryons congelés
Conduite à tenir ?

1. Irradiation 18 gy
2. Surveillance
3. Orchidectomie
Mr L.

- Surveillance → 07/07: Choix du patient
  Modification des zones hypo-échogènes sous forme nodulaire à l’échographie.
- Examen: TT 1cm
- Scanner thoraco-abdomino pelvien normal.
- Marqueurs négatifs
  - Conduite à tenir ?
Orchidectomie Gauche

• 09/07 Orchidectomie par voie inguinale
• Congélation de pulpe testiculaire: pas de spz
• Histologie : séminome pur
• Androgénothérapie substitutive
• Surveillance
• Réimplantation des embryons: échec.