# Assisted reproduction using testicular tissue

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## Outline

- Clinical applications of testicular sperm for ART
- Overall reproductive results with surgically retrieved sperm
- Results in specific conditions
- The limits of gamete function in extremely damaged testes
- Child health and safety issues
- What needs to be improved



## Clinical applications of ART using testicular sperm

- Obstruction
  - Failure of vasovasostomy
  - Epididymal obstruction
  - CBAVD
  - Postinflamatory
- Transport
  - Psychogenic anejaculation
  - Spinal cord injury
  - Lymphadenectomy
- Function
  - Total asthenozoospermia
  - High DNA damage

- Production
  - Idiopathic azoospermia
  - Cryptorchidism
  - Iatrogenic (chemotherapy radiotherapy)
  - Yq microdeletions
  - Klinefelter's syndrome
  - Hypogonadotrophic hypogonadism

## The special features of testicular sperm

- None or minimal motility
- Defective morphology
- Unable to undergo capacitation and acrosome reaction
- No binding to ZP
- Incomplete protein transition (histones > protamines)
- Incomplete chromatin condensation
- Abnormal epigenetic profile

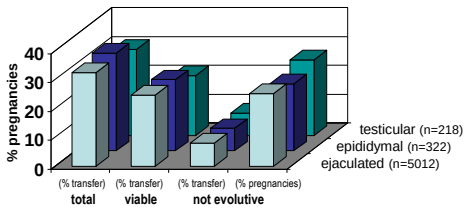


- Clinical applications of testicular sperm for ART
- Overall reproductive results with surgically retrieved sperm



## Reproductive results of surgically retrieved sperm

*Early reports of pregnancies*




*Tarlatzis (1998)*

## Meta-analysis of reproductive outcomes in OA and NOA

| Patients OA/NOA | OR (95% CI)* for pregnancy rates | | P | References |
|---|---|---|---|---|
| 48/26 | 1.3 | (0.7 – 2.6) | 0.49 | *Ghanem (2005)* |
| 52/123 | 1.1 | (0.6 – 2.0) | 0.76 | *Friedler (2002)* |
| 103/119 | 3.7 | (1.6 – 7.4) | <0.01 | *Mansour (1997)* |
| 129/40 | 1.3 | (0.8 – 2.4) | 0.25 | *Palermo (1999)* |
| 139/54 | 1.0 | (0.5 – 1.7) | 1.10 | *De Croo (2000)* |
| 471/362 | 1.53 | (0.83 – 2.74) | 0.17 | Overall result |

*OR >1 favours OA
*OR <1 favours NOA

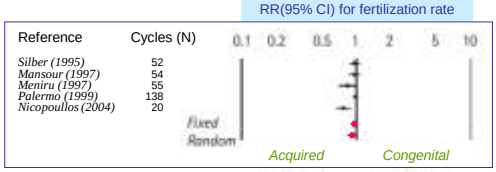
Higher fertilization rate in OA patients (65%) than in NOA patients (54%)

*Ghanem (2005)*

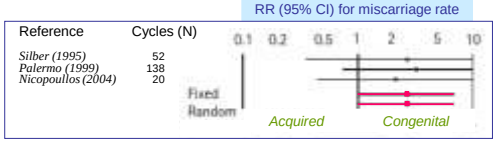


## Cause of obstruction and ART outcome

RR(95% CI) for fertilization rate




RR (95% CI) for miscarriage rate




*Nicopoullos (2004)*



## Sperm aneuploidy and source of spermatozoa

sperm aneuploidy rate (%)

| Testicular | Epididymal | Ejaculated (normal) | References |
|---|---|---|---|
| 51.6* | 7.7*§ | 2.3 | *Bernardini (2000)* |
| 1.4 | -- | 0.9 | *Martin (2000)* |
| 19.6* | 8.2*§ | 1.6 | *Levron (2001)* |
| 11.4* | 1.8§ | 1.5 | *Palermo (2001)* |
| 4.3* | 2.6* | 0.9 | *Burrello (2002)* |
| 8.2 | 5.6 | -- | *Mateizel (2002)* |
| 1.3 | -- | 0.4 | *Martin (2003)* |
| 5.9* | 0.8 | 0.5 | *Rodrigo (2004)* |

*p<0.05 vs ejaculated
§p<0.05 vs testicular

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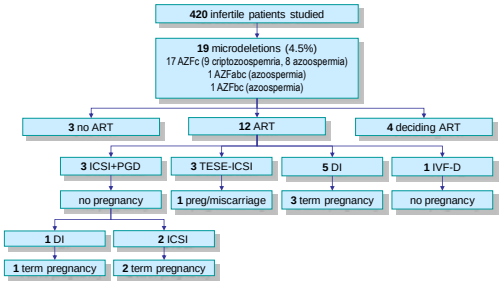
- Overall reproductive results with surgically retrieved sperm
- Results in specific conditions



## The choice and outcome of fertility treatment in 19 patients with Yq microdeletions




*García et al (2009)*



## Sperm findings and reproductive treatments in men with Yq microdeletions

| Patients n | SC >0[a] % | TESE + n/t (%)[b] | ART[c] (n) | CPR[d] n (%) | References |
|---|---|---|---|---|---|
| 48 | 33 | 14/21 (66) | 26 | 11 (42) | *Oates (2002)* |
| 38 | 26 | 10/28 (36) | 16 | 7 (43) | *Stouffs (2005)* |
| 39 | 38 | 7/16 (44) | 6 | 1 (16) | *Simoni (2008)* |
| 12 | 33 | 7/12 (58) | 13 | 9 (75) | *Palermo (2008)* |
| 63 | 38 | 6/27 (22) | 23 | 18 (78) | *Patrat (2010)* |

a: sperm concentration higher than 0 in semen
b: patients with positive sperm in testes/total attempted (%)
c: patients treated by ICSI with testicular or ejaculated sperm
d: pregnancies (cumulative clinical pregnancy rate per couple)

Overall cumulative PR per couple initially screened : 46/200 (23%)

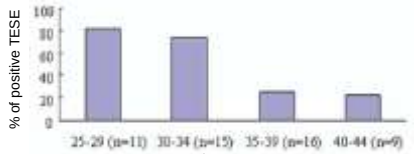
## TESE-ICSI in patients with non-mosaic Klinefelter's syndrome

| Cycles n | TESE +[a] n (%) | CPR[b] n (%) | References |
|---|---|---|---|
| 10 | 4 (40) | 0 (0) | *Tournaye (1996)* |
| 20 | 8 (40) | 4 (50) | *Levron (2000)* |
| 12 | 5 (42) | 5 (50) | *Friedler (2001)* |
| 11 | 6 (55) | 2 (33) | *Ulug (2003)* |
| 54 | 39 (72) | 22 (56) | *Schiff (2005)* |
| 17 | 6 (35) | 7 (77) | *Kyono (2007)* |
| 39 | 22 (56) | 7 (39) | *Yarali (2009)* |
| 68 | 45 (66) | 33 (53) | *Ramasami (2009)* |

a: patients with some sperm in testes (%)
b: clinical pregnancies (% per transfer)



## Age and residual spermatogenesis in Klinefelter syndrome




| | sperm positive (n=26) | sperm negative (n=25) | P |
|---|---|---|---|
| Age (years) | 31 | 38 | <0.001 |
| Testicular volume (mL) | 2.8 | 2.7 | NS |
| FSH (mUI/mL) | 29 | 27 | NS |
| Testosterone (ng(mL) | 2.9 | 2.2 | NS |

*Okada (2005)*



## Testicular sperm in hypogonadotrophic hypogonadism with persistent azoospermia

| Cycles (n) | FR (%) | ET (mean) | CP [n (%)] | Miscarriages (n) | References |
|---|---|---|---|---|---|
| 1 | 50 | 3 | 0 | -- | *Meseguer (2004)* |
| 17 | 42 | -- | 3 (17.6) | 0 | *Fahmy (2004)* |
| 6 | 45 | 3 | 3 (50) | 1 | *Arkasu (2009)* |

FR: fertilization rate
ET: embryos transferred
CP: clinical pregnancies (% per cycle)

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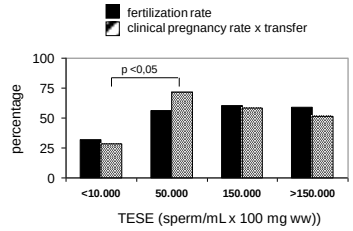
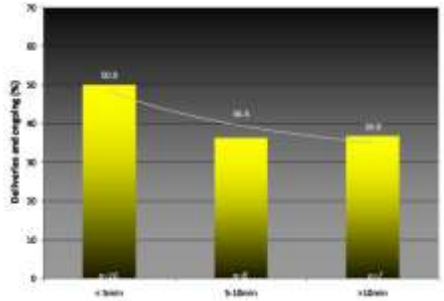
## Lower pregnancy rate in very poor testicular sperm yield




*Garcia (2008)*

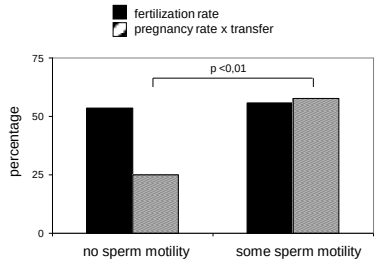


## Developmental competence of testicular spermatozoa in extreme NOA



*Chen (2009)*

## Lower pregnancy rate with totally immotile testicular sperm




*Garcia (2008)*



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## Malformations and the origin of non-ejaculated sperm

| | OA | NOA | Other |
|---|---|---|---|
| Children born (n) | 282 | 76 | 54 |
| Multiple pregnancies (%) | 38 | 37 | 52 |
| Major malformations (%) | 4.2 | 0 | 3.7 |

| Total Malformations | Testicular sperm | Epididymal sperm |
|---|---|---|
| Males | 5/159 (3.1%)* | 2/38 (5.2%) |
| Females | 2/169 (1.2%) | 3/56 (5.3%) |
| Sex ratio** | 47% | 40% |

* 3 hypospadias (1.6%) 5-fold higher compared to expected 0.28%
** ICSI with ejaculated sperm (53%)
general population (51% )

*Fedder (2007)*

## Birth defects using surgically retrieved sperm

| Fetal karyotypes | | | |
|---|---|---|---|
| Testicular sperm | Ejaculated sperm | RR (95% CI) | References |
| 3/63 (4.8) | 45/1469 (3.1) | 1.53 (0.49 – 4.79) | *Bonduelle (2002a)* |
| 2/128 (1.5) | 10/504 (1.9) | 0.79 (0.18 – 3.57) | *Jozviak (2004)* |

| Major malformations | | | |
|---|---|---|---|
| Testicular sperm | Ejaculated sperm | RR (95% CI) | References |
| 6/206 (2.9) | 84/2477 (3.4) | 0.86 (0.38 – 1.84) | *Bonduelle (2002b)* |
| 3/147 (2.0) | 139/4248 (3.3) | 0.62 (0.20 – 1.93) | *Kallen (2005)* |
| 21/229 (9.2) | 248/2944 (8.4) | 1.09 (0.71 – 1.66) | *Ludwig (2002)* |
| 1/87 (1.1) | 33/1774 (1.9) | 0.62 (0.009 – 4.47) | *Palermo (2000)* |
| 0/31 (0.0) | 39/934 (4.2) | 0 | *Wennerholm (2000)* |

*Woldringh (2010)* 22

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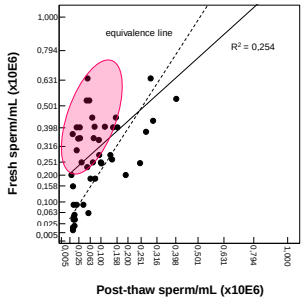
## Freezing and the availability of testicular sperm




*Garcia (2008)* 24

## Molecular markers used to predict the presence of sperm in testicular tissue

| | Sensitivity | Specificity | Reference |
|---|---|---|---|
| Telomerase | 87.5 | 100 | *Yamamoto (1999)* |
| DAZ | 83 | 100 | *Kuo (2004)* |
| DAZL | 78 | 94 | *Lin (2005)* |
| BOULE | 100 | 100 | *Lin (2005)* |
| CDC25A | 84 | 100 | *Cheng (2006)* |
| MSH4 | 80 | 100 | *Terribas (2010)* |

Do not avoid testicular biopsy
Dependent on the sampling procedure



## Summary and final remarks (1)

- Surgically retrieved sperm have good reproductive potential
- Similar ART results with sperm from epididymal and testicular origin
- No detrimental effect of freezing sperm
- More miscarriages are observed in pregnancies with non-ejaculated sperm
- Almost half of NOA patients with constitutional conditions can be treated with good chances of pregnancy



## Summary and final remarks (2)

- Age has a detrimental effect on residual spermatogenesis of KS, but not in other congenital problems
- Sperm function worsens in extremely impaired spermatogenesis
- Testicular or epididymal sperm do not increase the risk of malformations compared with ejaculated sperm
- There is a need for accurate and non-invasive markers of spermatogenic reserve

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