Non-Obstructive Azoospermia

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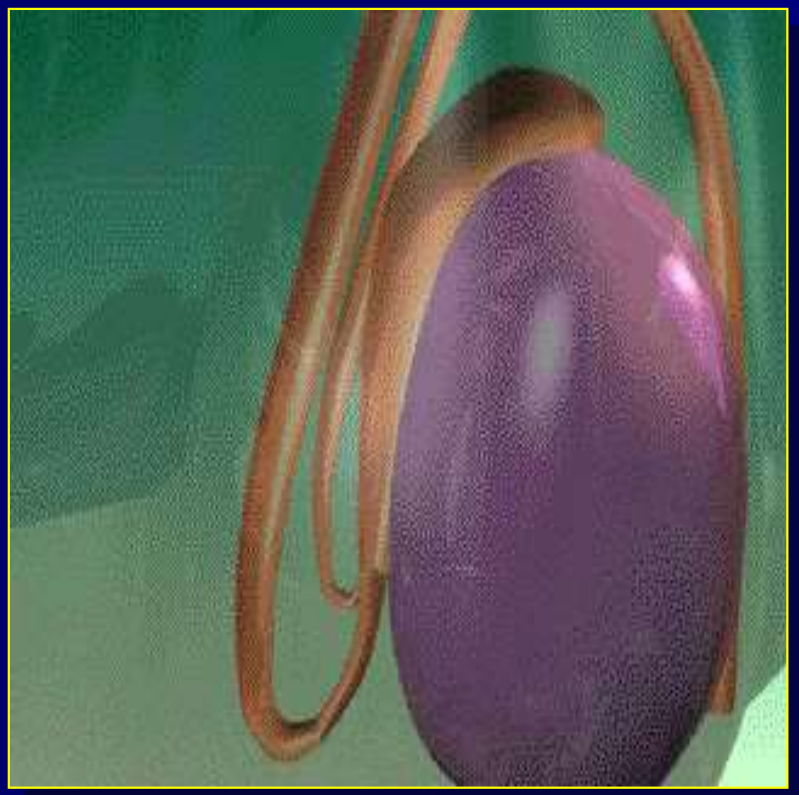

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Azoospermia

- Absence of sperm in ejaculate .
- Azoospermia is present in about 8% of infertile males (WHO. 1997)
- Diagnosis of azoospermia is made after examination of at least 2 semen samples obtained greater than 4 weeks apart .

Causes of Azoospermia

- Pretesticular failure
- Testicualr failure
- Post-testicular failure

Pretesticular failure

- Genetic abnormality
  - **Kallmann's syndrome**
  - **Prader-Willi syndrome**
  - **Cerebral ataxia with HH**
- Idiopathic HH
- Isolated LH deficeincy
- Isolated FSH deficiency
- Prolactin excess

Testicular failure

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- Genetic abnormality
  - Klinfelter's syndrome: nonmosaic, mosaic
  - XYY syndrome
  - 46 XX male syndrome
  - Yq AZF gene deletion
- Varicocele
- Bilateral anorchism, cryptorchidism
- Sertoli cell only syndrome
- Gonadotoxin : drug, radiation, chemical
- Orchitis

Evaluation

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- **History**
  - Infertility : duration, pregnancy
  - Developmental
  - Medical, surgical
  - Sexual
  - Family
- **Physical exam.**
- **Semen analysis**
- **Endocrine test**

Childhood and Developemental

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- Crytorchidism, testicualr torsion,
- Mumps orchitis
- Herniorrhaphy
- Onset of puberty
- Secondary sexual development
  - Onset axillary, pubic hair, start of shaving
- Onset of masturbation

Medical history

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- **Medical history**
  - Systemic illness: hepatic, renal failure
  - Gonadotoxins
    - **sulfasalazine, cimetidine, nitrofuratoin, chemotherapeutic, anabolic androgen**
    - **Thermal injury**
    - **Smoking, alcohol,marijuana**
- **Surgical history**
  - Herniorrhaphy, badder neck, orchiectomy, retroperitoneal surgery

Physical examination

- General appearance
- Gynecomastia
- Axillary, pubic hair
- Testis volume, consistency
- Epididymis induration
- Varicocele
- Digital rectal examination

Semen analysis

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- **At least 2 times analysis**
- Secretory azoospermia
  - **Pellet inspected after centrifugation at 1,500-2,000 rpm for 10min**
- **If ejaculatory vol < 1ml**
  - **Postejaculatory urine should be examined**

Ultrasound examination

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- **Scrotal US**
  - Testis volume
  - Varicocele
  - Testis tumor
- **Transrectal US**
  - Low volume azoospermia without absence of testicular atrophy
  - Palpable abnormality on DRE

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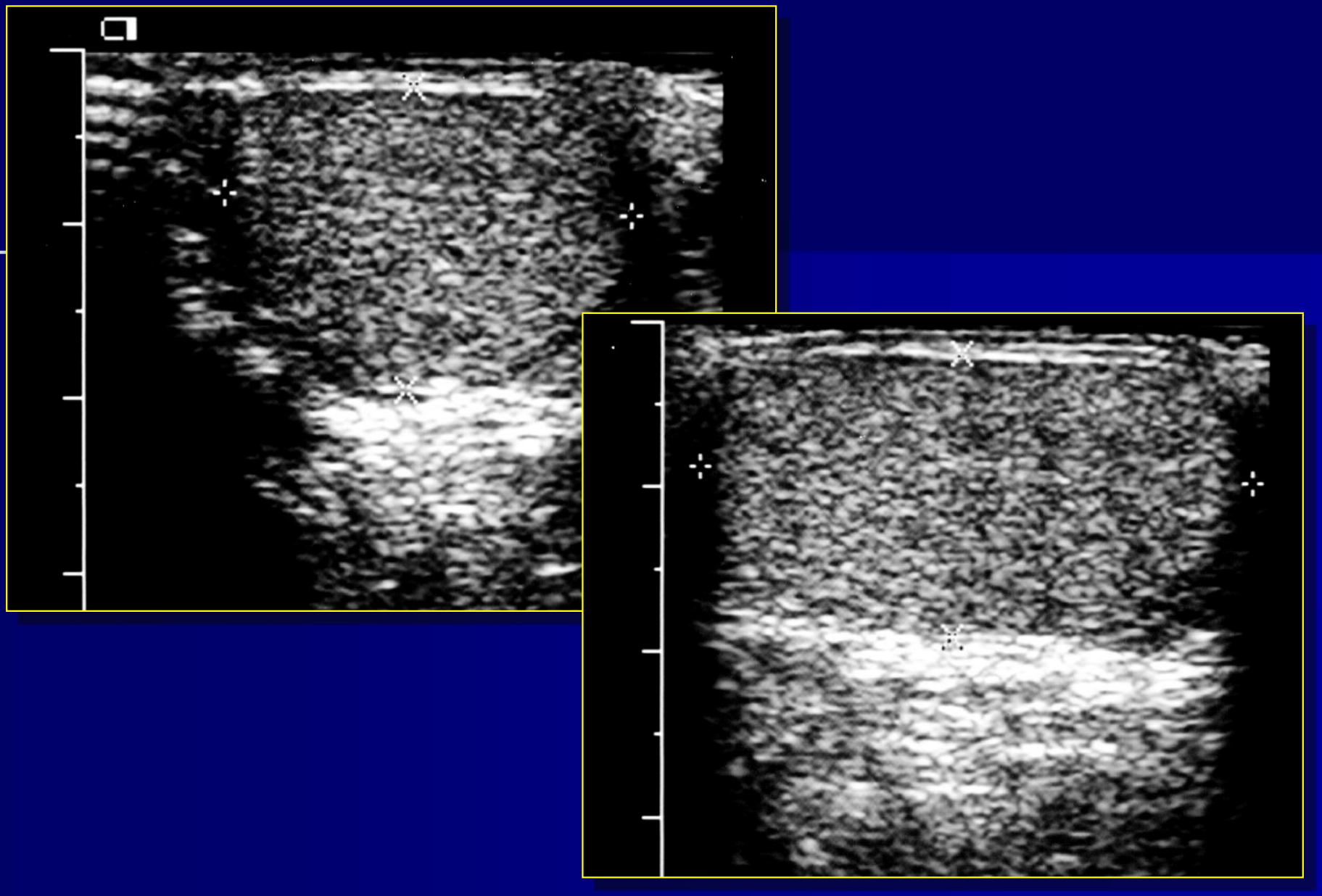

Volume(cc) = length x width x AP depth x 0.52

Hormonal status in clinical Dx

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Clinical status	FSH	LH	T
Germ cell aplagia	↑	Normal	Normal
Testicualr failure	↑	↑	Normal or ↓
HH	↓	↓	↓

Genetic evaluation of NOA

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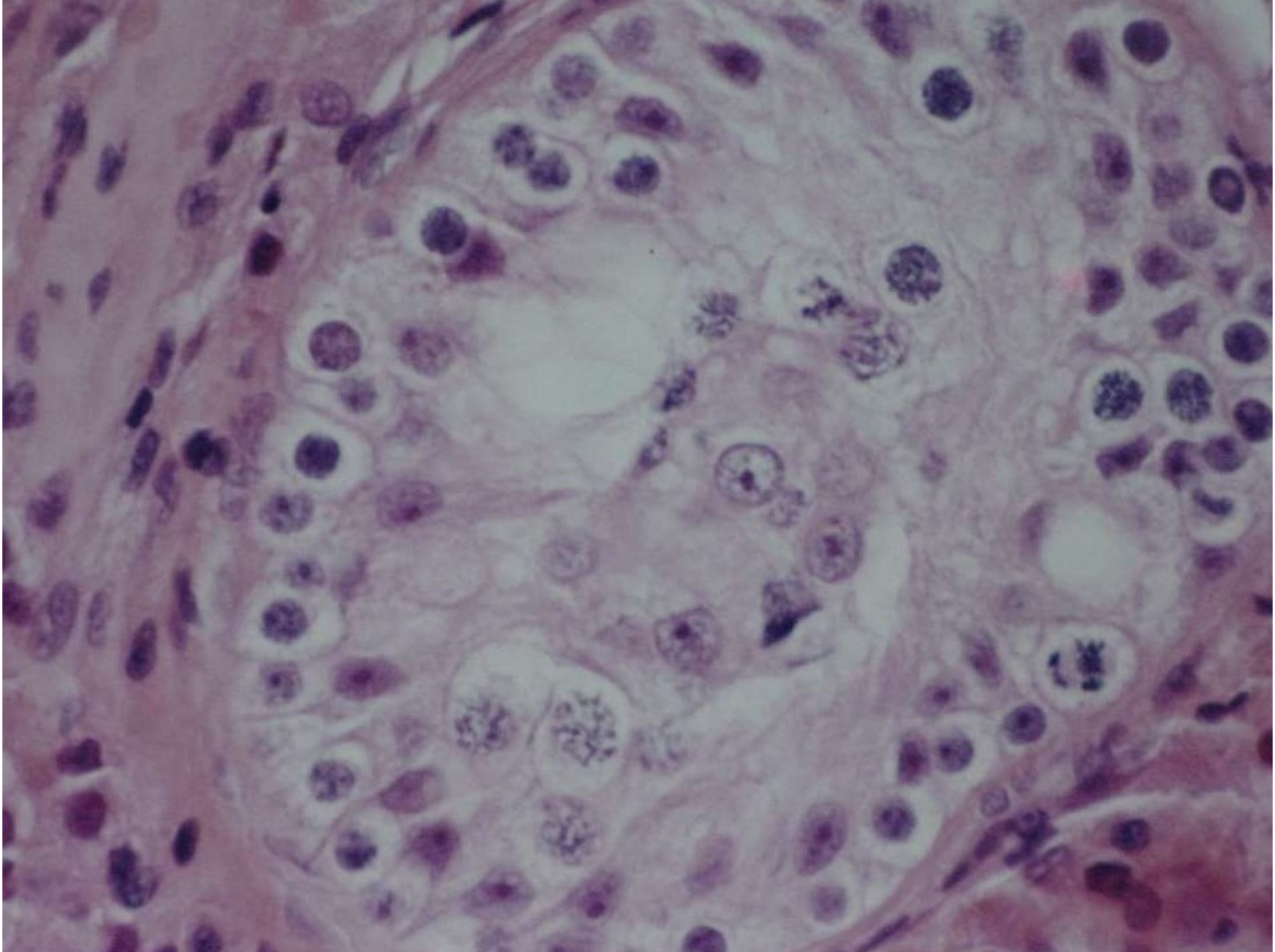
- **Sex chromosomal disorder**
  - Klinfelter's syndrome(1/500) :15% of NOA
  - XYY male(1/1,000), XX male(1/20,000)
- **Yq deletion : 10-20% of NOA**
- **X-linked :**
  - Kallamann's syndrome
  - Androgen receptor deficiency
  - Kennedy syndrome (spinal-bulabar muscular atrophy)
- **Autosomal defect**
  - Prader-Willi syndrome
  - Androgen synthesis deficiency



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MODERN TRENDS

Edward E. Wallach, M.D.
Associate Editor

Assisted reproductive techniques in patients with Klinefelter syndrome: a critical review

Dominik Denschlag, M.D.,[a] *Clemens Tempfer, M.D.,*[a] *Myriam Kunze, M.D.*[a] *Gerhard Wolff, M.D.,*[b] *and Christoph Keck, M.D.*[a]

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Objective: To summarize the existing experience with the use and success rate of assisted reproductive techniques (ART), in particular testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI), in Klinefelter patients.

Design: A systematic review of the literature, including all published case reports to date.

Patient(s): Thirty-nine reported successful pregnancies fathered by nonmosaic Klinefelter patients.

Main Outcome Measure(s): The overall risk of transmitting a chromosomal abnormality to the offspring of Klinefelter patients.

Result(s): In nonmosaic and mosaic Klinefelter patients, chromosomally normal sperm cells can be extracted from testicular tissue and used for ICSI.

Conclusion(s): The application of ART to Klinefelter patients can be recommended as a method to achieve reproduction in this selected infertility patient cohort. (Fertil Steril® 2004;82:775–9. ©2004 by American Society for Reproductive Medicine.)

Key Words: Klinefelter syndrome, male infertility, ICSI, TESE, genetic counseling

TABLE 1

Pregnancies induced by ICSI with sperm from nonmosaic Klinefelter patients.

First author, year (reference)	Sperm origin	Pregnancy outcome	Comment
Staessen, 1996 (31)	TESE	Biochemical pregnancy	
Bourne, 1997 (32)	Ejaculation	Twin birth	Frozen sperm
Hinney, 1997 (33)	Ejaculation	1st trimester abortion	
Tournaye, 1997 (34)	TESE	2× singleton birth	
Palermo, 1998 (35)	TESE	Singleton and twin birth	
Reubinoff, 1998 (23)	TESE-FNA	Singleton birth	
Nodar, 1999 (36)	TESE	Twin birth	
Ron-El, 1999 (37)	TESE	Singleton birth	
Kitamura, 2000 (38)	TESE	Singleton birth	
Levron, 2000 (14)	TESE	2× singleton, one twin, and one triplet birth	
Ron-El, 2000 (39)	TESE	Twin birth	Reduced triplet due to 47,XXY
Ron-El, 2000 (40)	TESE	Twin birth	Frozen sperm
Greco, 2001 (41)	TESE	Twin birth	
Kyono, 2001 (42)	TESE	Clinical pregnancy–2nd trimester	
Poulakis, 2001 (43)	TESE	2× singleton births	
Crüger, 2001 (44)	Ejaculation	Singleton birth	
Friedler, 2001 (45)	TESE	Singleton birth	2× twin and one singleton birth[a]
Rosenlund, 2002 (46)	TESE	Singleton birth	Frozen sperm and blastocyst
Bergere, 2002 (13)	TESE	Singleton birth	
Yamamoto, 2002 (10)	TESE	4× singleton and twin birth	
Tachdjian, 2003 (12)	Ejaculation	Twin birth	

[a] The other reported pregnancies (two twin and one singleton) have already been documented in earlier publications (33, 35, 36).

Klinefelter's Syndrome and ICSI

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	Palermo	Halabi
Testicular Biopsies	58	36
Sperm Recovered	42 (72.4%)	13 (36%)
Oocytes		
Injected	462	282
Fertilized	259 (56.1%)	105 (38%)
Deliveries	18 (31%)	1 (8%)
Children	21	1

Origin of Sperm in Non-Mosaic 47,XXY (I)

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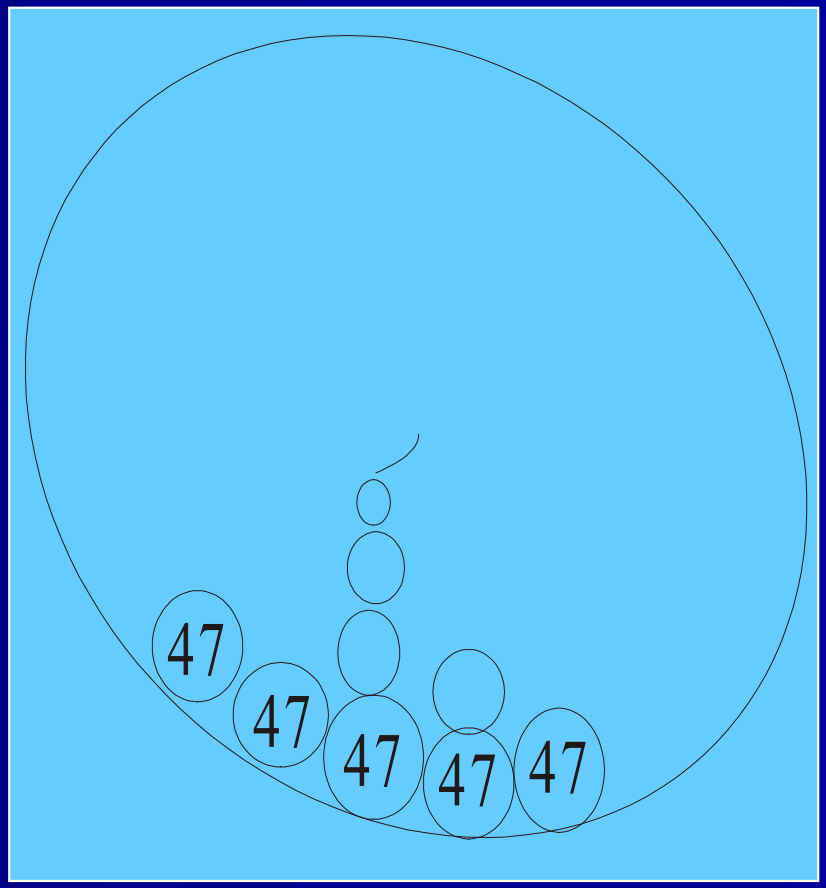
- XXY cell may enter and complete meiosis . Based on indirect evidence : presence of 24XX , 24XY spermatozoa in ejaculate potentially issued from XXY spermatogonia.



Origin of Sperm in Non-Mosaic 47,XXY (II)

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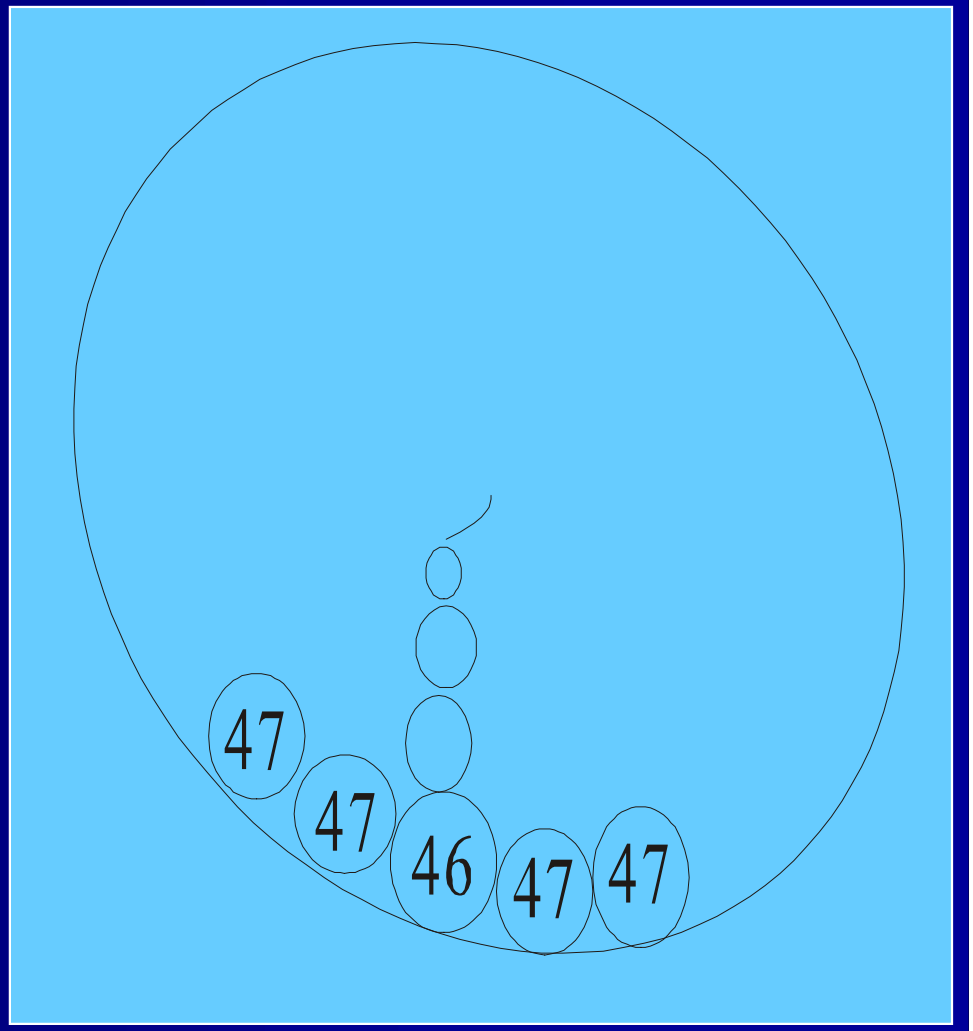
- Testicular mosaicism
- Testicular sex chromosome mosaicism may owe its importance for spermatogenesis to the fact that XY rather than XXY cell lines enter meiosis



Kline Felter Syndrom:Summary

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- TESE and ICSI can be successful in non-mosaic Klinefelter's patients.
- The genetic risks resulting from injection of such spermatozoa should be discussed with each couple.

Y Chromosome Gene Deletions

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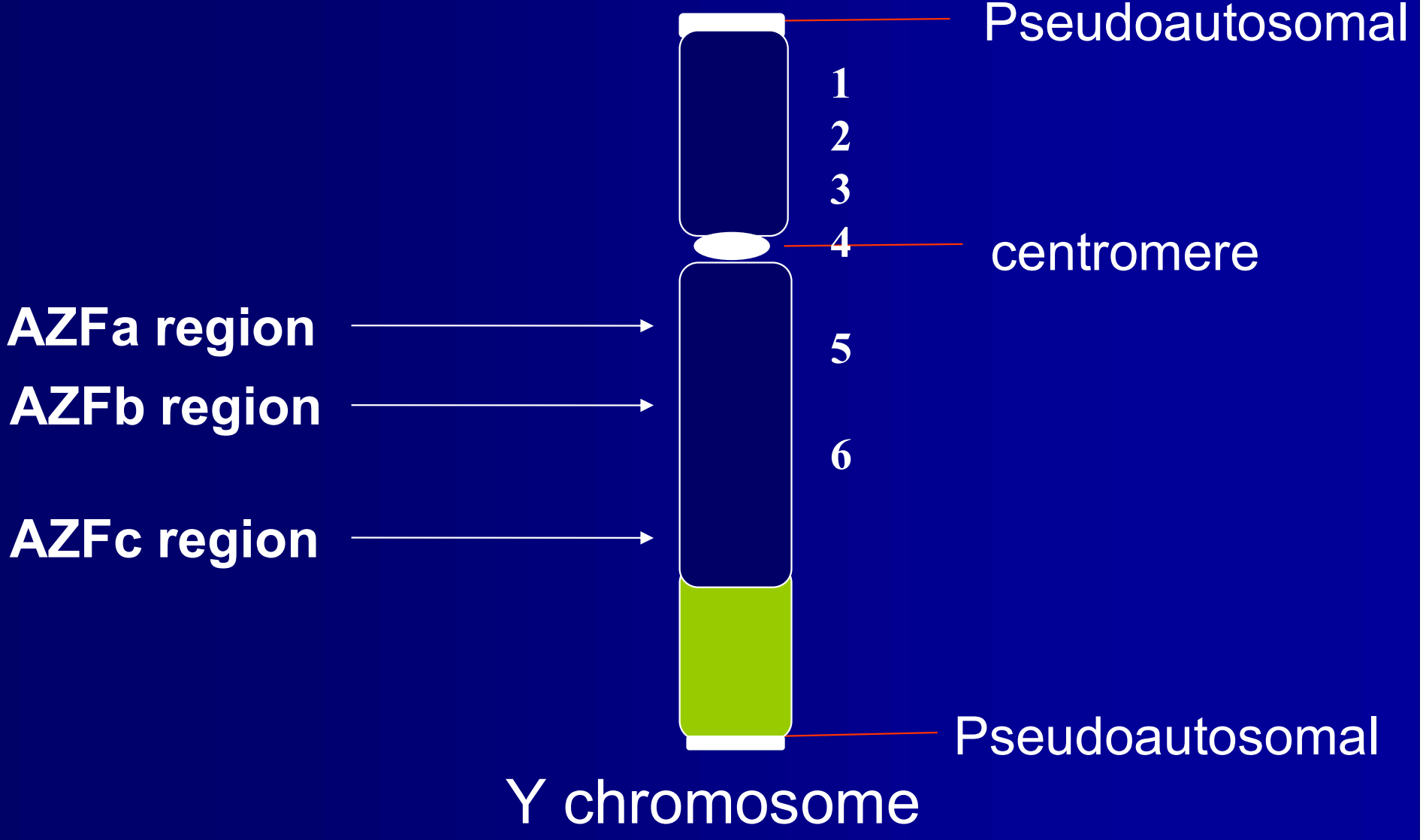

Genetics Counselling in Couples with Gene Deletion

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- Risk of transmitting microdeletion to their male offspring.
- Pre-implantation genetic diagnosis (PGD) and gender selection (girl) as an alternative.

Genetics Counselling in Couples with Gene Deletion

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- General information regarding the association of spermatogenesis defects and Y microdeletions.
- Treatment of microdeletion per se is not currently available.
- Microdeletions do not always preclude the potential for treatment with testicular sperm extraction and ICSI.

Chromosomal Abnormalities in the Spermatozoa of 46,XY Males

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Source	N	# of Cells Analyzed	Number of Sperm with (%)				
			Total Abnl	Nulli.	Autosome Disomy	Sex Chrom. Disomy	Diploid
Testicular (Non-Obst Azo)[a]	5	490	11.4	3.7	2	4.3	1.4
Epididymal (Obstr Azo)[b]	8	6,675	1.79	0.43	0.51	0.61	0.24
Ejaculated (Control	14	25,150	1.56	0.27	0.51	0.45	0.33

Palermo *et al Hum Reprod* 2002; 17:570-5

a>b>c; P<0.00001

Sertoli Cell Only Syndrome SCOS

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- Tournaye et al, 2004 reported successful retrieval in :

  86% of cases of in complete SCOS.

  19% of cases of complete SCOS.

Sertoli Cell Only Syndrome (SCOS)

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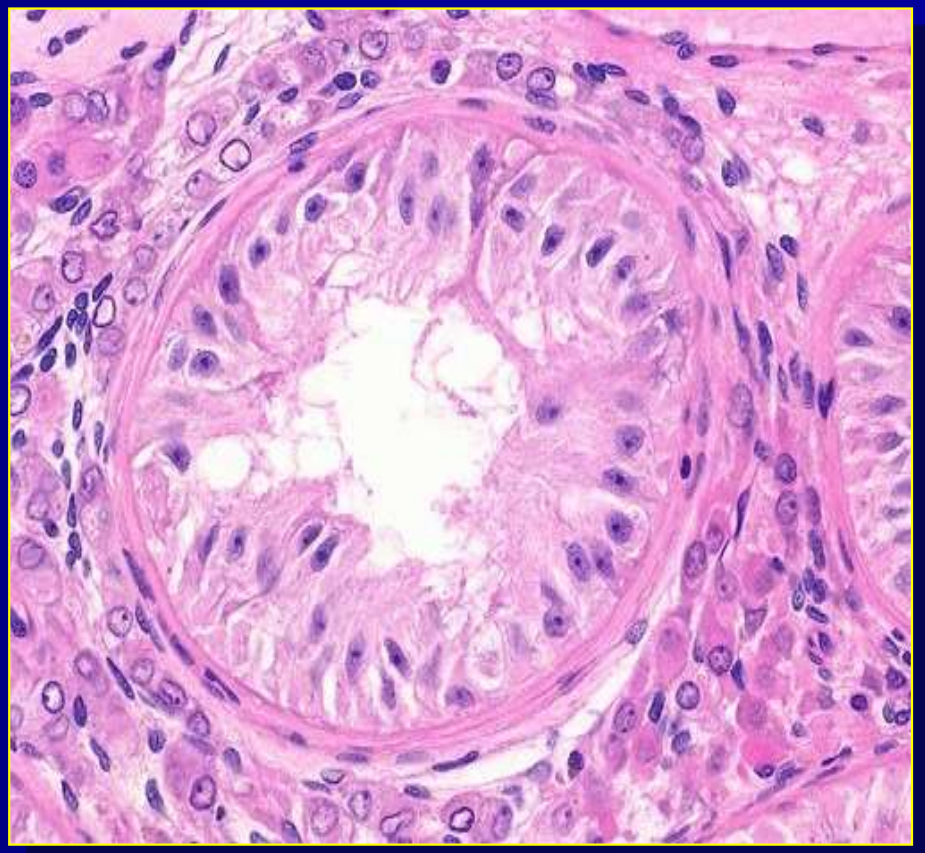
There are islands of spermatogenesis in testicular pulp even in worst prognosis histological Conditions, Such as SCOS.



Treatment of NOA

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- Therapeutic challenge .
- A variety of surgical and medical approaches have been used in attempts to improve the fertility potential in males with NOA.

- Varicocelectomy.
- Hormonal therapy.
- Clonidine therapy.
- Aromatase inhibitors.

Varicocelectomy

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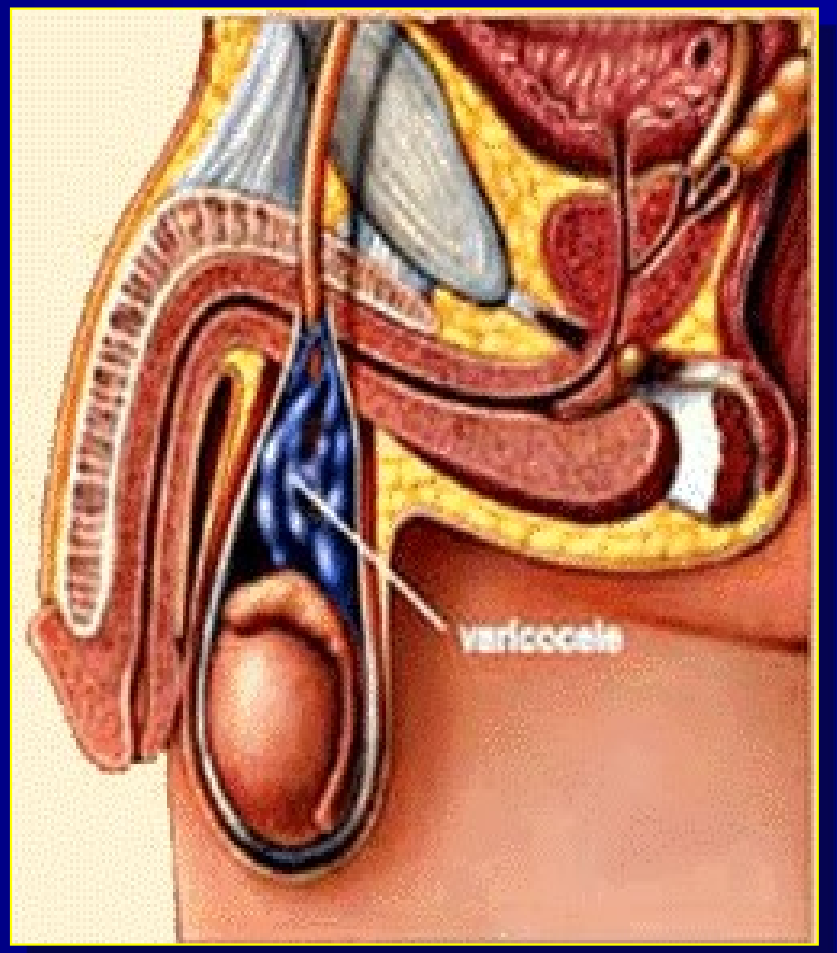
- Debate.
- Some believe that most of these men still require TESE.
- More recent work:
  - -Improves sperm retrieval in TESE (North et al, 2005)
  - -Return of sperm to the ejaculate (Pasqualotto et al, 2004)



Varicocelectomy

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TABLE IV —*Sperm occurrence in ejaculate following varicocelectomy in NOA patients.*

Authors	No. of azoospermic patients	Sperm occurrence in ejaculate post-varicocelectomy	%
Negri *et al.* (1998) [74]	6	2	33.3
Matthews *et al.* (1998) [75]	22	12	55
Kim *et al.* (1999) [76]	28	12	43
Kadioğlu *et al.* (2001) [77]	24	5	21
Pasqualotto *et al.* (2003) [78]	15	7	47
Kruse *et al.* (2003) [79]	1	1	
Cakan *et al.* (2004) [80]	13	3	23
Schlegel *et al.* (2004) [81]	31	7	22
Aponte *et al.* (2004) [82]	39	21	54
Total	179	70	39.1

Hormonal Therapy

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- Empiric treatment ⇒ ↑ Sperm Production
- Recent works: FSH treatment before TESE improved sperm retrieval in :
  - Normogonadotropic NOA patients (Aydos et al, 2005)
  - Hypogonadotropic Hypogonadism (Fahmy et al, 2004)

Hormonal Therapy

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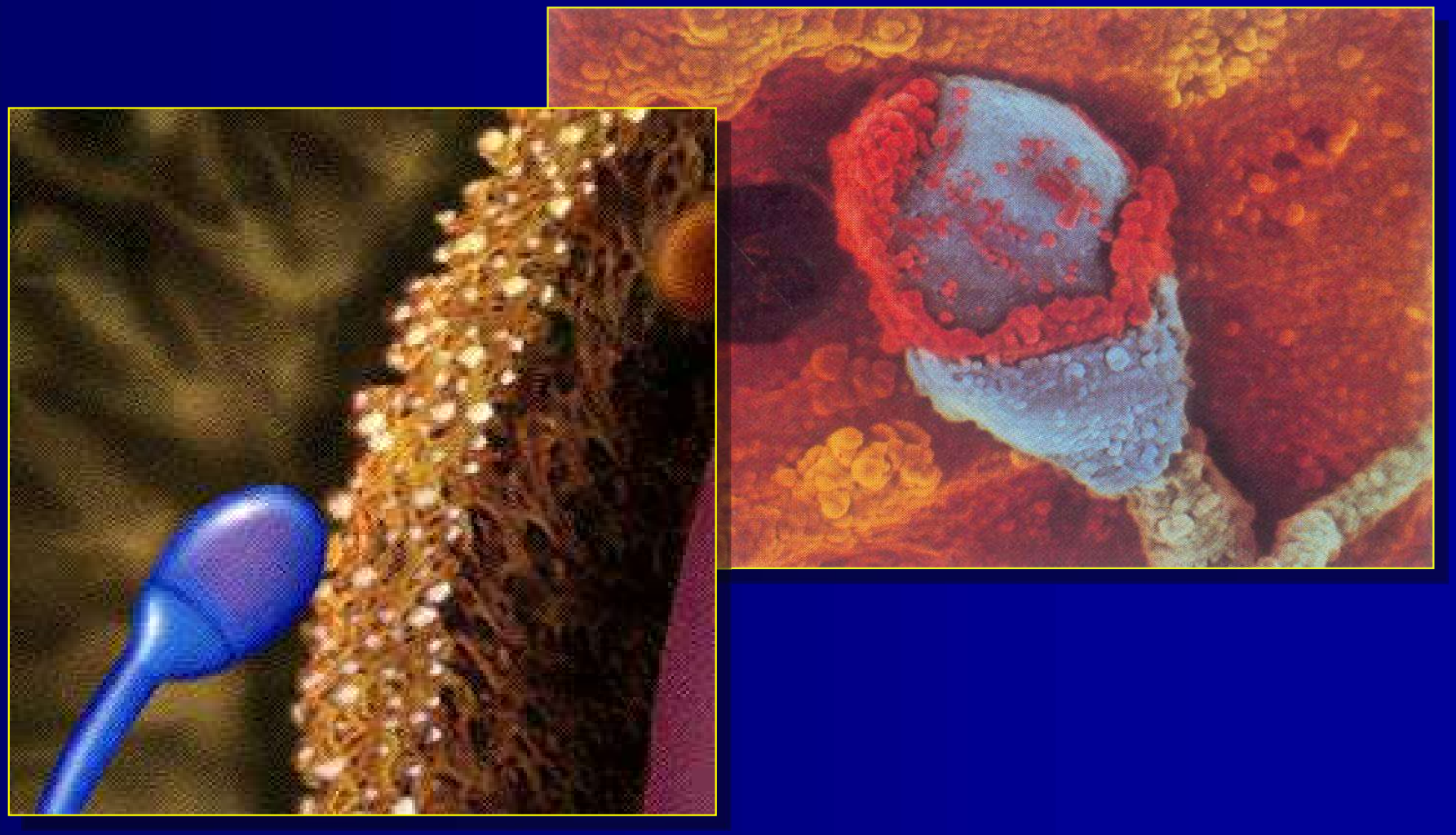

Clonidine Therapy

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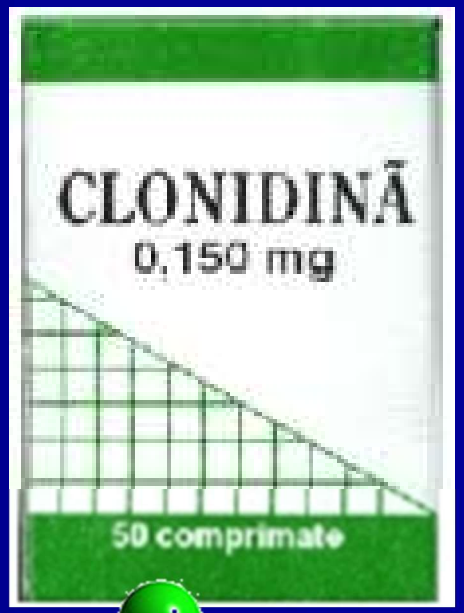
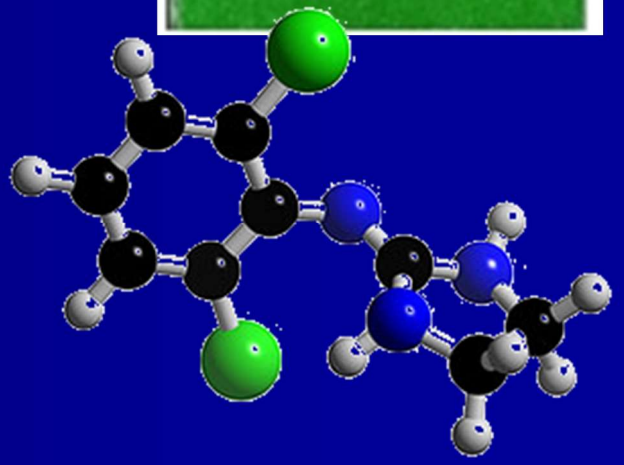
- Partial GH ↓ Sptd arrest.
- Clonidine ↑ GH.
- Clonidine therapy:
  - Return of sperm to ejaculate.
  - Initiated pregnancy.





Aromatase Inhibitors

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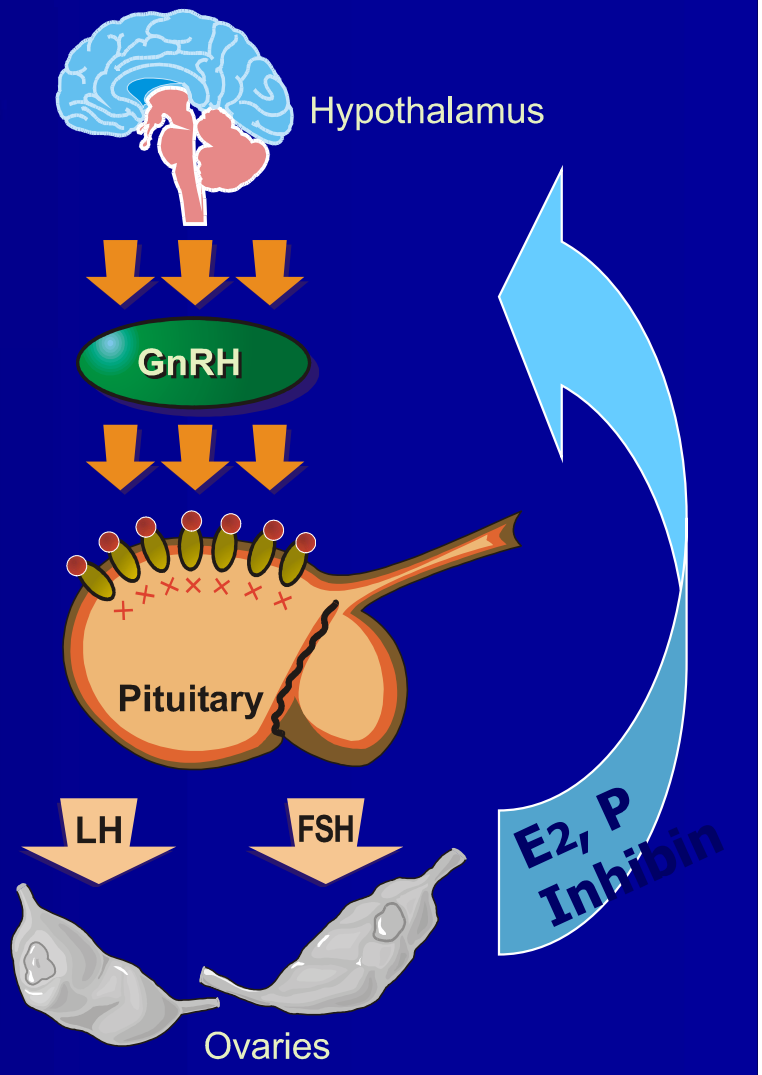
- Some men with NOA → abnormal T/E2.
- Aromatase inhibitors (testolactone & anastrozole)
  - Restore T/E2
  - Return of sperm to ejaculate (paviovich et al, 2004)



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Medical & Surgical Treatment

- No specific treatment is available.
- Before TESE/ICSI:

  - Treatment of correctable abnormalities
    - Repair of large varicocele.
    - Hormonal abnormalities.
    - Avoidance of :
      - Exogenous androgens.
      - Gonadotoxins.
    - 3-6 months

Medical & Surgical Treatment

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If female age is advanced, the benefit of treatment must be balanced against the rapidly declining female fertility potential

Intracytoplasmic Sperm Injection

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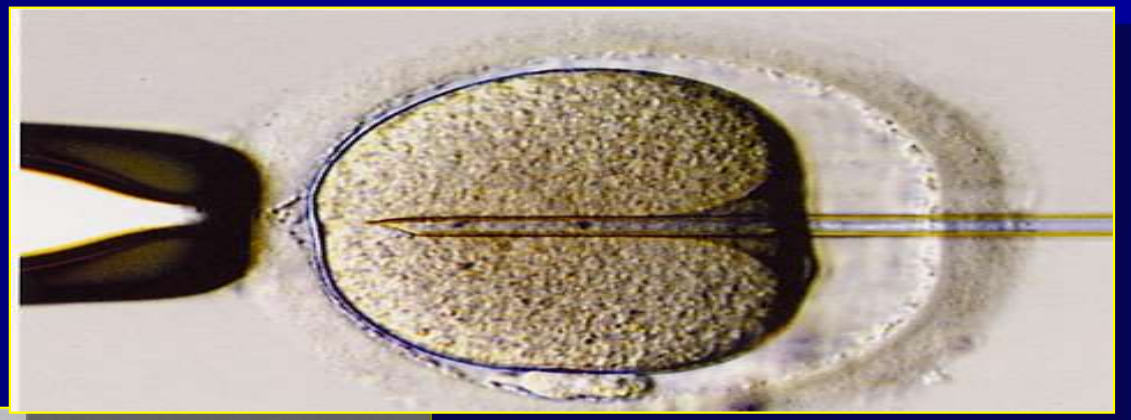
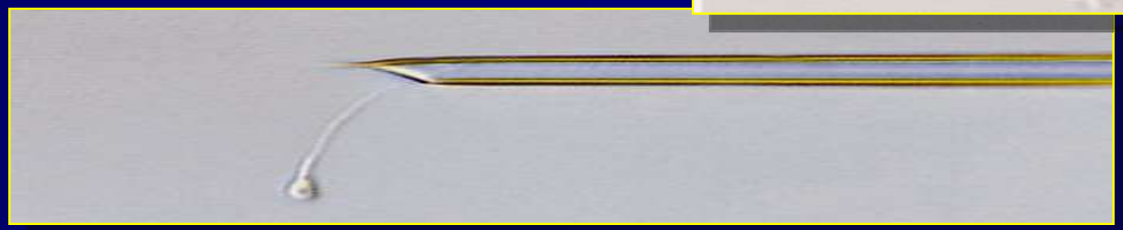
- ICSI ⇒ real advance in severe male factor infertility (Palermo et al, 1992)
- Testicular sperm was used with success in NOA (SIlber et al, 1996)





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Sperm Recovery Techniques

Testicular Sperm Extraction (TESE)

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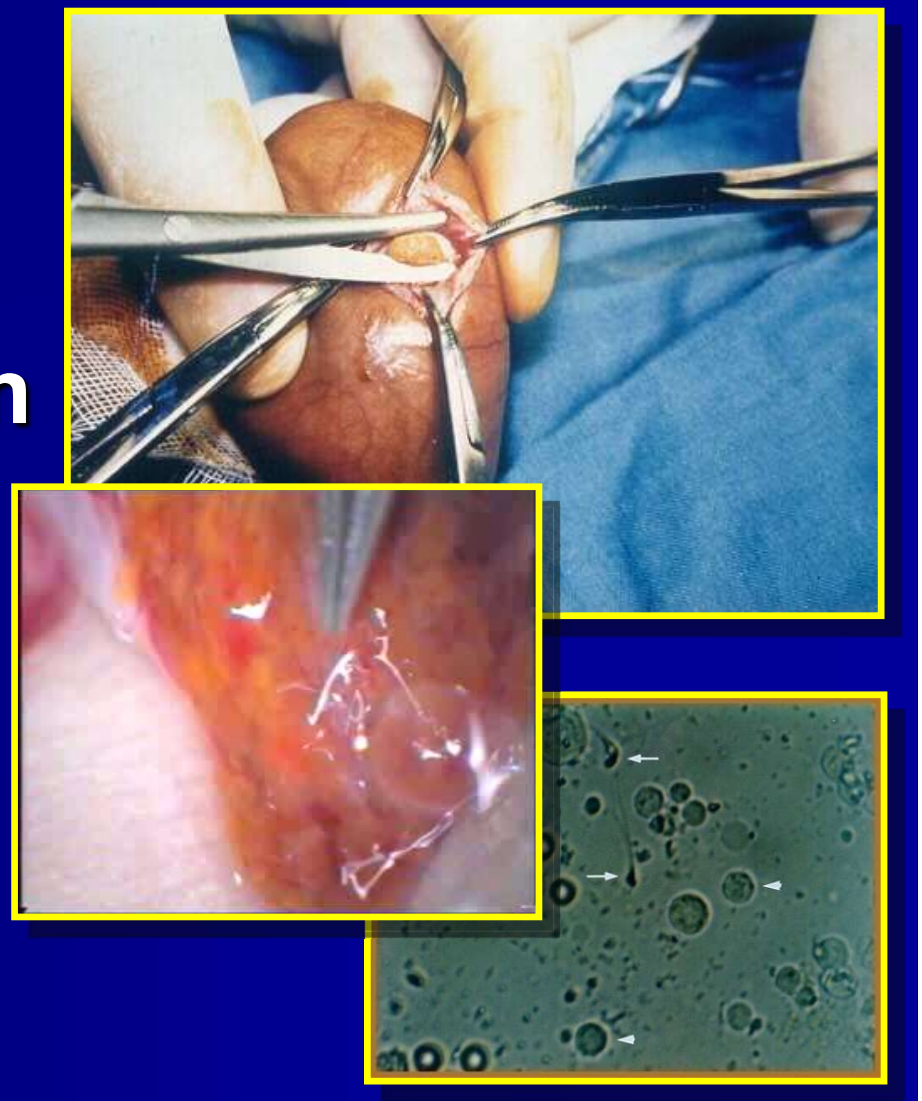
- Identification of vessels
- Absence of sperm in one site doesn't preclude the presence in others.
- Multiple site TESE.



Fine Needle Aspiration

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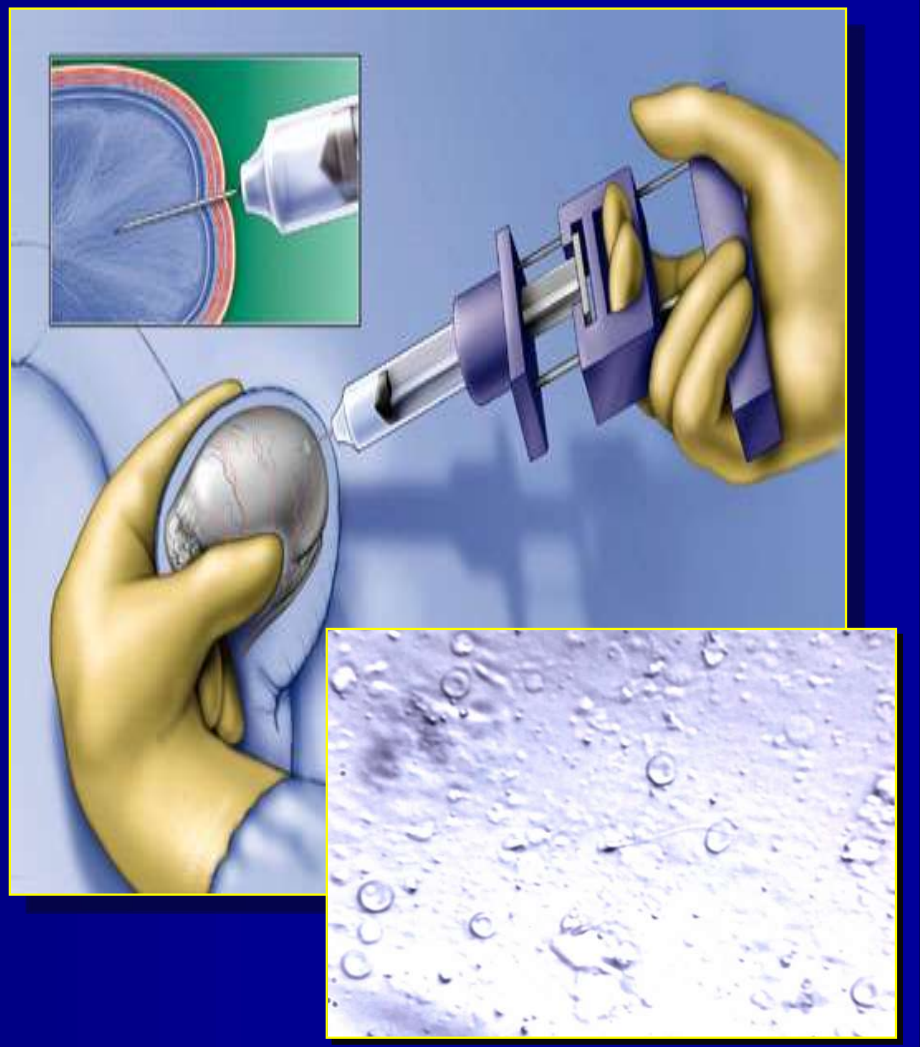
- Less invasive recovery technique.
- Decrease side effects of open TESE.
- Results are comparable to open TESE in OA.
- Less efficient than open TESE in NOA.



Microsurgical TESE

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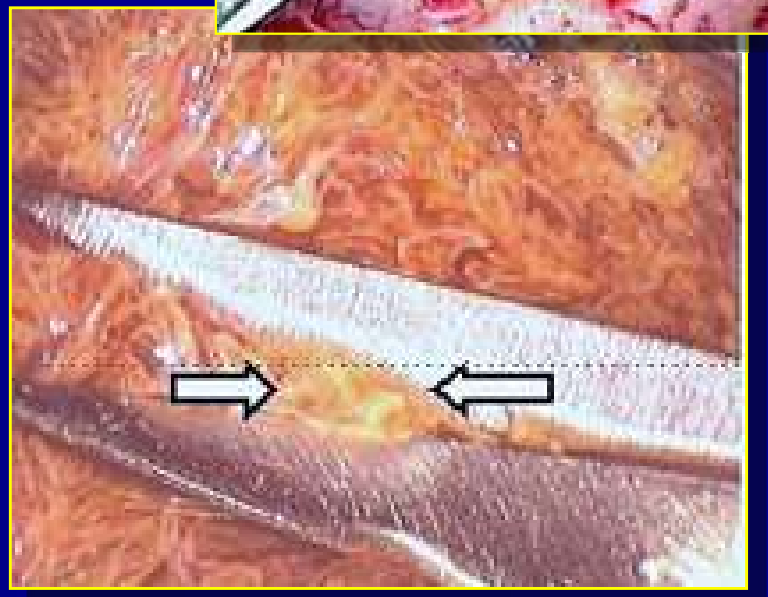
- Proposed to minimize testicular damage
  - ↑sperm recovery.
  - ↓search time.
- Open multiple
- microsurgical TESE:

Recovery rate (50%) in NOA.



Classification of Testicular FNA

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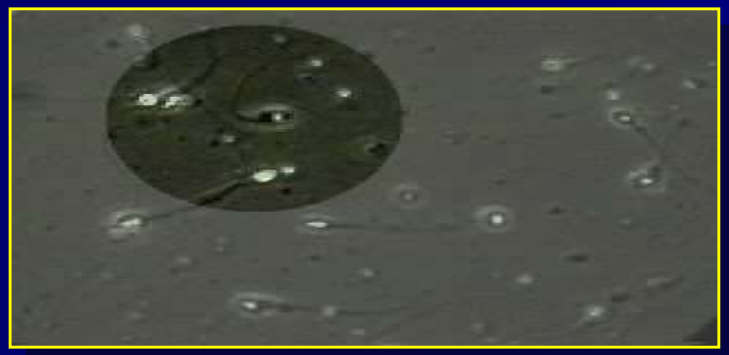
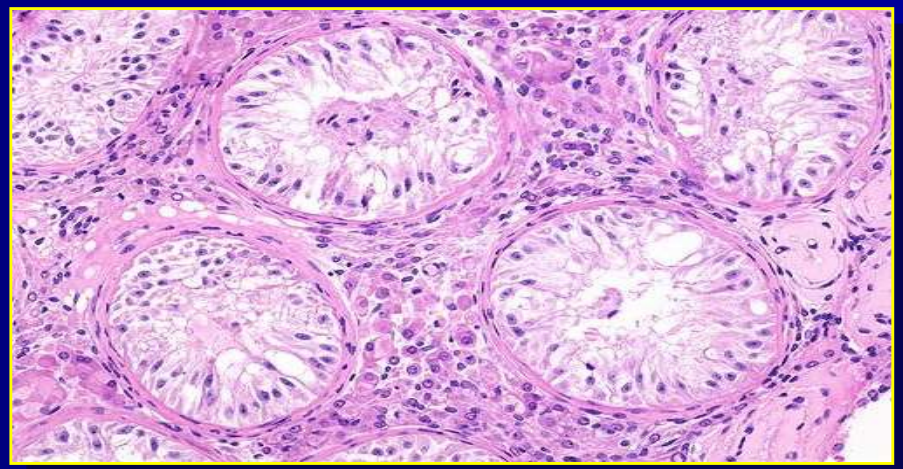
- A1 : (Sperm Count > 500) Obstructive
- A2 : (Count > 300) Non Obstructive
- B : Spermatid maturation arrest .
- C : Sertoli cells only syndrome .
- D : Sclerosis .





Adverse Effects of TESE

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- Infection.
- Hematoma.
- ↑ ASA.
- ? Testosteron level

} Recent works, Not signficant

Can a successful sperm recovery be predicted?

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- TESE ⇒ successful in 50%.
- Adverse effects of TESE.
- Unsuccessful TESE
  - Emotional and financial implications.

?

Predictive factors are Important

Biochemical Markers

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- FSH
- Inhibin – B
- Anti-mullerian hormone
- Total Testesteron

- ILG-1
- Nitrite & Nitrote
- Stem cell factor
- Others

FSH

Inhibin B

T. Testo

Predictive Factors

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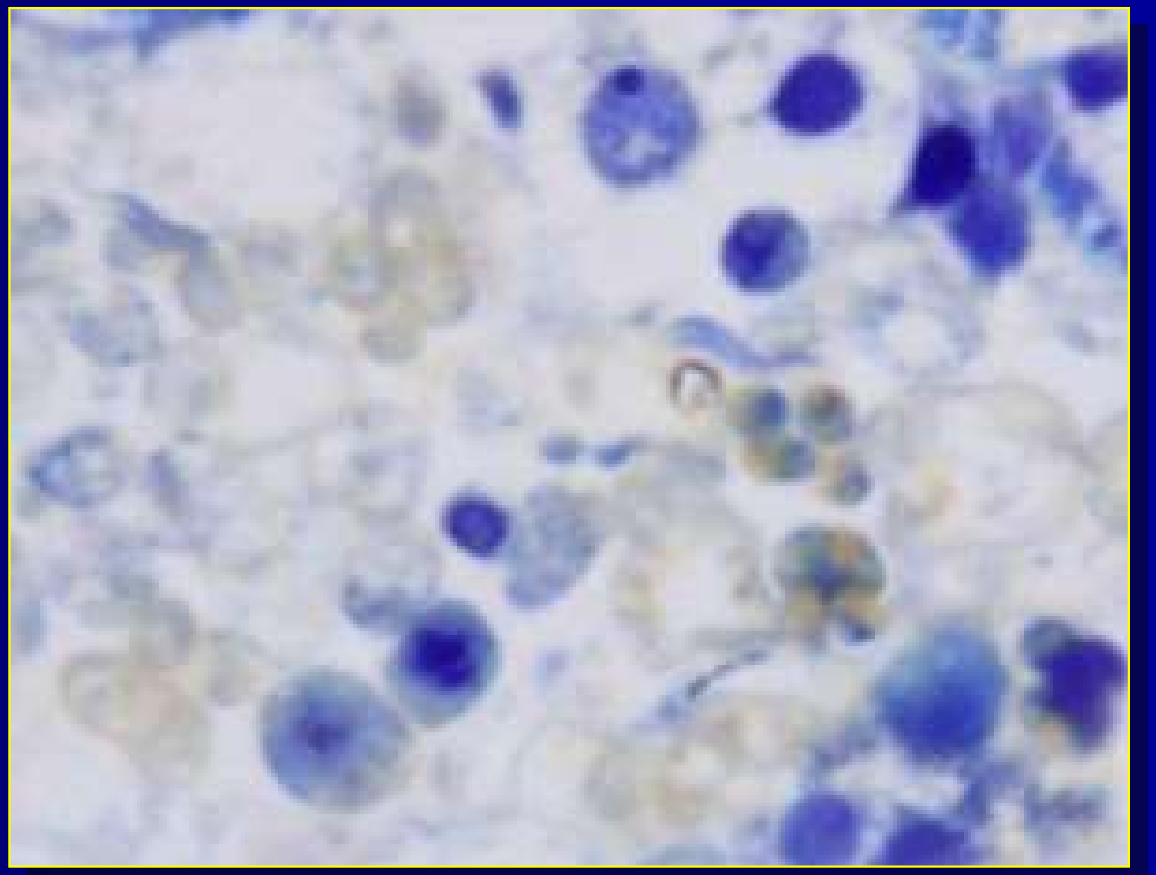
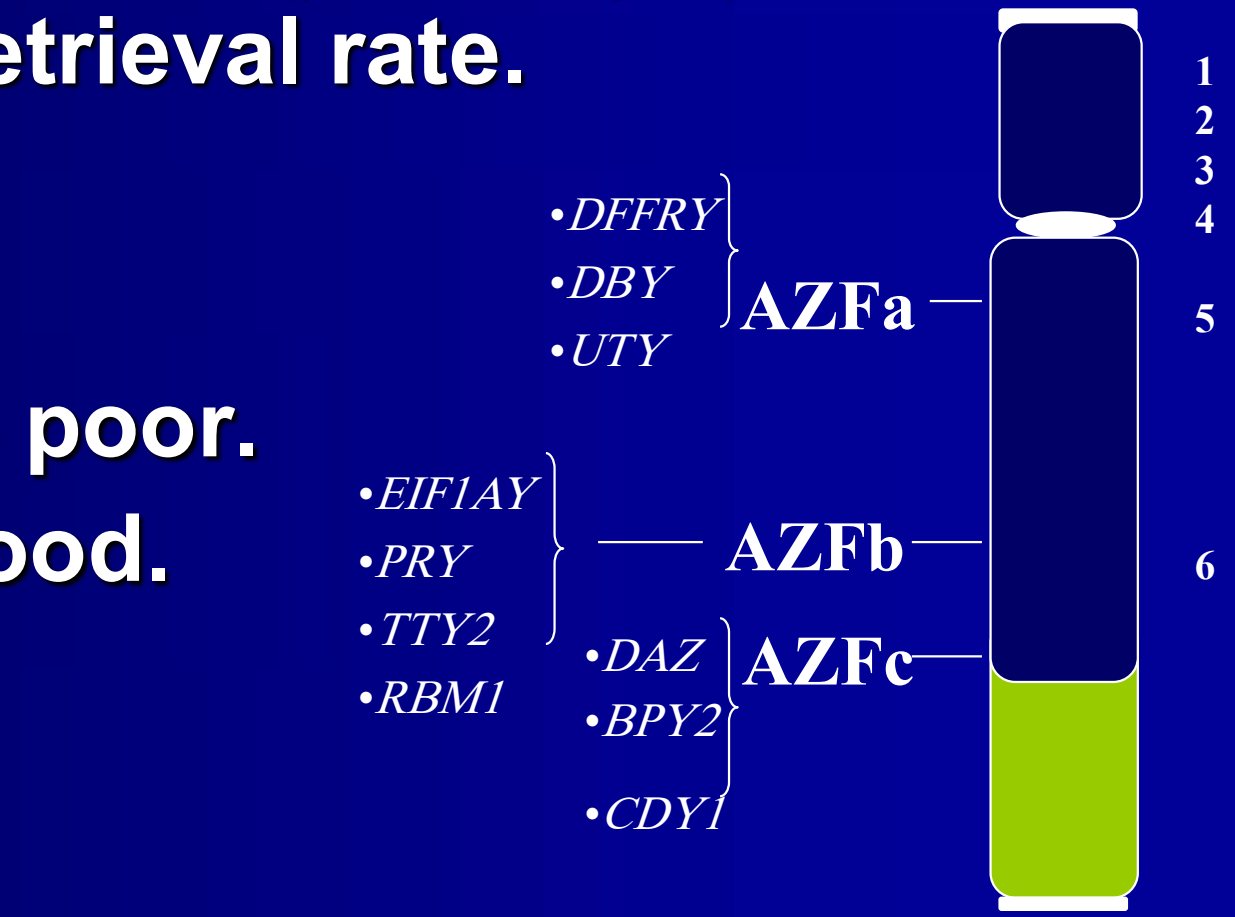
- Round spermatid in semen (by MGG stain)



Genetic Markers

- Y microdeletions, AZF a, b, c ⇒ different retrieval rate.
- AZFa, b ⇒ poor.
- AZFc ⇒ good.



Predictive markers

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- No reliable predictive data are currently available.
- Patients should not be excluded for TESE on the basis of clinical parameters
  - Small testis size
  - High FSH level

They should be told that there is a one to one chance of sperm recovery irrespective of the clinical situation

Outcome of ICSI using testicular sperm in NOA men

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- Based on the present data, couples may be counseled that their average chances of achieving delivery within two ICSI cycles with freshly retrieved sperm are 20% and after three cycles of more than 30% (Nicopullos et. al,.2004 ; Vernaeve et al,. 2005)

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The use of Frozen-Thawed Sperm

Preliminary diagnostic TESE & cryopreservation is the procedure of choice in NOA.

Advantages :

- **Avoid pointless ovarian stimulation.**
- **Avoidance of repeated testicular surgery.**

Low Restrictive Criteria

- In order to counteract the reasonable risk of not finding sperm or only immotile sperm, upon thawing, scheduling fresh surgery as back-up is advocated.
- The use of totally immotile sperm after thawing should be discouraged.

The Use of Immature Germ Cells

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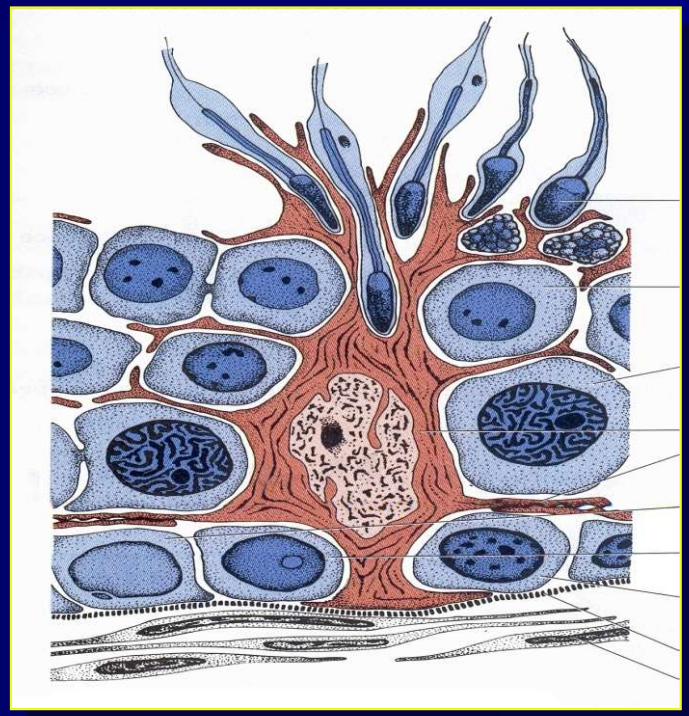
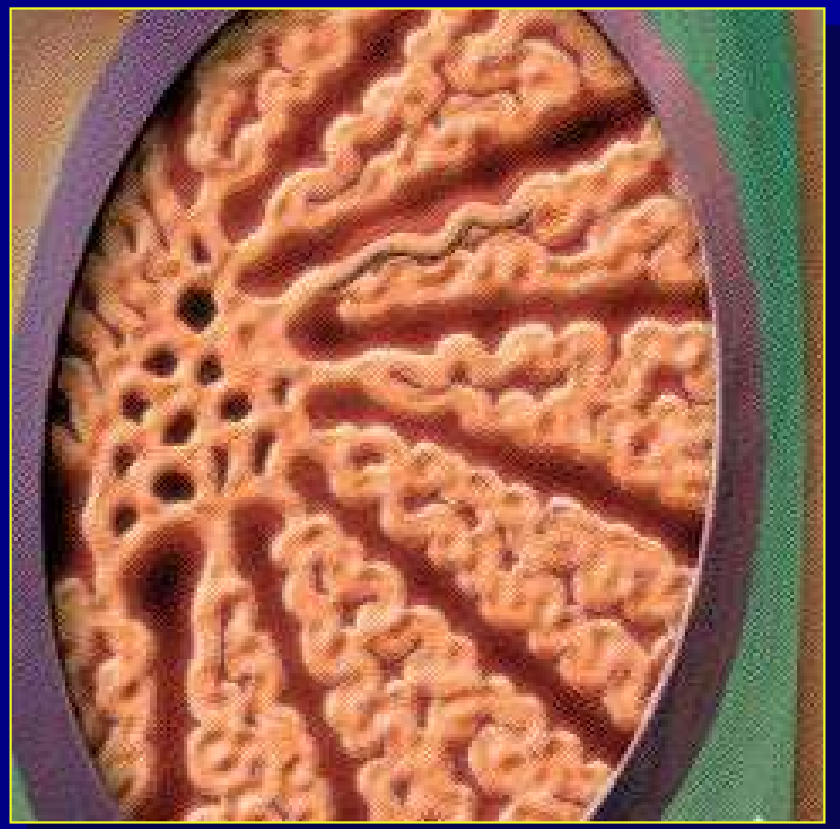
- ICSI ⇒ spermatozoa or late elongated spermatid (up to Sd2 on Clermont classification)





ART with Spermatids

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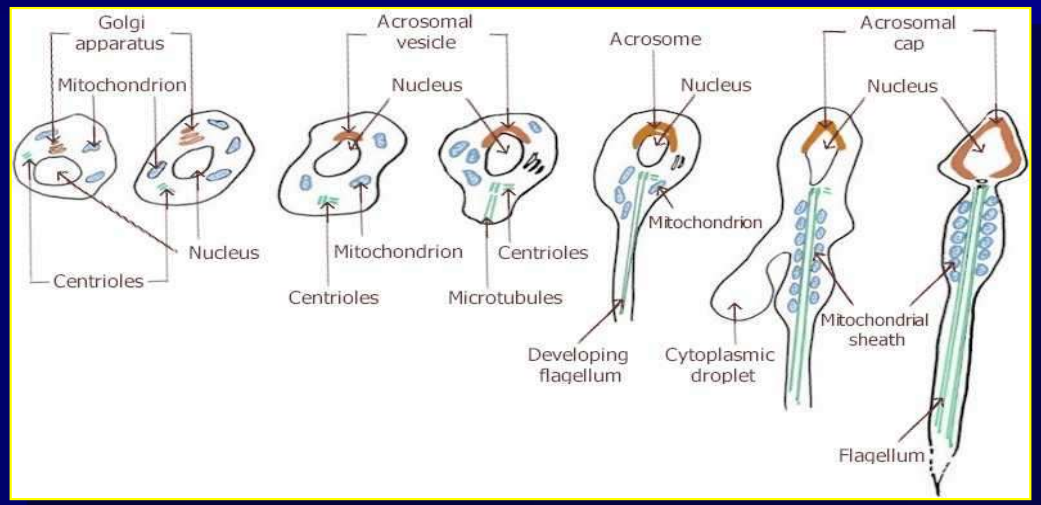
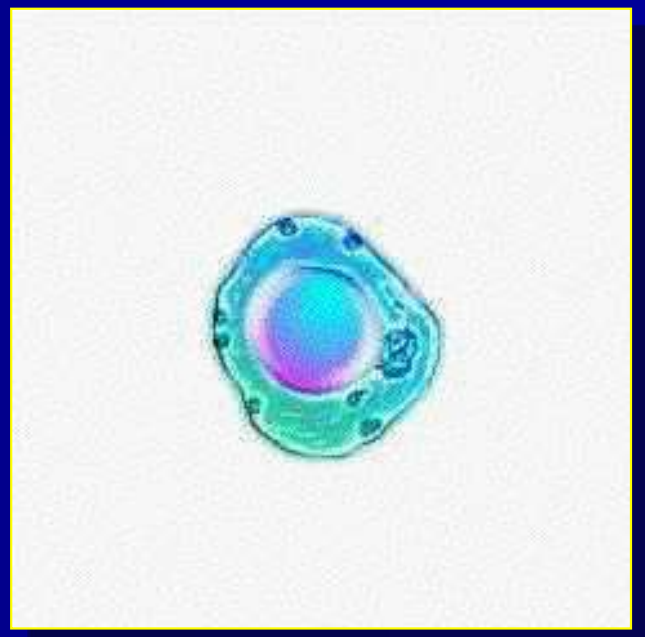
- ICSI using round spermatids has been proposed as a mean of overcoming sterility in men when no testicular sperm or elongated spermatids can be retrieved.





ART with Spermatids, Debate

- Is there any target group?
- It has been also postulated that genomic imprinting may be less complete when spermatids are used.
- Since only few pregnancies have been reported ???

ART with Spermatids : conclusion

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These Techniques are not successful approaches in treatment of NOA

ART with Secondary Spermatocyte

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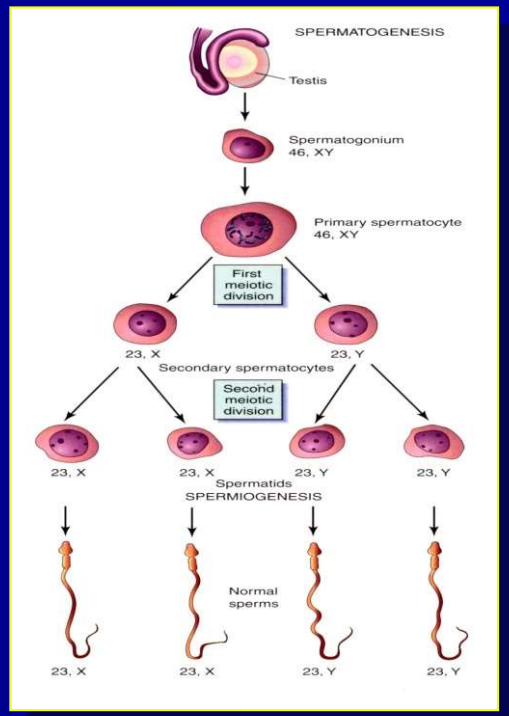
- A pregnancy has been reported after ICSI with secondary spermatocytes (Sofikitis et al., 1998)
- However, this approach has never been confirmed.



In-vitro Maturation of Germ Cells

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- There have been reports on the possibility of overcoming maturation arrest at C1 with the use in-vitro culture system.
- Spermatogenesis was resumed and few atypical elongated spermatids were detected.
- ICSI with the in-vitro developed elongated spermatids resulted in birth of two normal babies

In-vitro Maturation of Germ Cells

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- Concerns about the risk of
  - ➢ Apoptotic DNA damage in developed germ cell.
  - ➢ Chromosomal abnormalities of embryos.
  - ➢ Incompleteness of genomic imprinting

↓

Based on these concerns

- Other groups have never reported this approach.
- Many authors consider this apporach as experimental (Tourraye, 2003 Vernaeve et al.,2005)

Preimplantation Genetic Diagnosis

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- **PGD is a very early form of prenatal diagnosis**
  - Embryos are biopsied during culture in-vitro.
  - Genetic diagnosis is carried out (PCR, FISH)
  - Embryos shown to be free of the genetic disease are transferred to the mother.

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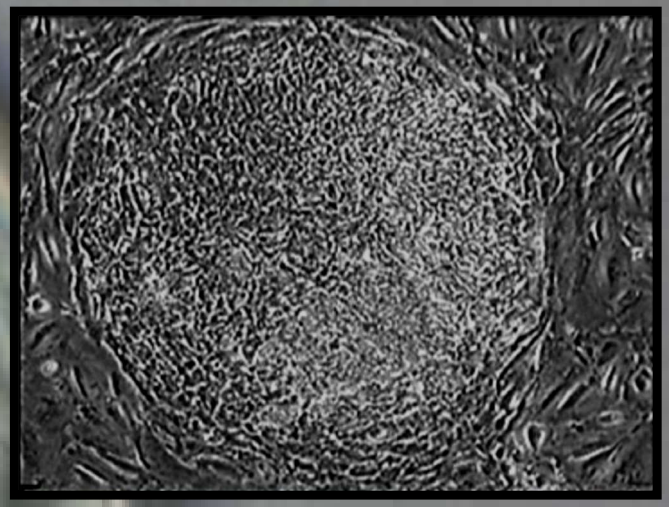
Future Prospects In the Treatment of NOA

Embryonic Stem Cells



Trophectoderm

ICM

Human Embryonic Stem Cells

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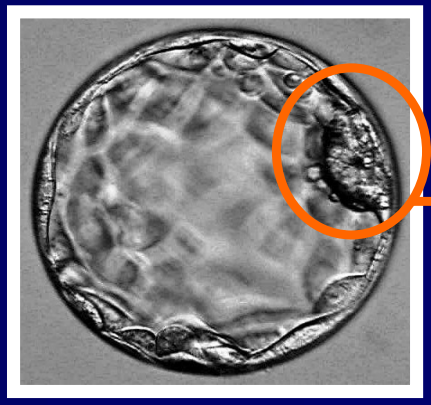
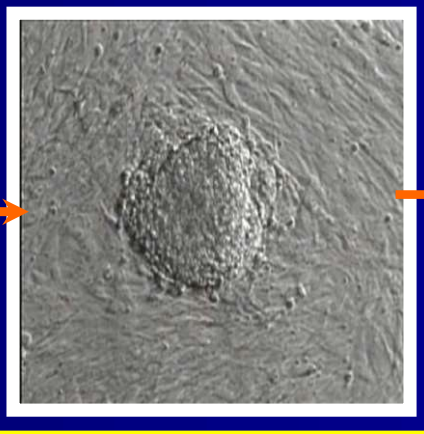


ICM isolation

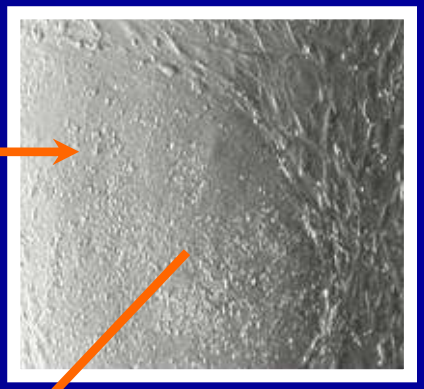
culture

Feeder cells

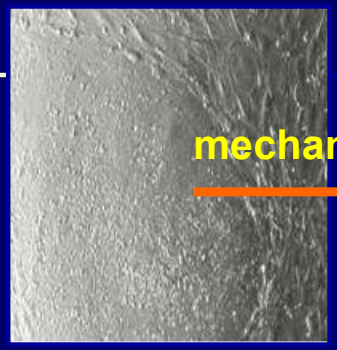


Culture

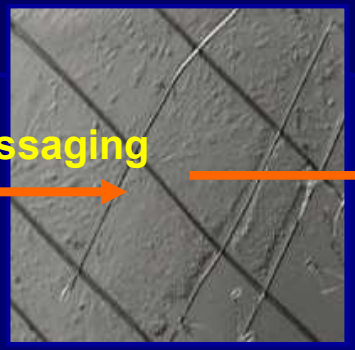
hESC colony



Human Embryonic Stem cells

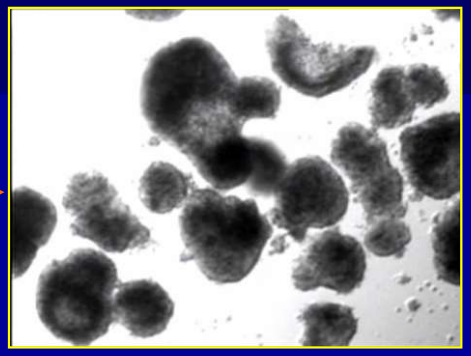


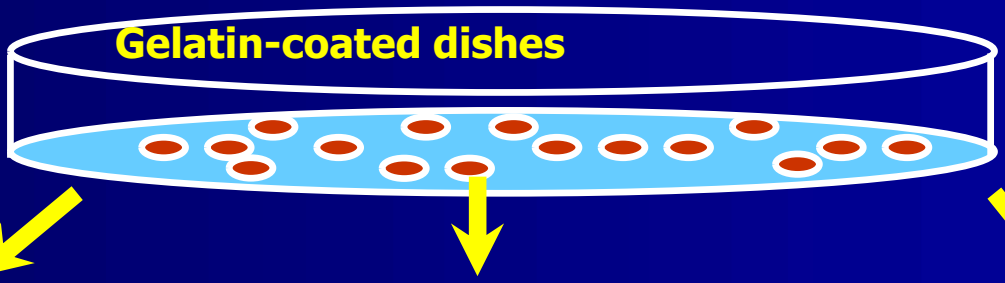
mechanical passaging

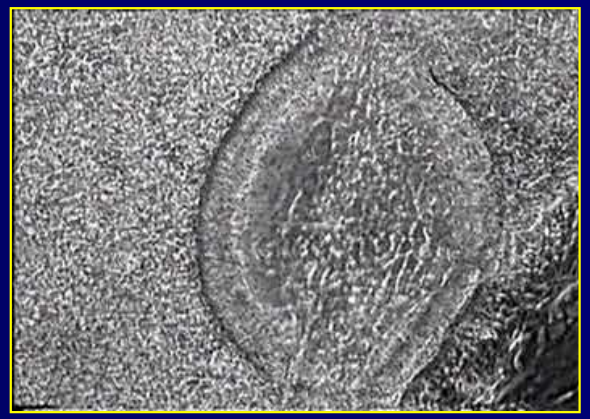


Cystic EB formation

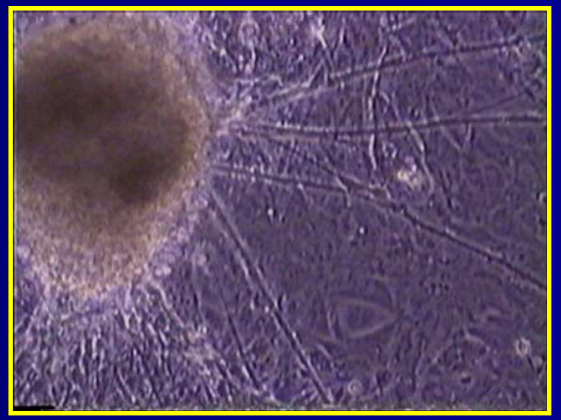
7-10 days of culture



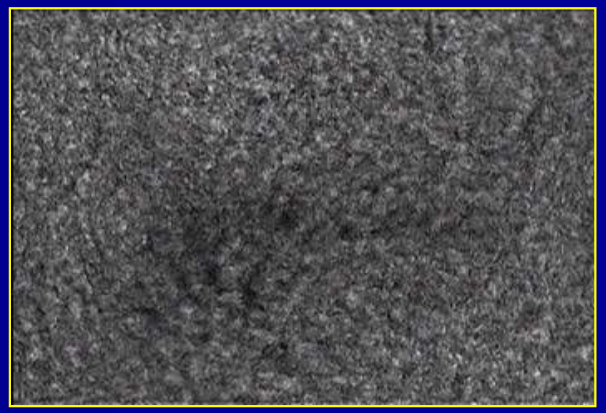




(endoderm)



(ectoderm)



(mesoderm)

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Somatic cell haploidization

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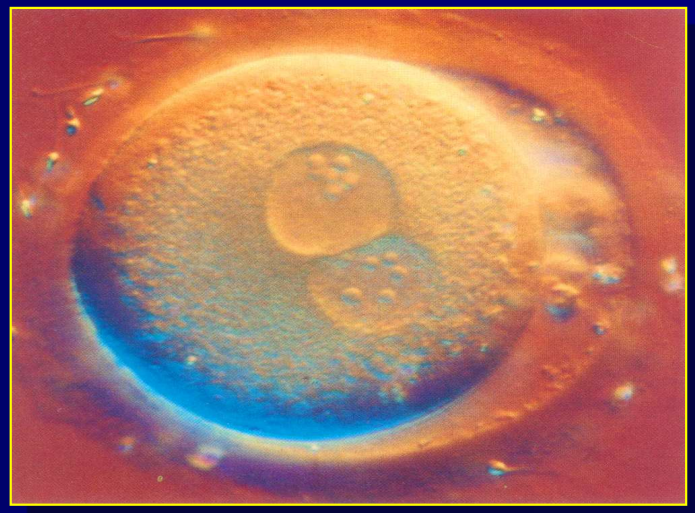
- Has been proposed as an experimental design to use somatic cell in substitution for the male gamete in cases with absent germ line (Tesarik, 2002; Tesarik and Mendoza, 2003)



Somatic Cell Haploidization

- Hypothesis: patients' somatic cell nuclei have to be introduced to a metaphase II oocyte leading to the formation of a triploid zygote, which is diploidized later leading to the formation of a diploid embryo.
- This construct should be activated by entirely artificial means.

Somatic Cell Haploidization

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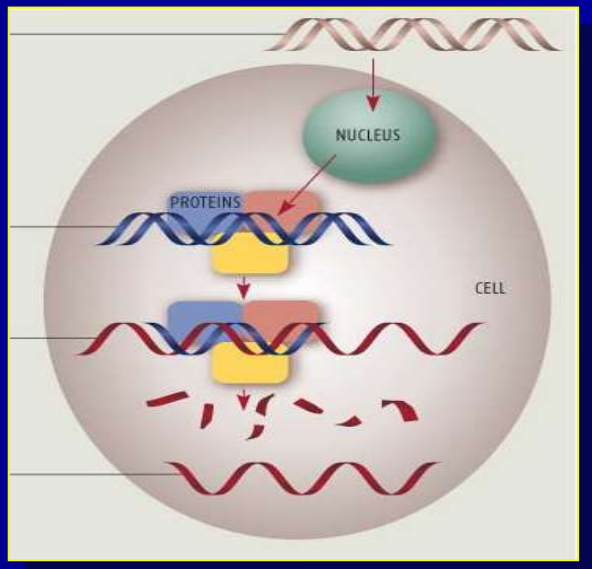
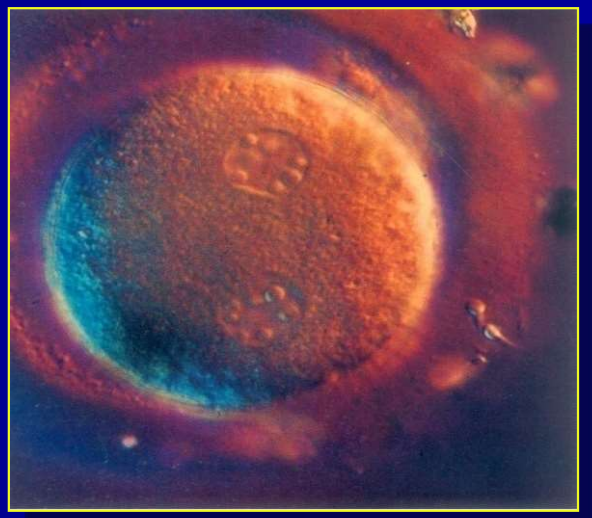
- Full data about this approach are still lacking.
- Together with the enormous therapeutic potentials this approach might have in the future, this situation should encourage .

-further intensive research.

-open religious and ethical discussion.





Spermatogonial Stem Cell Transplantation

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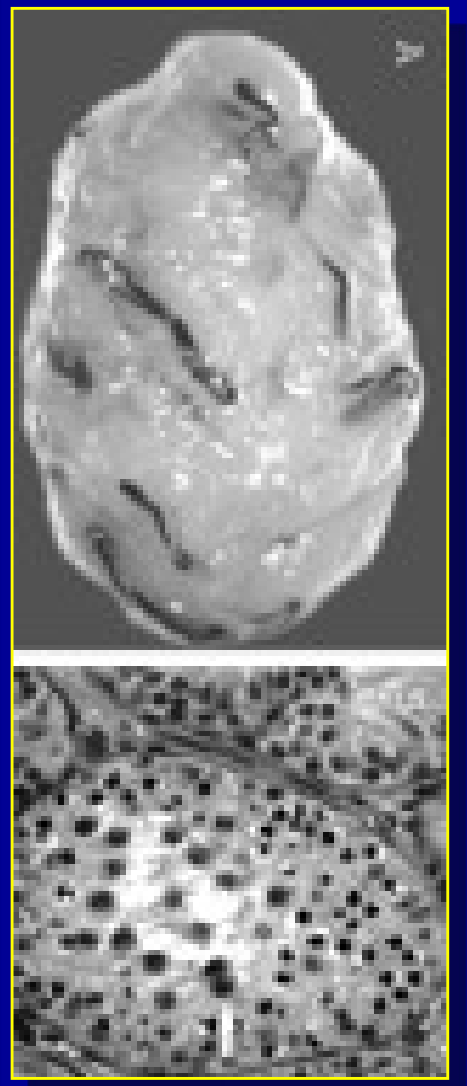
- By the end of this decade 1 in 250 young men will be childhood cancer survival.
- For these patients infertility has often been an accepted consequence of their life saving treatment.
- Clinical application will involve pre-pubertal males facing systemic chemotherapy with sterilizing side effects



Testicular Transplantation

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- Pre –treatment testicular biopsy.
- Isolation spermatogonial stem cell.
- Cultured in-vitro.
- Upon completion of treatment with no evidence of recurrence auto-transplantaion with their own cultured spermatogonia.
- Subsequent repopulation of their testis with germinal tissue would result in spermatogenesis and fertility (Khaira et al., 2004)

Gene Therapy

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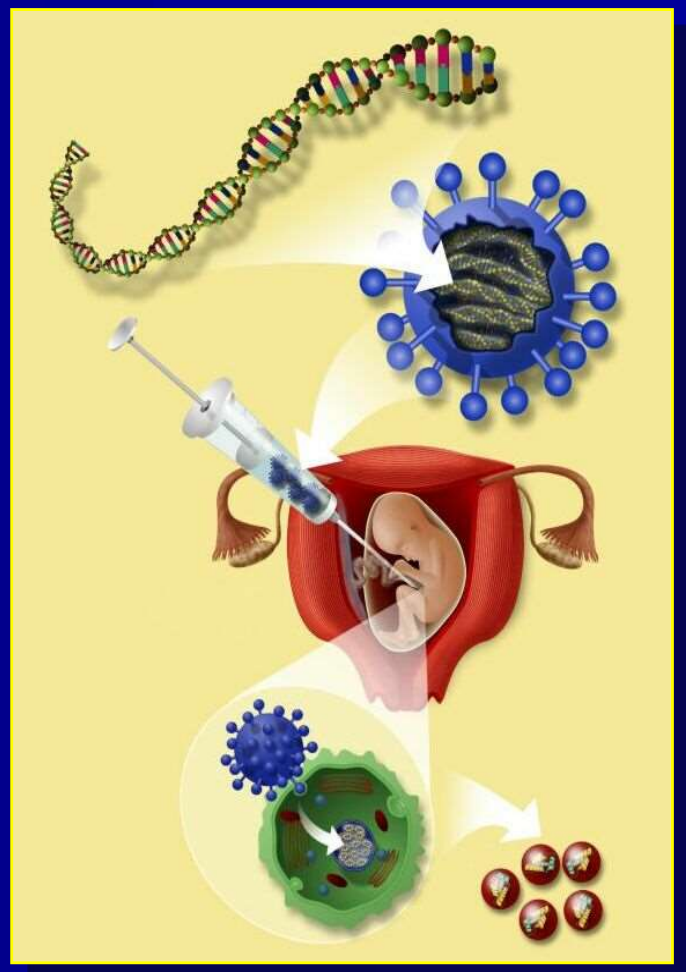
- Recently retroviral transduction of male germ line sperma-togonia has been reported and a prtential roadblock to gene therapy for male infertility has been overcome (Tenebaum et al,. 2005)



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Conclusion

- NOA may be a local presentation of systemic illnesses.
- A complete careful evaluation is important for identification of etiology of male infertility which may open new approaches regarding prevention and treatment.

Conclusion

Although current medical management of NOA remains hindered by a lack of efficacious treatments, rapid advances in understanding the genetics of male infertility will most likely change this scenario in the years to come.

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Conclusion

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Just as the possibility of ICSI was thought to be inconceivable several decades ago, the advent of current and future discoveries will present the possibility for achievements that now seem incredulous

Acknowledgement

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Thank You