PGT for translocations: Lessons learned from 760 cycles

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Reminder French legal situation

- PGT allowed in France since our first bioethics law in 1994
- Restriction: couples with a known risk of transmitting a monogenic disease or if one of the partners carries a chromosomal rearrangement.
- Diagnosis is limited to the only disease that could be transmitted.
- This implies that in case of translocation the diagnosis is only performed on the chromosomes involved in the translocation in concerned.
- Impossible to use any other technique than FISH
- PGT-A is therefore forbidden in our country.

Introduction

- Patients bearing a balanced translocation present an increased risk of obstetrical complications (recurrent abortion, live born foetuses affected by a chromosomal syndrome).
- PGT offers couples the chance to have an unaffected child, without facing TOP.
- A major issue is the need for a large number of embryos to increase the chance to get a balanced one since the unbalanced embryos rate varies from 67% to over 80%.

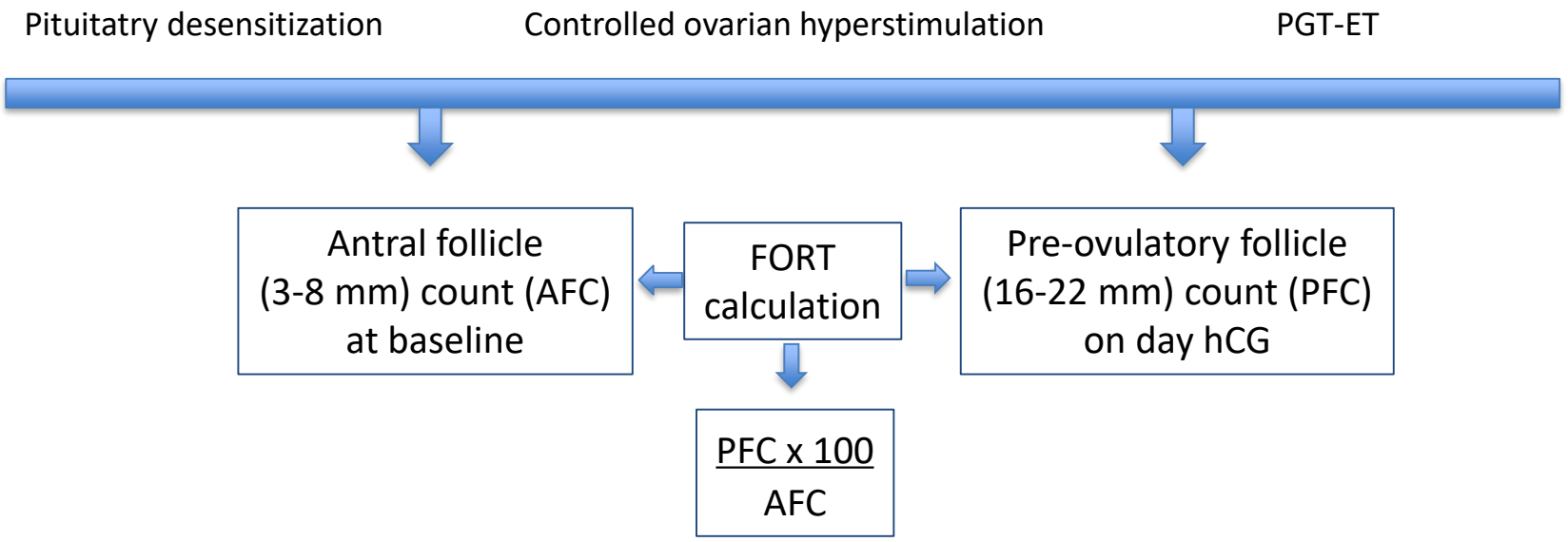
Sermon *et al.*, 2004; Ko *et al.*, 2010; Harper *et al.*, 2012; Scriven *et al.*, 2013; Moutou *et al.*, 2014; Kato *et al.*, 2016

Introduction

- Autosomal translocations are associated with an increased risk of sperm quality alteration (Sasagawa et al., 1993; Penna Videaú et al., 2001; Rao et al., 2005; Mayeur et al., 2019)
- In women, cytogenetic studies are more often focused on POF whose main causes are linked to the X chromosome (Burton et al., 2000, Schlessinger et al., 2002, Kummer et al., 2009, Jiao X et al., 2012; Kim et al., 2015)
- Some have raised the hypothesis that autosomal translocations could affect the ovarian function less drastically. So far, two studies addressed this issue by merely analysing the strength of ovarian response to COS in female translocation carriers compared to controls (Chen et al., 2005; Dechanet et al., 2011).

Introduction

- To objectively evaluate antral follicle responsiveness to exogenous FSH, the follicular output rate (FORT) may be helpful.
- FORT provides an assessment of antral follicle responsiveness to FSH independently of the follicular cohort size before treatment.



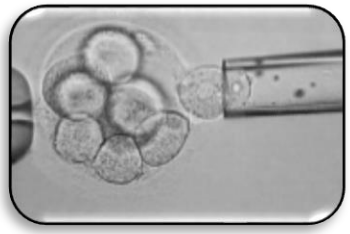
Genro et al., 2011; Gallot et al., 2012; Grynberg et al 2016

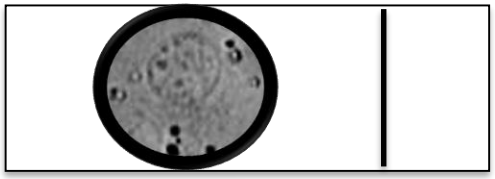
Objectives

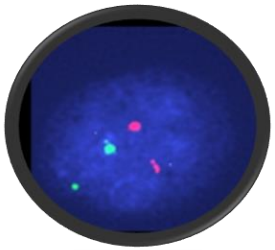
- Evaluate if there is a relationship between the Follicular Output Rate (FORT) and the female chromosomal status
- Evaluate if the number of metaphase II oocytes or biopsied embryos could be predictive of the chance to reach an embryo transfer.

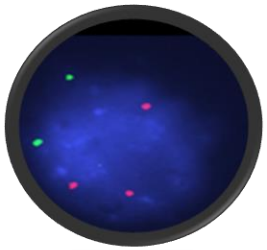
Materials & Methods

- Monocentric retrospective observational study during ten years (January 2006 - April 2017).
- 343 couples underwent PGT for translocation
- Patients with a translocation involving sex chromosomes have been excluded (12 patients)
- 331 couples were finally included in this study in 760 COS cycles
- Embryos biopsy was performed on day 3 or 4
- FISH analysis after blastomeres spreading on slides







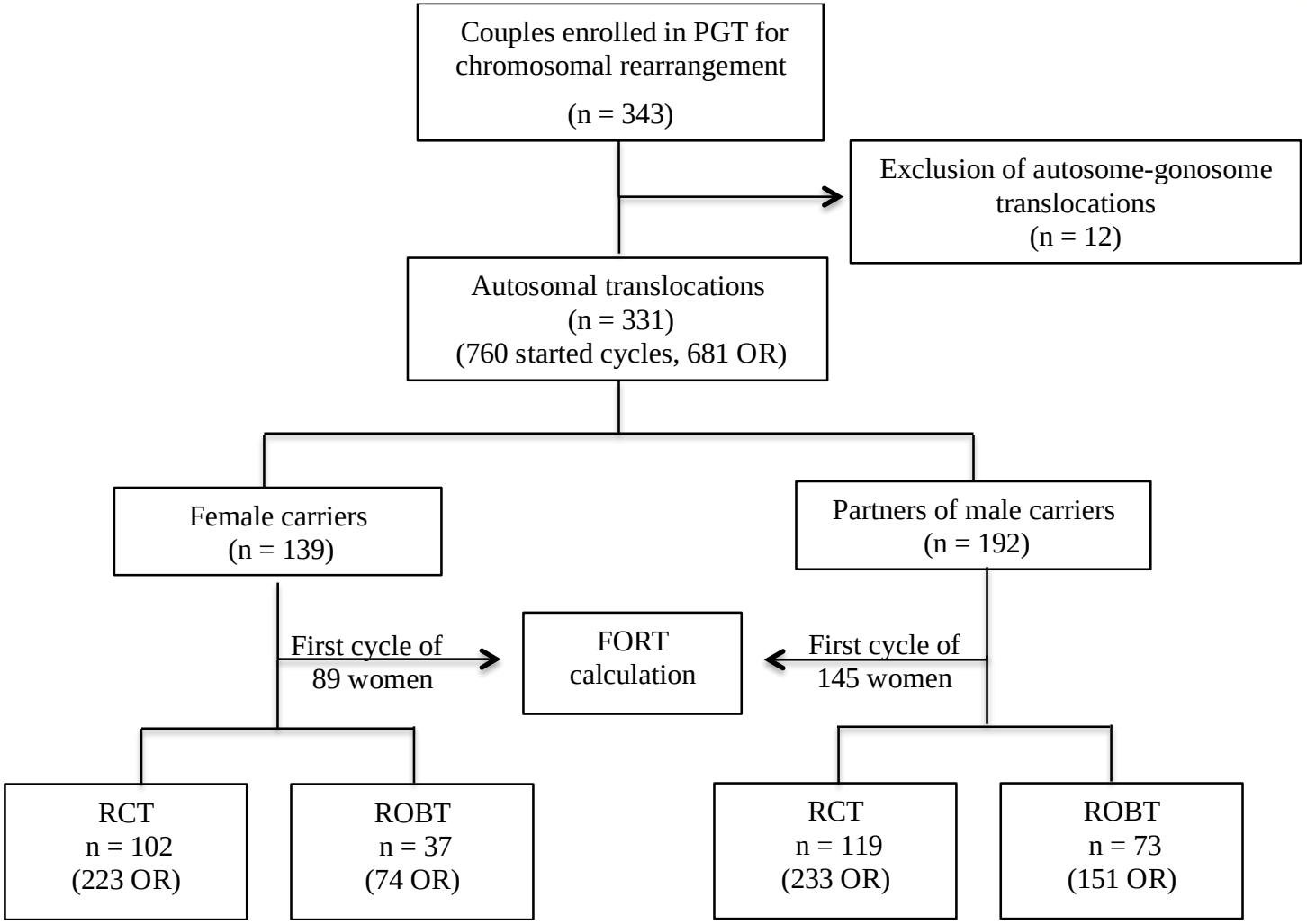


FORT Calculation

First cycle of a sub population of women (89 female translocation carriers and 145 partners of male translocation carriers) that met the following inclusion criteria:

- ovaries without morphological abnormalities such as cysts, endometriomas, etc.
- regular menstrual cycles
- AFC at baseline <25
- BMI ranging from 17 to 29 kg/m2

Flow Chart



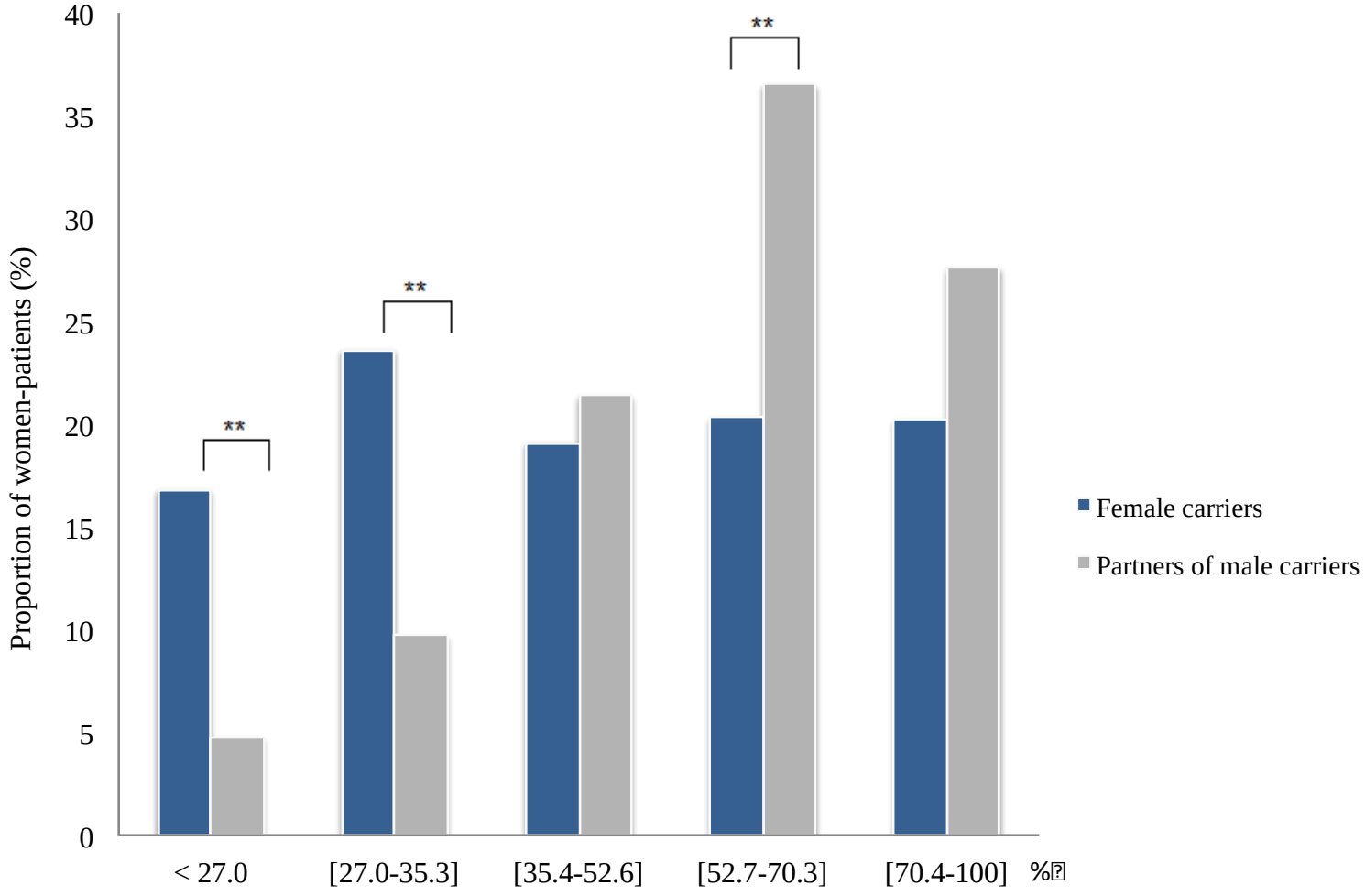
To answer to our second aim, we studied the PGT-ET outcomes of 681 OR from 139 female carriers compared to 192 partners of male carriers.

Response to exogenus FSH assessed by FORT

	Female carriers (n=89)	Male carriers (n=145)	*P* value
Age (years)	32.6±0.4	33.3±0.3	NS
BMI (kg/m^2)	23.5±0.6	23.5±0.4	NS
AMH (ng/mL)	3.1±0.2	3.7±0.2	NS
AFC (3-8 mm)	17.5±0.5	15.2±0.4	NS
Agonist protocol (%)	50 (56.2)	68 (46.9)	NS
Antagonist protocol (%)	39 (43.8)	77 (53.1)	NS
Duration of COS (days)	10.9±0.2	10.9±0.1	NS
Total dose of FSH (IU)	2883.0±110.2	2754.0±68.3	NS
Estradiol level on dhCG (pg/mL)	2501±102.1	2956.0±297.0	NS
PFC (16-22 mm)	9.9±0.4	11.8±0.3	<0.0001
FORT (%)	48.1±2.6	59.4±1.	<0.001

PFC values and FORT were significantly decreased in female translocation carriers suggesting diminished antral follicle sensitivity to FSH.

Proportion of female carriers and partners of male carriers in defined FORT categories



FORT Percentile	< 20th	20th – 40th	40th – 60th	60th – 80th	>80th

karyotypes for female carriers with a FORT under the 20th percentile (<27.0 %)

Translocation	AFC	FORT (%)	Metaphase II oocytes	Biopsied embryos	Outcome
46,XX,t(17;18)(p13;p31)	23	13.0	5	2	No ET
46,XX,t(6;20)(p21;q13)	21	14.2	13	3	No PR
46,XX,t(1;17)(q42;q25)	21	14.2	13	4	No ET
46,XX,t(8;16)(q11;q12)	13	15.3	1	1	No ET
46,XX,t(6;10)(q15;p10)	19	15.7	10	6	No PR
46,XX,t(3.1)(q21;q32)	24	16.6	8	5	No ET
45,XX,t(13;14)(q10;q10)	22	18.1	3	1	No ET
46,XX,t(2;16)(q14;q24)	20	20.0	7	0	No ET
45,XX,t(13;14)(q10;q10)	24	20.8	7	4	No PR
45,XX,t(14;21)(q10;q10)	9	22.2	7	2	No PR
46,XX,t(4;21)(q32;q21)	22	22.7	8	3	No ET
46,XX,t(11;22)(q23;q11)	21	23.8	2	1	No ET
46,XX,t(6;20;13)(p12;p12;q33)	24	25.0	6	5	No ET
46,XX,t(4;8)(p16;p23)	23	26.0	10	4	No ET
46,XX,t(9;11)(q22;q22)	23	26.1	11	7	No ET
Mean±SEM	*20.6±1.1*	*19.6±1.2*	*7.4±0.9*	*3.2±0.5*	*NA*

12 RCPT
3 ROBT

No redundant break point

No ET 11 patients
No PR 4 patients

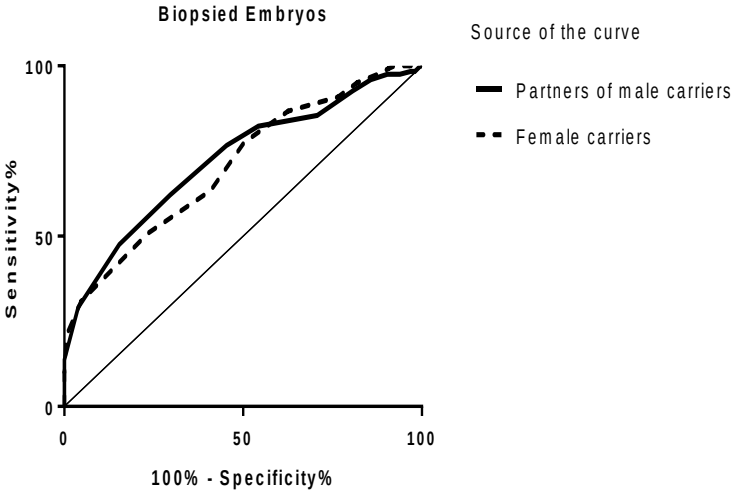
PGT-ET outcomes in female or male translocation carriers

	Female carriers 139 couples 336 cycles	Male carriers 192 couples 424 cycles	*P* value
OR (n)	297	384	
Number of oocytes retrieved[a]	11.7±0.3	12.1±0.2	NS
Number of metaphase II oocytes[a]	9.7±0.2	10.0±0.2	NS
Number of Biopsied embryos[a]	5.1±0.2	4.7±0.1	NS
Number of balanced embryos[a]	1.2±0.1	1.6±0.1	<0.001
Unbalanced embryo rate (%)	75.9	65.5	<0.0001
Number of embryo transfers (% per OR)	176 (59.2)	260 (67.7)	<0.05
Number of embryos transferred[a]	0.85±0.04	1.05±0.04	<0.01
Number of pregnancies (n)	57	75	
Clinical pregnancy per OR (%)	19.2	19.5	NS
Clinical pregnancy per ET (%)	32.4	28.8	NS
Number of live Births (n)	50	57	
Live Birth Rate per OR (%)	16.8	14.8	NS
Live Birth Rate per ET (%)	28.4	21.9	NS

[a] mean ± SEM

Parameters influencing the chance to reach an ET

		AUCs	CI	Sensitivity (%)	Specificity (%)	Cutoff level
Number of metaphase II oocytes	Males	0.595	0.535-0.655	NA	NA	NA
	Females	0.627	0.563-0.691	NA	NA	NA
Number of biopsied embryos	Males	0.724	0.668-0.708	82	45	5.5
	Females	0.707	0.646-0.767	87	37	6.5



The number of mature oocytes is weakly informative

While the number of biopsied embryos seems to be more predictive

The cut-off value for biopsied embryos was higher in female carriers

Conclusion: Lessons learned

- diminished capacity of small antral follicles to respond to exogenous FSH
- the proportion of female carriers with a very low or low FORT values (<35.3%) was higher than in controls
- no consequence on PGT-ET outcomes was observed
- albeit female translocation carriers with a FORT value fewer than 27%, we failed to obtain ET or PR
- female aging could progressively be an additional negative factor since aging greatly influences the number of small antral follicles available before treatment

Conclusion: Lessons learned

- Embryo-unbalanced rate was significantly higher in female translocation carriers compared to controls

=> discontinuous gradient centrifugation to select spermatozoa before ICSI could decrease the proportion of chromosomally unbalanced spermatozoa (Perrin et al., 2011; Rouen et al., 2013)

=> male meiosis is different from female with an increased incidence of 3:1 and 4:0 segregation in females carriers compared to males (Tease *et al.*, 2002; Ko *et al.*, 2010)

Conclusion: Lessons learned

- The number of metaphase II oocytes is weakly informative for evaluating the chance to reach PGT-ET

- For the number of biopsied embryos : the true positive rate (sensitivity) was >0.8 meaning that if we succeed to biopsy a sufficient number of embryos, the chance to reach ET is fairly good.
- If the number of embryos suitable for biopsy does not reach the calculated cut-off, should we propose an accumulation of vitrified embryos? the true negative rate (specificity) is low…..

- in view of our results we do advise to be more demanding with female carriers than with partners of male carriers

Limits of our study

- Retrospective
- Confounding factors are missing (smoking, drinking, environmental exposure)
- French legal issue : PGT performed by the use of FISH => inter-chromosomal effect could not be assessed

Acknowledgment

- Couples who consent to the use of their medical data

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