Testicular tissue freezing

Impact of the freezing procedure and the treatment received on the quality of the tissue after thawing

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Testicular tissue cryopreservation

Impact of freezing and treatment received on tissue quality

Nothing to declare

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Epidemiology & Context

Each year in France

2 550 children and adolescents are diagnosed with cancer (INCA, 2016)

Cure rates > 80%

Toxicity on germ cells

Wyns *et al.*, 2010

Fertility preservation

In adult men

Sperm cryopreservation

Before gonadotoxic treatment

In pre-pubertal boys

Testicular tissue freezing (TTF) (Picton *et al.*, 2015)

Experimental

Indications

- Before highly gonadotoxic treatment
- Mostly before hematopoietic stem cell transplantation (HSCT)

Literature Review

201 cases of testicular tissue freezing

Controlled slow freezing ± seeding

- 2/16 without seeding (Bahadur *et al.*, 2000; Sadri-Ardekani *et al.*, 2016)
- 2/16 vitrification (Curaba *et al.*, 2011; Poels *et al.*, 2013)

2/16 explore the tissue quality after thawing

(Kvist *et al.*, 2006; Keros *et al.*, 2007)

No study assessed the impact of treatment on tissue quality

Bahadur *et al.*, 2000; Kvist *et al.*, 2006; Keros *et al.*, 2007; Wyns *et al.*, 2007; Wyns *et al.*, 2008; Ginsberg *et al.*, 2010; Curaba *et al.*, 2011; Wyns *et al.*, 2011; Babayev *et al.*, 2013; Poels *et al.*, 2013; Goossens *et al.*, 2013; Ginsberg *et al.*, 2014; Pietzak *et al.*, 2015; Sadri-Ardekani *et al.*, 2016; De Michele *et al.*, 2017; Ho *et al.*, 2017

Material & Methods

Aim of the study

To assess the impact

- **Freezing** on the structural quality of the testicular tissue after thawing
- **Cancer treatment** received prior to testicular tissue freezing
- Effect of age or pathology on fresh testicular tissue

Study design

Multicentric national prospective study (PROSPERMA) (8 CECOS centers)

+

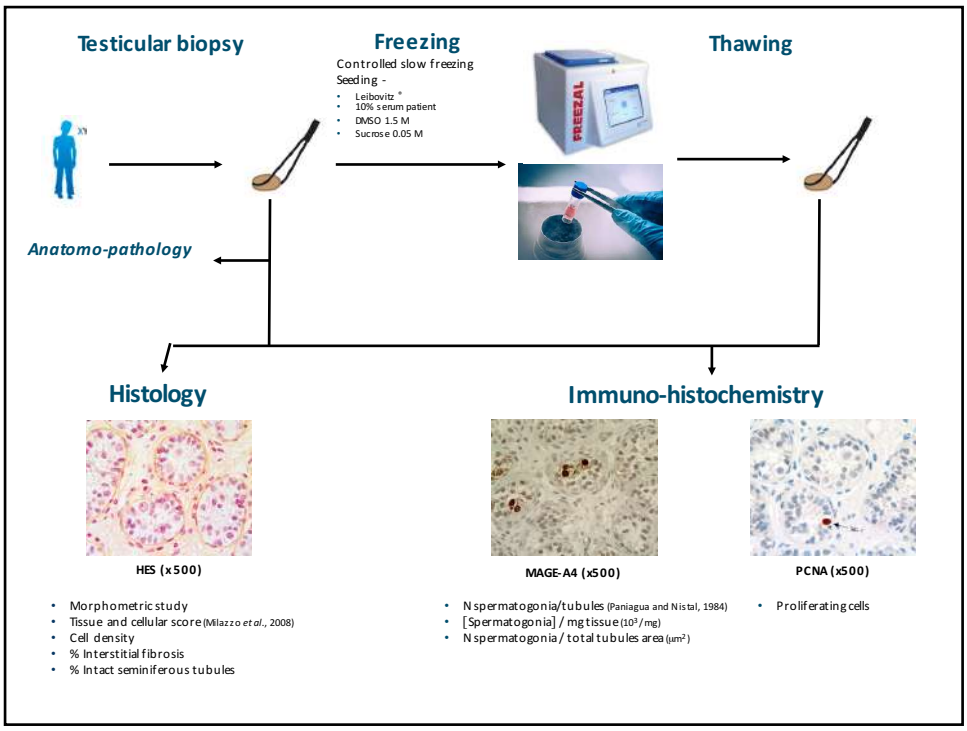
Retrospective Rouen University Hospital Cohort (RUHC)

Inclusion criteria

Patient less than 17 years (2006-2016)

Testicular tissue freezing

- Before HSCT for malignant disease
- After chemotherapy or radiotherapy with low or moderate fertility toxicity



Materials & Methods

Chemotherapy

Cumulative dose received per square meter (mg/m^2)

Cyclophosphamide Equivalent Dose (CED) mg/m^2 (Green *et al.*, 2014)

Algorithm establishing a reference scale of alkylating agents, according to Cyclophosphamide

Constitution of 5 groups

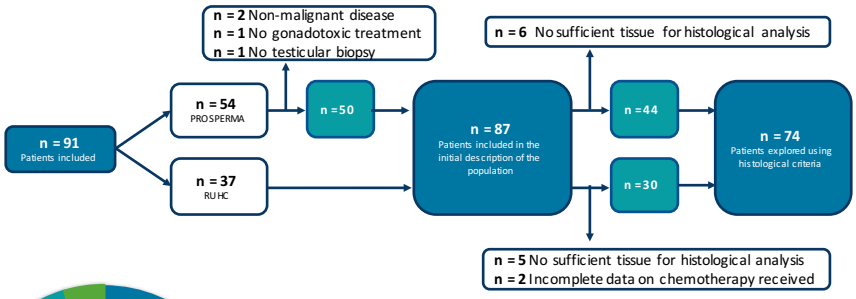
- Patients without any alkylating agent
- Patients with alkylating agents but not included in the CED
- Patients with CED < 4000 mg/m^2
- Patients with CED between 4000 and 8000 mg/m^2
- Patient with CED > 8000 mg/m^2

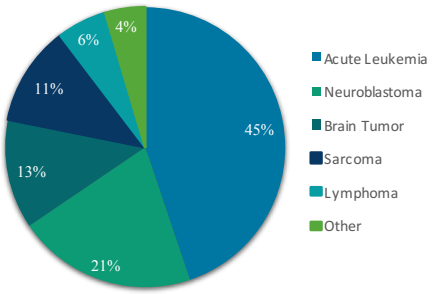
Cumulative dose of Anthracycline (DCA) (Poganitsch-Korhonen *et al.*, 2017)

Anthracycline isotoxicity conversion factor

- Factor 1 Doxorubicine
- Factor 0,833 Daunorubicine

Results & Discussion





Representative distribution according to national cancer data

- Neuroblastoma 21% vs 8,4% (Desandes *et al.*, 2016)
- Stage IV
- Chemotherapy intensive before HSCT (Talleur *et al.*, 2017)

Results & Discussion

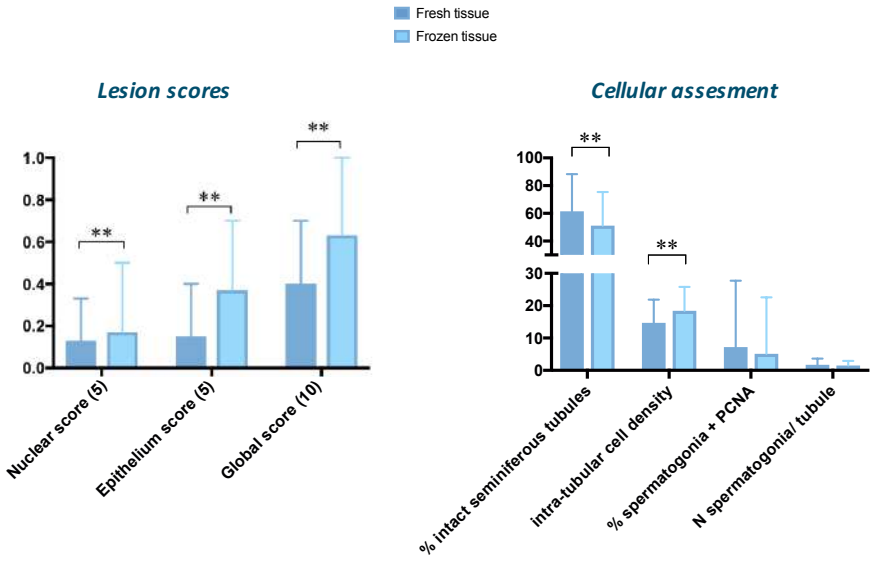
Characteristics of the population

Variables Mean ± sd (min ; max)	Total population n= 87	PROSPERMA n= 50	RUHC n= 37	Value of *p*
Age (years) at the time of consent	7 ± 4 (0 ; 16)	6 ± 4 (0 ; 16)	8 ± 5 (0 ; 16)	0.2224
Body surface (m^2)	0.9 ± 0.4 (0.4 ; 1.8)	0.9 ± 0.4 (0.4 ; 1.8)	0.9 ± 0.4 (0.4 ; 1.8)	0.4362
Body mass index (kg/m^2)	16.4 ± 3.2 (11.6 ; 26.4)	16.3 ± 3 (12.2 ; 26.4)	16.5 ± 3.4 (11.6 ; 26.4)	0.6872

Received treatment

	Cumulative dose of Anthracycline		
	n	Median [1er Q ; 3ème Q] mg/m^2	Value of *p* modality (< 4000 = réf.)
CED < 4000	15	160.6 [99.6 ; 236.3]	*Ref.*
4000 < CED < 8000	17	122.1 [97.2 ; 207.2]	0.5583
CED ≥ 8000	13	296.5 [166.3 ; 313.3]	**0.0097**
Value of *p* (effect)	-	**0.0028**	-

Impact of freezing



Impact of freezing

Good preservation of seminiferous tubule architecture

Moderate tissue damage (Gobal tissue lesion score < 1)

≅ (Milazzo *et al.*, 2008; Milazzo *et al.*, 2010; Sadri-Ardekani *et al.*, 2016)

Number of spermatogonia per seminiferous tubule

Ability of spermatogonia to proliferate

≠ Poels *et al.* (2013)

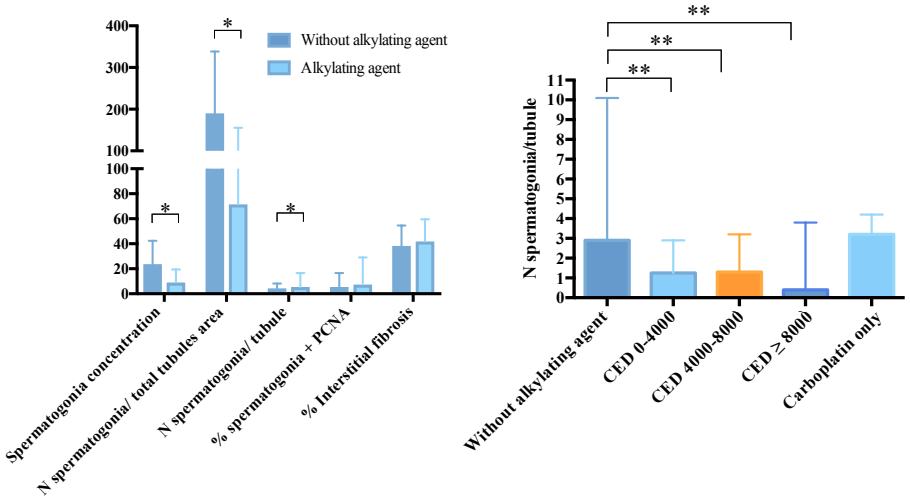
PCNA expression unchanged (Milazzo *et al.*, 2010)

⚠ Low staining for many young patients

Artefactual increase in cell density

Incomplete rehydration

Effect of alkylating agent



Impact of treatment

Decrease of spermatogonia number

Upon introduction of alkylating agents except platinum salts

- ≅ (Nurmio *et al.*, 2009 ; Green *et al.*, 2014b ; Poganitsch-Korhonen *et al.*, 2017)
- No correlation with the CED
  - ≠ Post-treatment sperm parameters (Green *et al.*, 2014b)

CED without moderate gonadotoxic alkylating agents (Platinum salts)

However, Cisplatin is highly gonadotoxic if doses > 600 mg/m^2 (Wyns *et al.*, 2010)

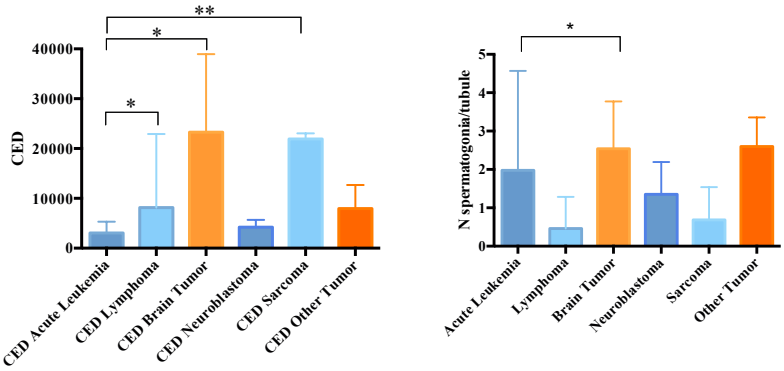
- Prosperma+RUHC => no patient with doses > 600 mg/m^2
- No effect of Cisplatin on sperm parameters in adult men survivor of childhood cancer (Green *et al.*, 2014a)

No additional effect of Anthracycline

≅ Poganitsch-Korhonen *et al.* (2017)

Difficult assessment of the specific impact of each molecule

Cancer effect



Patients with Brain Tumors

- Higher number of intra-tubular spermatogonia
- 8/10 Carboplatin only (Wyns *et al.*, 2010)

Impact of cancer difficult to evaluate

- Cancer effect related to treatment effect

Age effect

No correlation with age

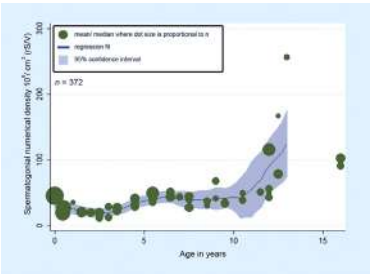
Number of spermatogonia per tubule and their proliferation ability

≅ Nurmio *et al.* (2009)

Analysis in subgroup of age

Variation in spermatogonia concentration

≅ Masliukaite *et al.* (2016); Poganitsch-Korhonen *et al.* (2017)



Masliukaite *et al.*, 2016

Conclusion

Optimal cryopreservation protocol of human testicular tissue

Controlled slow freezing without seeding
(Bahadur *et al.*, 2000; Sadri-Ardekani *et al.*, 2016)

Spermatogonial stem cell toxicity of alkylating agents except platinum salts

⚠ CED> 8000 mg/m^2

Proposal of testicular tissue freezing

⚠ CED > 4000 mg/m^2

In memory to Jean-Pierre Milazzo