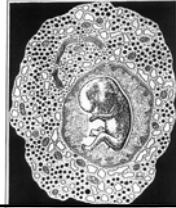




Testicular biopsy - a valuable source of spermatozoa

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UNIVERSITY OF ZAGREB



1669



AZOOSPERMIA CASE



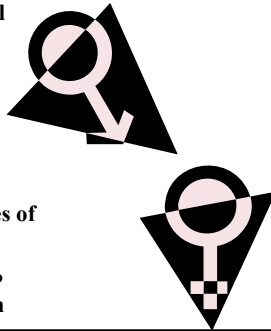
- 34 y. patient complaining on infertility problems;
- married for 3 years, the couple was not able to conceive;
- p. had usual diseases in the childhood, including parotitis without any sequelae;
- no history of infertility in the family (brother with 2 children);
- female partner was in good health, also without any history of infertility in her family;

AZOOSPERMIA CASE



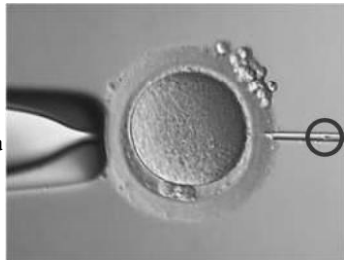
INFERTILITY

- Multicentric epidemiological studies:
- Ejaculate volume ↓
- Sperm concentration ↓
- Sperm motility ↓
- EU: 15% couples infertile
- Croatia: 8.000 newborns missing per anum/130 classes of elementary school
- Male factor infertility: 7-8%
- 50-70% aetiology not known



INTRACYTOPLASMIC SPERM INJECTION into the oocyte (ICSI)

- “Revolution in the treatment of male infertility”
- Patients with extreme oligospermia & azoospermia
- One sperm sufficient



What diagnostic parameters should be assessed BEFORE the biopsy?

- Anamnesis & status (testis consistency, volume)
- Detailed ejaculate analysis
- Hormones: FSH, LH, prolactin, testosterone, inhibin-B
- PSA
- Urine analysis (retrograde ejaculation)
- Microbiology (ejaculate, urethral smear, hepatitis, AIDS)
- Karyotype
- Y chromosome microdeletions
- Cystic fibrosis (in situ hybridization)



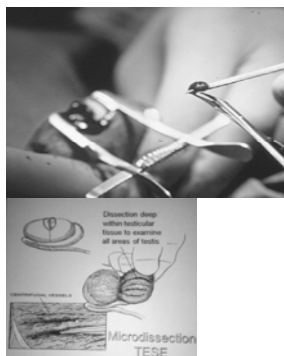
WHEN TO PERFORM BIOPSY OF THE TESTIS? (ASRM/ESHRE)

- Bilateral aplasia of d. deferens
- Idiopathic normogonadotrophic azoospermia
- Hypergonadotrophic azoospermia
- Ejaculation failure (resistant to treatment)
- Cryptozoospermia
- Necrozoospermia
- Inoperable posttesticular obstruction
- Operation of posttesticular obstruction



HOW TO DO IT?

- “Open” biopsy of the testis (+microsurgical exploration)
- Multiple biopsies form various sites (recommended)
- Histological analysis (recommended)



BIOPSY PROCEDURE



EMBEDDING MEDIA

PARAFFIN

- simple
- fast
- section thickness 4-7 μ m
- diagnostic standard?
- IHC
- in situ* hybridization

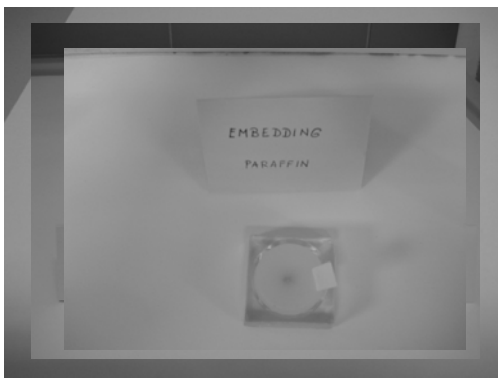
EPON

- polymerization 2-3 days
- section thickness $\leq 1\mu$ m
- diagnostic standard

UNICRYL

- polymerization 2-3 days
- section thickness $\leq 1\mu$ m
- IHC
- *in situ* hybridization

PARAFFIN TECHNIQUE



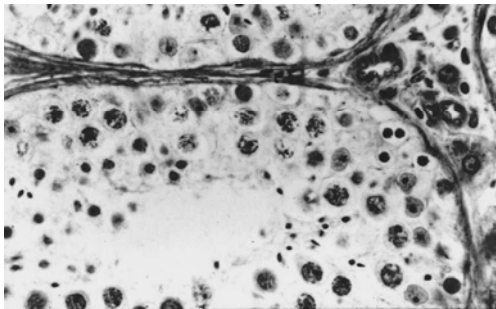
PARAFFIN SECTIONS



PARAFFIN TECHNIQUE

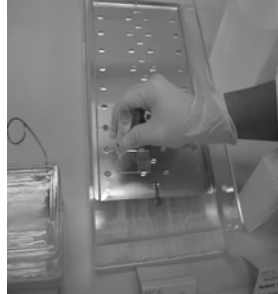
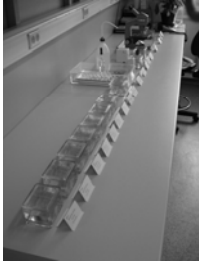


HUMAN TESTIS Paraffin Section

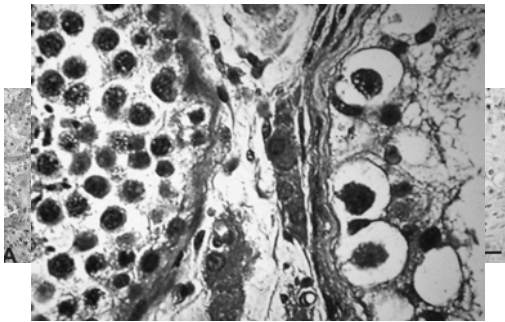


IHC

PALP
OCT-4

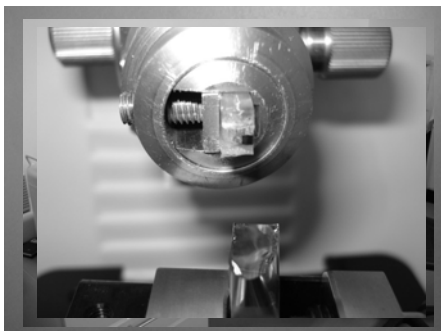


Ca in situ

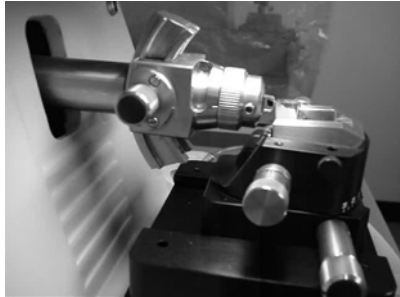


Sonne et al., 2004; Damjanov et al., 2009

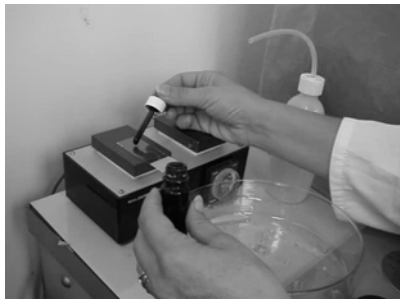
PLASTIC EMBEDDING



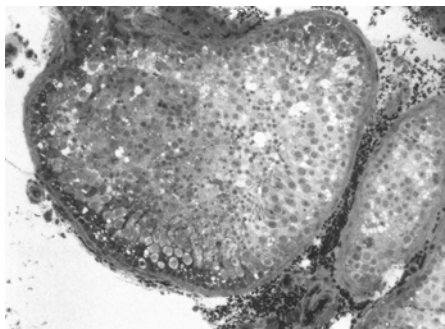
SEMI-THIN SECTIONS



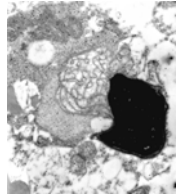
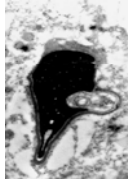
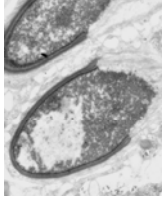
SEMI-THIN SECTIONS



HUMAN TESTIS
Semi-thin Section



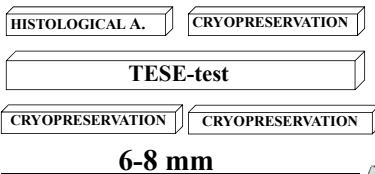
TEM



HISTOLOGICAL EVALUATION OF TESTICULAR BIOPSY

SCORE	MORPHOLOGY
1	Tubular sclerosis
2	Sertoli cells only
3	Spermatogonia only
4	Arrest at primary spermatocyte, no spermatids
5	Many spermatocytes, no spermatids
6	No late spermatids, arrest at spermatid stage
7	No late spermatids, but many early spermatids
8	Few late spermatids
9	Many late spermatids, disorganized tubular epithelium
10	Full spermatogenesis

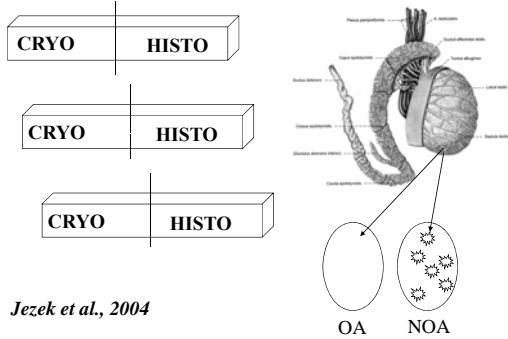
OPEN TESTICULAR BIOPSY “Sandwich Pattern”



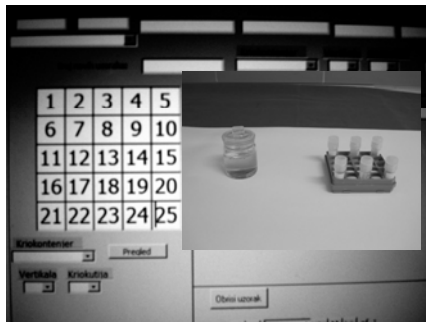
Jezeq et al., 1998



“PIECE *per* PIECE”



BANK OF CRYOPRESERVED TESTICULAR BIOPSIES



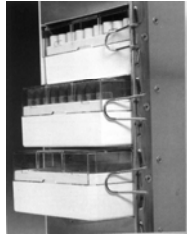
BIOPSY CRYOPRESERVATION

- Transport medium
- Freezing medium
- Programmed freezing (step wise)
- Usually 3 pieces/testis

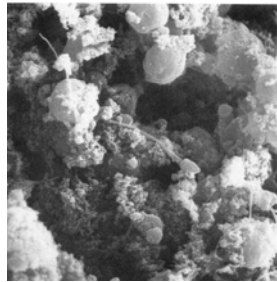
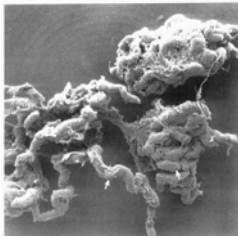


SPERM EXTRACTION

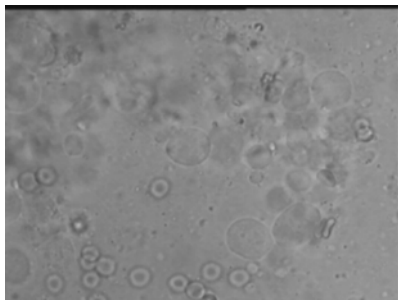
- Water bath incubation (37°C)
- Incubation with collagenase (CO₂ incubator)
- Biopsy maceration with sterile needles
- Centrifugation
- Sperm incubation in oil prior ICSI
- ICSI



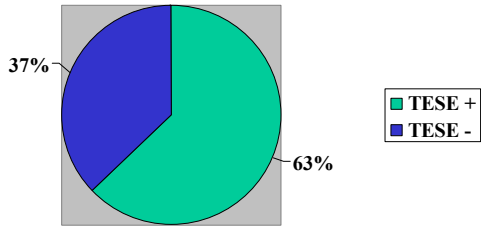
EFFECT OF COLLAGENASE



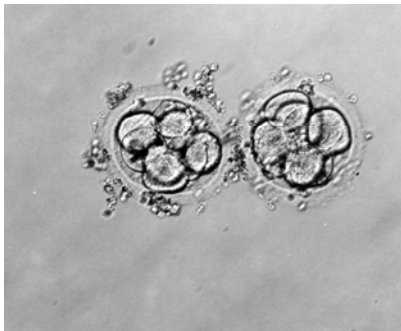
THAWING



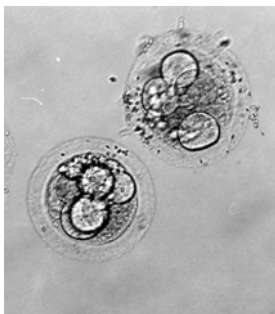
TESE RATIO
(N= 108, non-obstructive azoospermia)



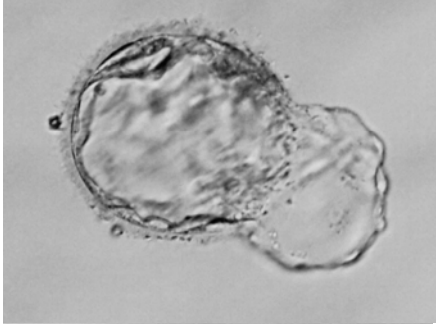
RESULT OF TESE/ICSI



FREEZING-THAWING OF EMBRYOS



BLASTOCYST CULTIVATION



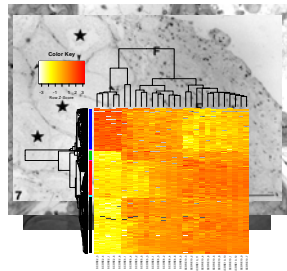
COMBINED TESE/ICSI USING CRYOPRESERVED TESTICULAR BIOPSIES

- No. of patients: 246
- No. of oocytes retrieved: 406 / 1.65 per patient
- Embryo transfer: 367 / 90% cycles
- Pregnancies: 75 / 18.5% per oocyte puncture / 20.4 % per transfer / 30.5% per patient
- Miscarriages: 9 / 12% from total No. of pregnancies
- Normal pregnancies: 37
- Delivery: 26 singletons; 7 twins + 1 triples



FUTURE DIRECTIONS

- Prevention (environment, life style, stress)
- Spermocyte, spermatogonia cultivation
- Stem cell isolation & cultivation
- DNA multiarray analysis, proteomics
- Genetic & protein markers for infertility



COLLABORATION

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Gregor Majdič
Marko Cotman



Some advantages and disadvantages of TESA and TESE methods:

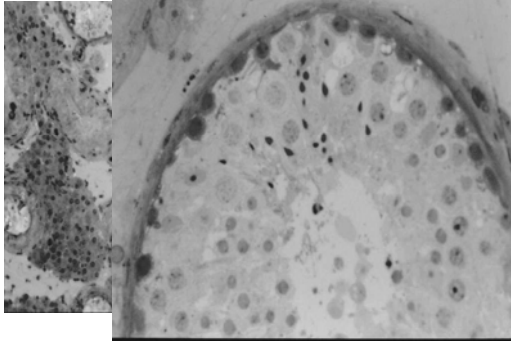
TESA

- Simple, fast, economic
- Local anaesthesia
- Good results in obstructive azoospermia treatment
- Cytological smear is used for diagnosis
- Multiple, must be repeated
- Sometimes no result despite of a positive cytology

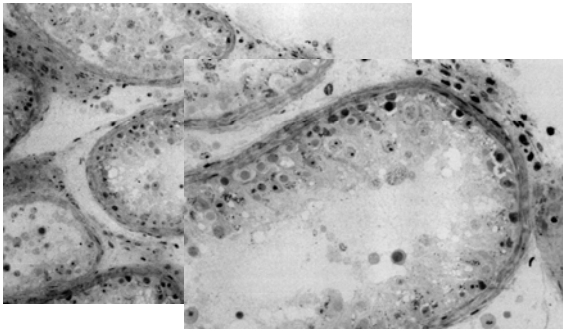
TESE

- Complex, expensive
- Local/general anaesthesia
- Method of choice in obstructive and non-obstructive azoospermia and TM of testis
- Semi-thin sections are used for diagnosis (Ca in situ diagnosis)
- Only one procedure is sufficient
- Treatment of a woman is time-independent from the operation of a man
- Pieces of biopsy can be frozen for possible repeated TESE /ICSI procedures

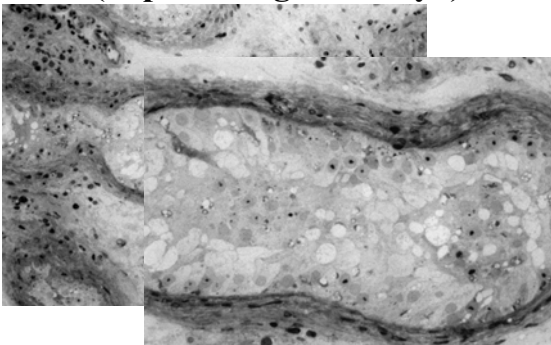
“MIXED ATROPHY”



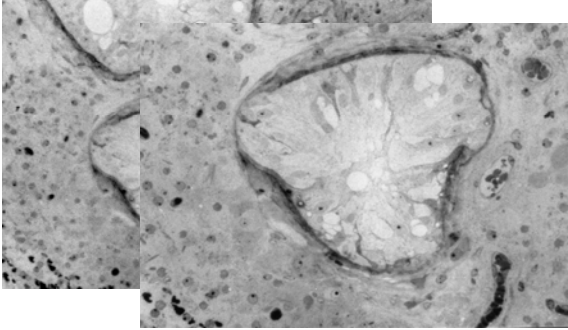
**SEMI-THIN SECTIONS
(Spermatid “stop”)**



**SEMI-THIN SECTIONS
(“Spermatogonia only”)**



**SEMI-THIN SECTIONS
("Sertoli cells only")**



**SEMI-THIN SECTIONS
(Tubular Fibrosis)**

