Bank your future: Insemination and semen cryopreservation

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Disclosure

I have no commercial or other activities that may reflect on the contents of this lecture

Lecture objectives

- Give an short overview of reasons for cryopreservation of semen
- Discuss the possible effects of cryopreservation on semen parameters and sperm function
- Discuss the use(fulness) of cryopreserved semen in practice

Reasons for cryopreservation of semen

- Fertility preservation in male cancer patients
- Donor inseminations
- Treatment of male and female subfertility
- Back-up for assisted reproductive procedures
- Fertility preservation before vasectomy
- Separation of X and Y bearing spermatozoa

Fertility preservation in male cancer patients

Effect of Chemotherapy (1)

- Interrupt cellular proliferation
  - Germinal epithelium cells more susceptible than Leydig cells
- May cause mutagenic changes in spermatozoa
  - Non-permanent increase in aneuploidy

Schmidt et al., Int J Androl, 2007

Effect of Chemotherapy (2)

- May negatively effect semen parameters
- May negatively effect sperm functions and DNA status
- Increased risk of azoospermia

Negative effect on semen parameters

Parameter	Patients	Controls
Adolescent cancer patients (n=205)		
Volume (ml)	1.6	3.0
Count (10^6/ml)	50.6	84.5
Motility (%)	45.1	68.5
Testicular cancer (n=83)		
Count (10^6/ml)	15.0	48.0
Leukemia		
Count (10^6/ml)	19.5	106.0
Motility (%)	45.0	64.0

Agarwal and Allamaneni, J Natl Cancer Inst Monogr, 2005

Negative effects on sperm function

- Most important negative effect is influence on sperm DNA integrity
  - Poor sperm DNA integrity leads to
    - Lower fertilisation rates
    - Poorer embryo quality
    - Lower pregnancy rates

Negative effect of cancer on sperm DNA

Test	Patients	Controls
Halosperm®[1]	34.3%	10.8%
TUNEL[2]	50±3.4%	40±3.1%
CMA3[3]	63.0	30.2
TUNEL[3]	21.0	9.7
Comet assay[4]	50.1	13.9
	(Abnormal values)	

1= Meseguer et al., Fertil Steril, 2007; 2 = Edelstein et al., Fertil Steril, 2007;
3 = Spermon et al., Hum Rerprod, 2006; 4 = O'Donovan, Andrologia, 2005

Fertility after cancer treatment

General

- Before treatment
  - 91.2% of patients fathered a child
- After treatment
  - 67.1% of patients fathered a child
- Five year cumulative pregnancy index (CPI) = 85%

Schmidt et al., Int J Androl, 2007

Effect of cancer treatment on semen parameters

With radiotherapy

- 1 year post treatment (normozoospermic men)
  - 16% were oligozoospermia
  - 20% were still azoospermia
- Spermatogenesis continues to improve over next 5 years

Simons et al., CA Cancer J Clin, 2005

Conservation of fertility by cryopreservation in cancer patients

Is a very important option for cancer patients

Other preservation options available for cancer patients especially young boys

- Testicular tissue banking
- Spermatogonial stem cell banking and in vitro maturation

Effect of semen cryopreservation on semen parameters and sperm functions

Effect of cryopreservation on semen parameters in cancer patients (n = 64)

	Before freezing		After freezing	
	Mean (SD)	Range	Mean (SD)	Range
Volume (ml)	2.8 ± 1.5	0.5 – 7.0	–	–
Count (10^6/ml)	39.4 ± 28.7	0.1 – 100.0	18.4 ± 13.5	4.5 – 40.0
Motility (% a+b)	45.3 ± 11.3	0.0 – 60.0	23.3 ± 12.1	0.0 – 40.0
Morphology (% normal)	5.8 ±5.2	1.0 – 16.0	–	–

Menkveld – Unpublished data

Effect of cryopreservation on spermatozoa and sperm structure and function

- Semen parameters
  - Reduced motility
  - Reduced vitality
- Structural and functional damage
  - Reduction of intact acrosomes
  - Reduction of acrosin activity
  - Alternations in acrosome structure
  - Shrinkage of nuclei and cytoplasmic membranes
  - Loss of plasma membrane integrity
- Thus reduction in fertilisation potential

Nijs et al, RMB Online, 2009

Effect of long-term semen cryopreservation on sperm DNA

- Short term storage for 1-5 years (n= 14)
- Long term storage for 9-13 years (n = 16)
- DNA integrity (TUNEL assay)
- Short term storage = 31% fragmentation
- Long term storage = 37% fragmentation
- Original DNA fragmentation = not known

Edelstein et al., Fertil Steril., 2009; 90(4):1327-1330

Effect of cryopreservation on sperm binding function

- Sperm-Hyaluronan Binding assay
  - HA binding before freezing= 68.5%
  - HA binding after freezing= 71.3%
- = No effect

Nijs et al., RMB Online 19(2):202-206,2009

Use(fulness) of cryopreserved semen in practice

Survey of use of semen cryopreservation in cancer patients

Australia and New Zealand (1)
- Paediatric Oncology Centres
- 12/13 responded
- 12 offered semen cryopreservation (SCP)
- Only 9/12 (75%) offered counselling

- USA (2)
  - Oncologists
  - 91% agree that SCP should be offered
  - 10% offers always
  - 27% offers sometimes

(1) Heath and Stern, 2006; (2) Rofeim and Gilbert, 2004

Use of cryopreserved semen samples in cancer patients

- Magelssen et al., 2005
  - 7% (29/393) of men used their samples ≥ 1 time
  - 55.1% (16/29) achieved pregnancy
  - 17% (67/393) achieved pregnancy without use of their CPS
  - 21% (205/966) patients without CPS achieved pregnancies
- Simons et al., 2005
  - Up to 30% but often <10% use their CPS

Use of cryopreserved semen samples in cancer patients

- Chung et al., 2004
  - Less then 5%
- Ragni et al., 2003 (15 year period)
  - 5.2% (36/686) used CPS for ART
  - Cumulative rates
    - 4, 8 and 12 years = 4.5%, 8.7% and 11.8%

Use of cryopreserved semen in cancer patients – Own experience

Background data

- Number of patients = 64
- Age (years) = 25.3 ± 5.4 (15.7 – 40.1)
- Samples/ patient = 2.4 ± 0.9 (1.0 – 5.0)
- Number of straws = 18.1 ± 10.0 (2.0 – 39.0)

Number of patients who used frozen semen samples

- 3/64 = 4.7%

Used for

- ICSI = 2
- IUI = 3
- GIFT = 1

Menkveld – unpublished data

Long-term use of cryopreserved semen

- Successful sperm storage for 28 years
  - Cryopreservation before cancer treatment
  - IUI with cryopreserved semen resulted in a live birth after 21 years
  - Second successful IUI with same stored sample resulted in a second live birth after 28 years

Feldschuh et al., Fertil Steril., 2005; 84(4):1017

Use of cryopreservation of semen for treatment of male subfertility

- Study design
  - Males frequently provided semen samples
  - Samples were cryopreserved
  - Best samples of each male was used for IUI + fresh sample
- Cumulative and average pregnancy rates per cycle
- Control group
  - Fresh semen samples only = 9/77(13%) and 4.1%
- Test group
  - Frozen + fresh sample = 19/73 (27.1%) and 9.1%

Aboulghar et al., Fertil Steril 56(6):1151-5,1991

Use of semen cryopreservation for sex selection

Separation of X and Y bearing spermatozoa

- Flow cytometry
  - 5871 sorts
  - 74.9% X and 25.1 Y
- Post sort purity
  - X sort = 87.9%
  - Y sort = 73.4%
- IUI cycles = 3629
  - Pregnancy rate = 15.6%
  - Miscarriage rate =15.7%
- Babies
  - X sort = 92.0%
  - Y sort = 81.5%

Karabinus, 2009

Conclusions

- Semen cryopreservation is an essential tool in assisted reproductive procedures
- IUI with cryopreserved (non donor) semen is mainly used for cancer patients
- In subfertile patients mainly used for
  - IVF
  - ICSI

  due to mostly poor semen quality in infertile males