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Genetics of male infertility

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Infertility

- **10-15% of couples world wide suffer from infertility or subfertility**
- **Male factor represents ~50% of these cases**
- **Genetic abnormalities account for ~15% of male infertility**
- **Natural selection prevents the transmission of mutations causing infertility**
- **This protective mechanism may be overcome by assisted reproduction techniques**
- **Identification of genetic causes - good practice for appropriate management of infertile couples**

Genetic causes of male infertility

- **Chromosomal abnormalities**
Klinefelter Sy. (XXY), XYY and XX males
- **Y chromosome deletions**
Y chromosome microdeletions, partial AZFc deletions
- **Gene mutations**
CFTR mutations
Androgen receptor gene mutations
- **Gene polymorphisms**
Y haplogroups
Androgen receptor (AR) gene exon 1 CAG repeat
POLG gene CAG repeat
Folate gene polymorphisms (MTHFR, MTR, MTRR)

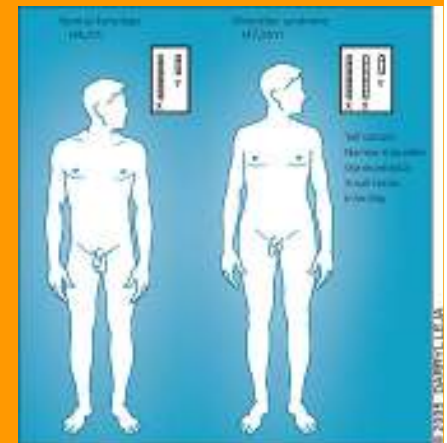
Chromosomal abnormalities

- **Incidence**

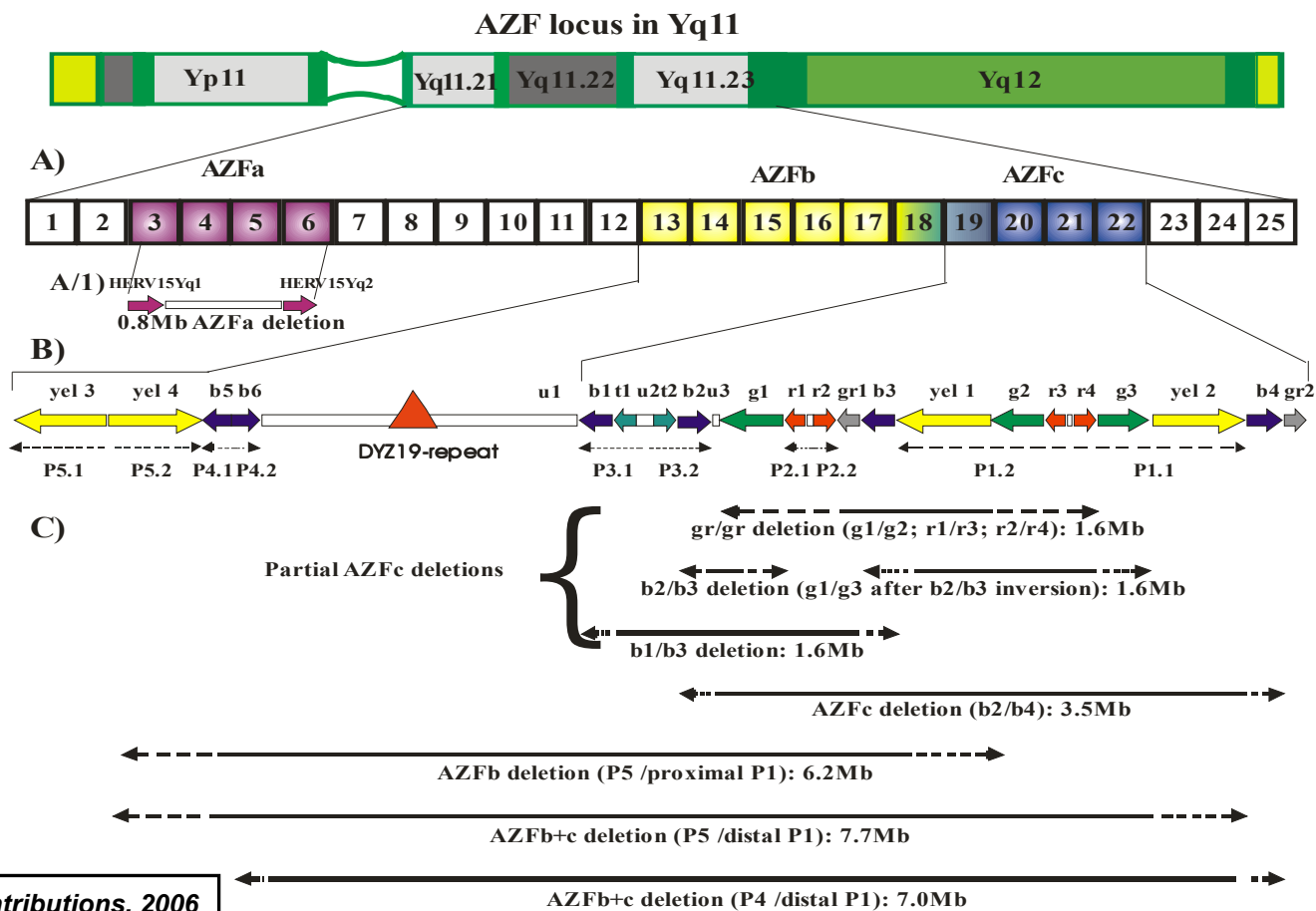
- **2 – 8% in infertile men**
- **15% in azoospermic males**

- **Sex chromosomal abnormalities are predominating**

- ***Klinefelter Sy (47,XXY)* usually azoospermic**
- ***47, XYY* – mainly fertile, variable sperm parameters (normo to severe oligozoospermia)**
- ***46,XX males* - always azoospermic**



Y chromosome - AZF region



Y microdeletions

- ***Prevalence of 10-15% in non-obstructive azoospermia and severe oligozoospermia***
- ***de novo events (in rare cases transmitted)***
- ***Variable phenotype of identical deletions (other modifier genes involved)***
- ***ICSI allows transmission of Y deletions and increases the risk of sex chromosome aneuploidy in the offspring***
- **Detection by PCR of STS markers**

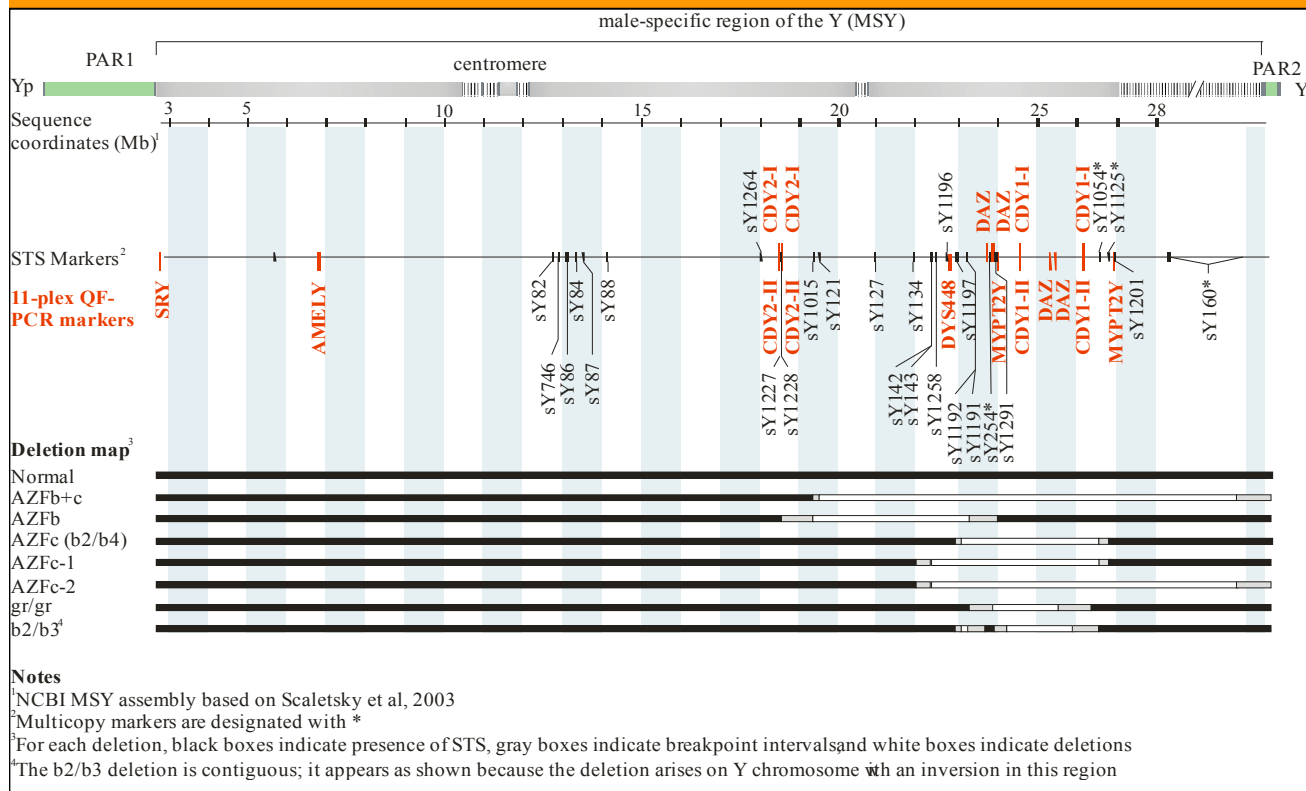
Y microdeletions detected among Macedonian infertile men



Partial AZFc deletions & duplications

- **Role of partial AZFc deletions is debated**
- **Remove half of the AZFc gene content (2 DAZ, 1 CDY, 1 BPY2)**
- **gr/gr, b2/b3**
- **Associated with a wide range of sperm counts (azoo to normozoospermia)**
- **Partial AZFc duplications (gr/gr, b2/b3)**
- **Detection by STS, gene dosage & gene copy type analysis**

QF-PCR analysis of sex chromosome aneuploidies and AZF deletions/duplications



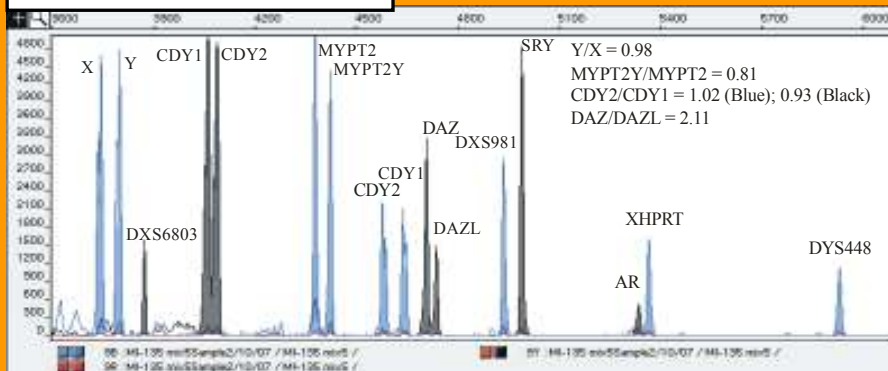
Marker	Chromosome location	Label	Repeat element	Allele size range in bp
AMEL	Xp22.1-p22.31 Yp11.2-p22.1	6-FAM	/	106 112
CDY - I	CDY 1 - Y (AZFc) CDY 2 - Y (AZFb)	HEX	/	134 137
DXS6803	X	HEX	tetra	/
MYPT2	MYP T2Y - Y (AZFc) MYPT2 - chr 1	6-FAM	/	181 176
CDY - II	CDY 1 - Y (AZFc) CDY 2 - Y (AZFb)	6-FAM	/	200 194
DAZ	DAZ - Y (AZFc) DAZL - chr 3	HEX	/	208 211/251*
SRV	Yp11.2-p22.1	HEX	/	248
DXS981	Xq 13.1	6-FAM	tetra	/
XHPRT	Xq 26.1	6-FAM	tetra	/
AR	Xq11.12	HEX	tri	/
DYS448	Yq11.2	6-FAM	hexa	/

Results of the 11-plex QF-PCR in 357 DNA samples

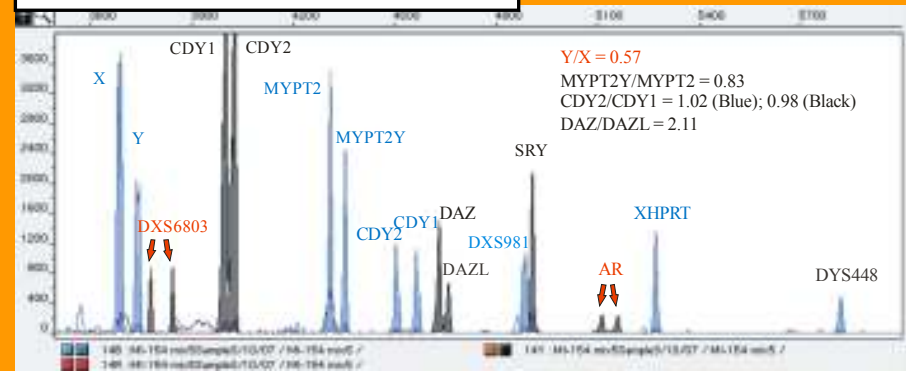
Marker	Normal values (range & average±StDev)	Sex chromosomal aneuploidies			AZF deletions					Partial AZFc deletions			AZFc duplications	
		XXY	XX	XYY	b 2/b4	AZFc-1	AZFc-2	AZF b	AZFb+c	gr/gr	b2/b3	gr/gr + b2/b4dup	gr/gr & b2/b4	b2/b3
AMEL Y/X ratio	0.85-1.20 1.02±0.07	0.50-0.58 0.54±0.03	no Y	1.89-2.04 1.97±0.11	normal	normal	normal	normal	0.23	normal	normal	normal	normal	normal
DAZ/DAZL (Y/chr 3) ratio	1.70-2.57 (2.01-2.89) 2.10±0.19 (2.48±0.19)	normal	no DAZ	4.55-5.35 4.95±0.57	no DAZ	no DAZ	no DAZ	0.97	no DAZ	0.86-1.57 1.00±0.21	0.90-1.36 1.16±0.24	2.29-3.37 2.95±0.58	2.70-6.09 3.9±0.80	3.20-7.51 5.52±2.44
MYPT2Y/MYPT2 (Y/chr 1) ratio	0.57-0.98 0.77±0.07	normal	no MYPT2Y	1.64-1.76 1.70±0.08	0.26-0.44 0.30±0.07	0,34	no MYPT2Y	normal	no MYPT2Y	0.30-0.46 0.36±0.04	normal	0.31-0.35 0.33±0.02	1.01-2.13 1.30±0.24	normal
CDY2/CDY1 - I (AZFb/AZFc) ratio	0.92-1.13 1.03±0.03	normal	no CDY1 no CDY2	normal	no CDY1	no CDY1	no CDY1	0.52	no CDY1 no CDY2	2.24-2.57 2.24±0.15	2.11-2.38 2.21±0.12	0.68-2.44 1.38±0.93	0.40-0.72 0.63±0.10	0.38-0.69 0.60±0.15
CDY2/CDY1 - II (AZFb/AZFc) ratio	0.82-1.08 0.94±0.05	normal	no CDY1 no CDY2	normal	no CDY1	no CDY1	no CDY1	0.42	no CDY1 no CDY2	1.31-2.63 1.87±0.30	1.67-2.11 1.86±0.16	0.54-1.90 1.23±0.70	0.36-0.80 0.64±0.10	0.52-0.70 0.61±0.09
DXS6803/DXS 981/ XHPRT/ AR	1 allele of each STR marker	1 or 2 alleles	1 or 2 alleles	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal
SRY	248 bp fragment	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal
DYS 448	1 allele	normal	no fragment	normal	normal	no fragment	no fragment	no fragment	no fragment	normal	normal	normal	normal	normal
No of individuals	274	10	2	2	6	1	1	1	1	11	5	3	32	4

Electrophoreograms of the 11-plex QF PCR

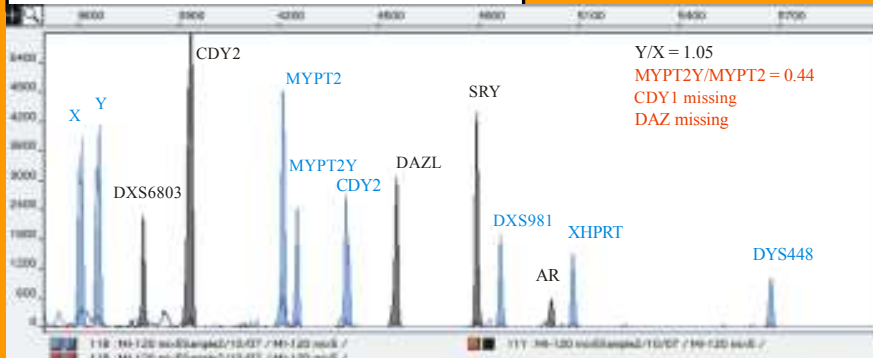
Normal sample



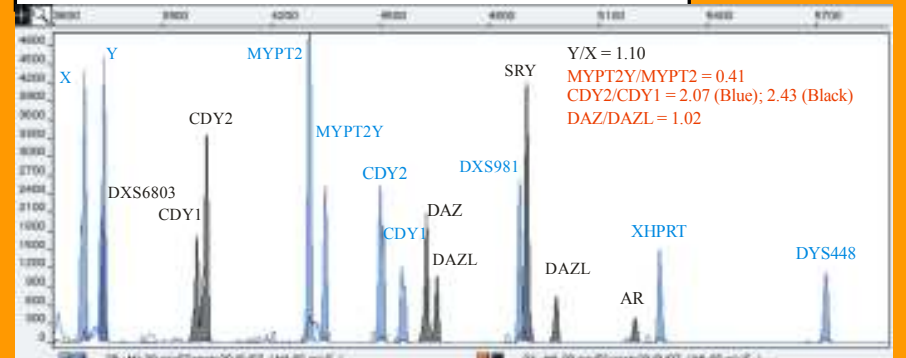
Klinefelter (XXY) Sy.



AZFc (b2b4) deletion



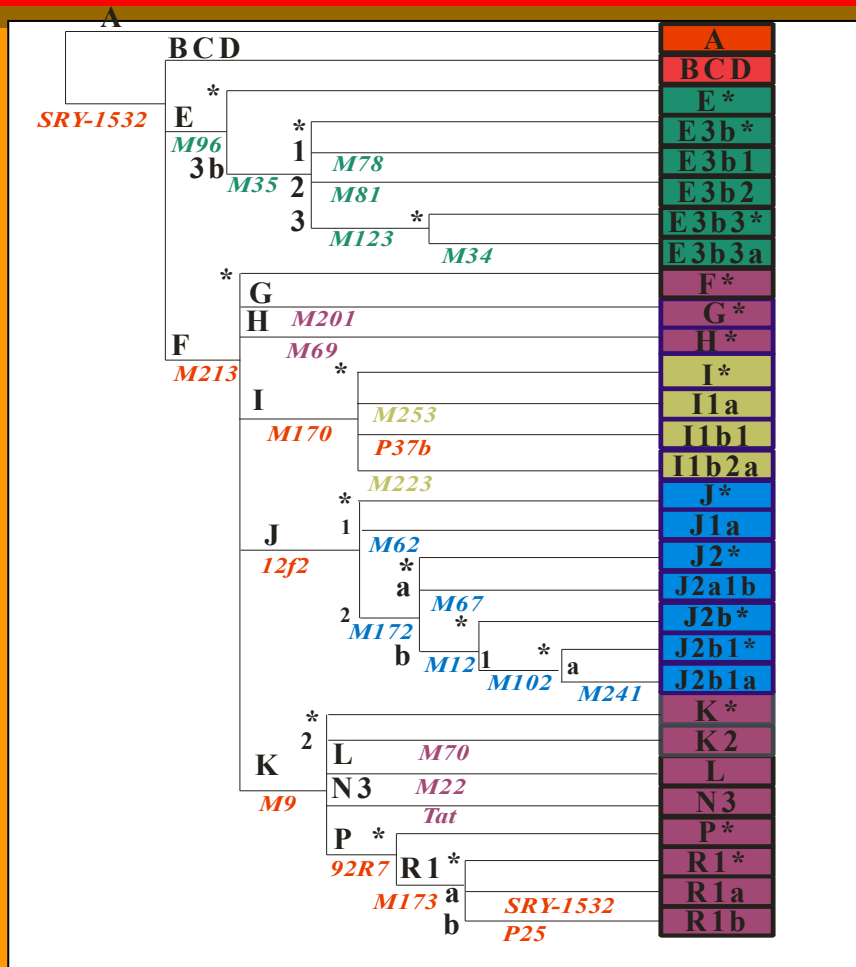
Partial AZFc (gr/gr) deletion



Distribution of chr. aneuploidies and AZF deletions/duplications

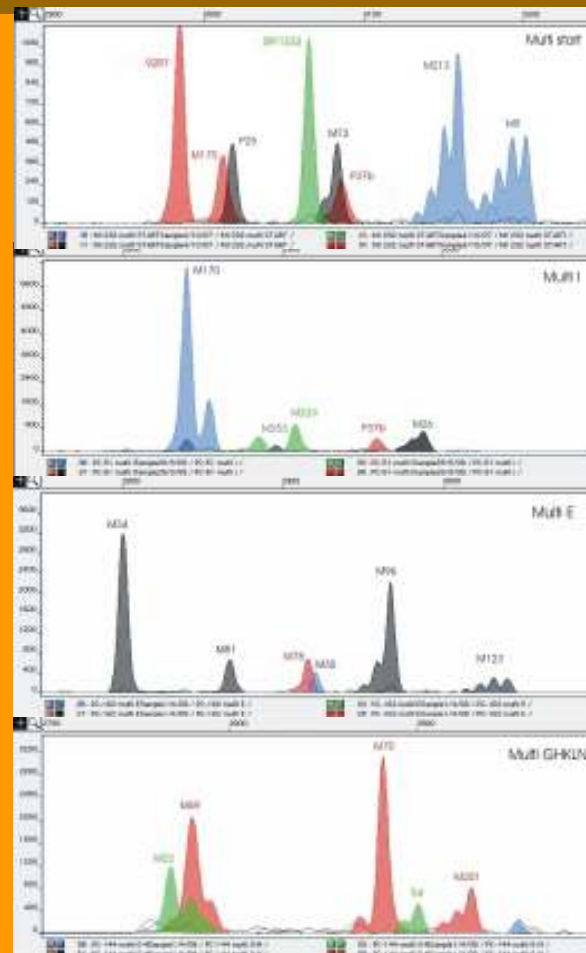
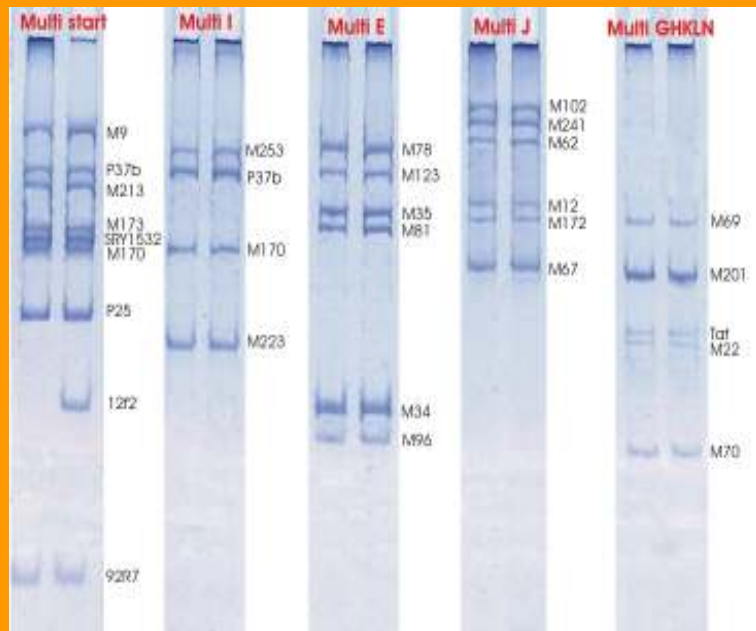
Condition	Infertile patients (n=205)				Fertile Controls (n=152)
	Azoospermia (n=89)	Severe oligozoospermia (n=54)	Mild oligozoospermia (n=27)	Normozoospermia (n=35)	
Chromosome aneuploidies					
XXY	10 (11.2%)				
XX	2 (2.2%)				
XYY	1 (1.1%)	1 (1.8%)			
AZF deletions					
AZFc (b2/b4)	5 (5.6%)	1 (1.8%)			
others (c1, c2, b, b+c)	4 (4.5%)				
Partial AZFc deletions					
gr/gr	2 (2.2%)	1 (1.8%)	1 (3.7%)	3 (8.6%)	4 (2.8%)
gr/gr + duplicat.				1 (2.9%)	2 (1.4%)
b2/b3	1 (1.1%)	2 (3.7%)	1 (3.7%)		1 (0.7%)
AZFc duplications					
gr/gr & b2/b4	5 (5.6%)	3 (5.6%)	4 (14.8%)	6 (17.1%)	14 (9.2%)
b2/b3		1 (1.8%)			3 (2.0%)

Y haplogroups and male infertility



- **Slowly mutating binary markers on the male specific region of the Y chromosome**
- **studying the origin of human populations and measuring the variability among them**
- **Y SNP typing in association with male specific and male associated diseases**
- **Association of Y haplogroups with infertility and Y deletions – contradictory results**

Y chromosome SNP typing by SNaPshot minisequencing in Macedonian infertile men



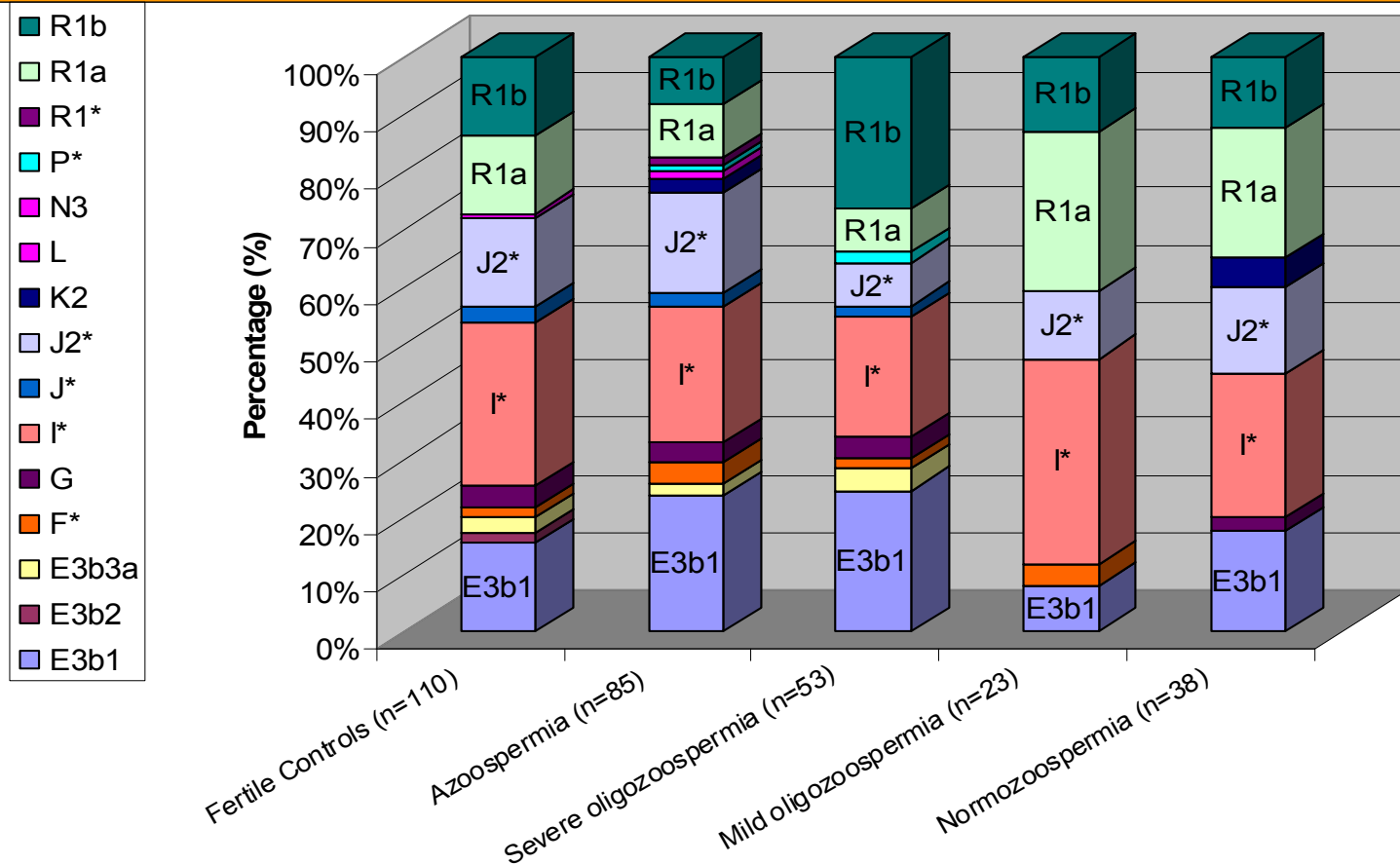
R1a-SRY1532

I1b2a-M223

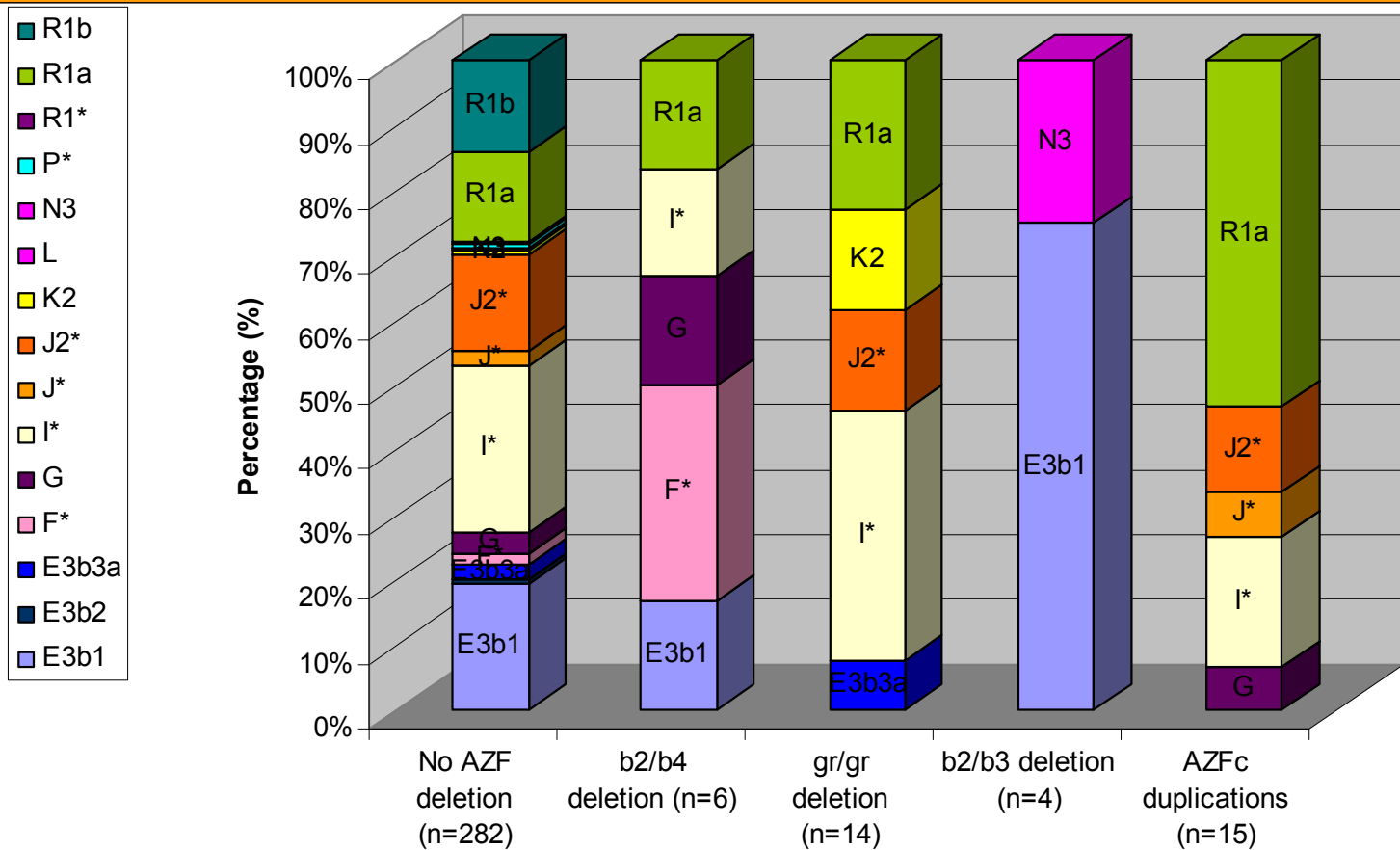
E3b1a-M78

G-M201

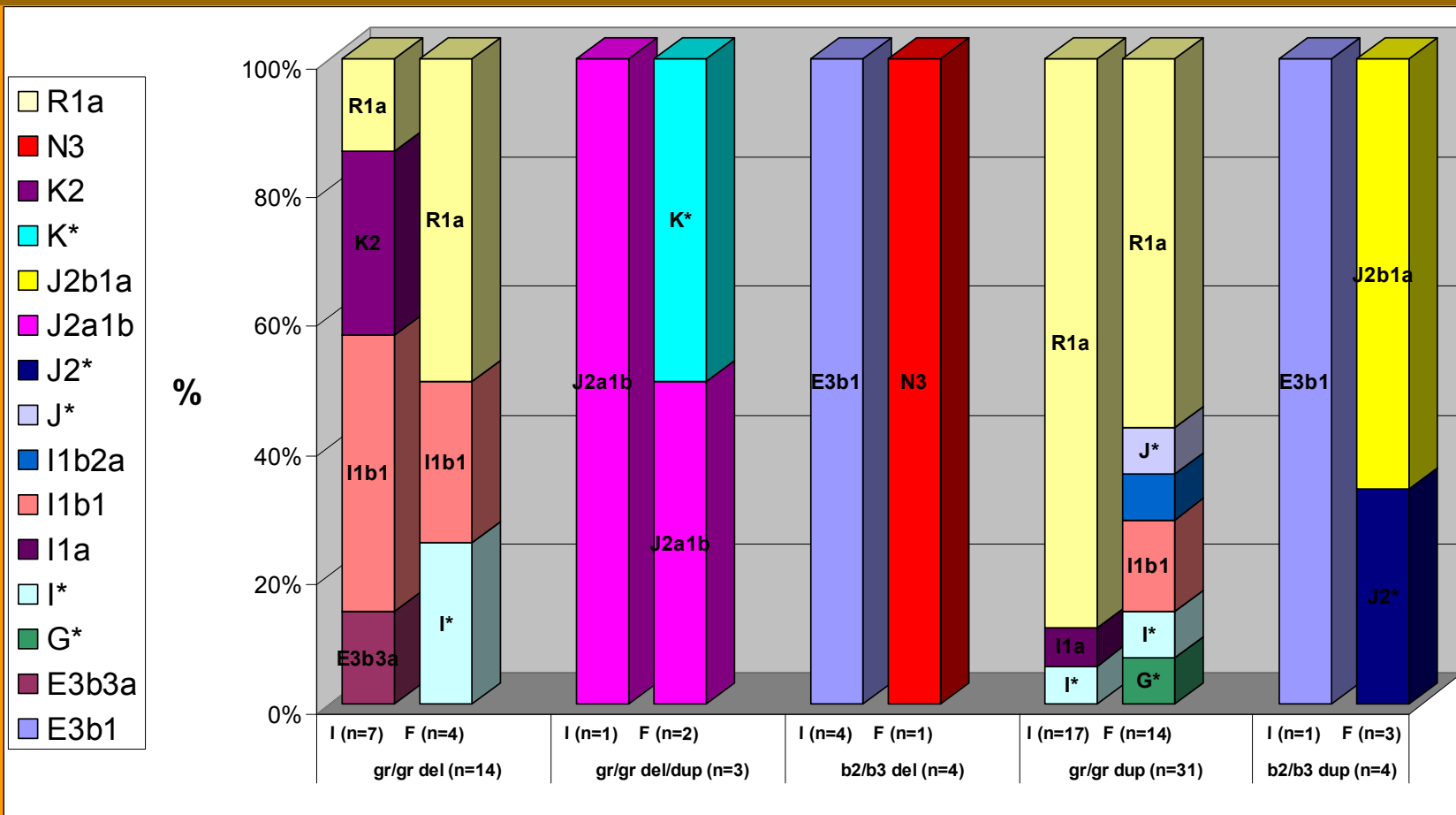
Y haplogroups in Macedonian infertile men



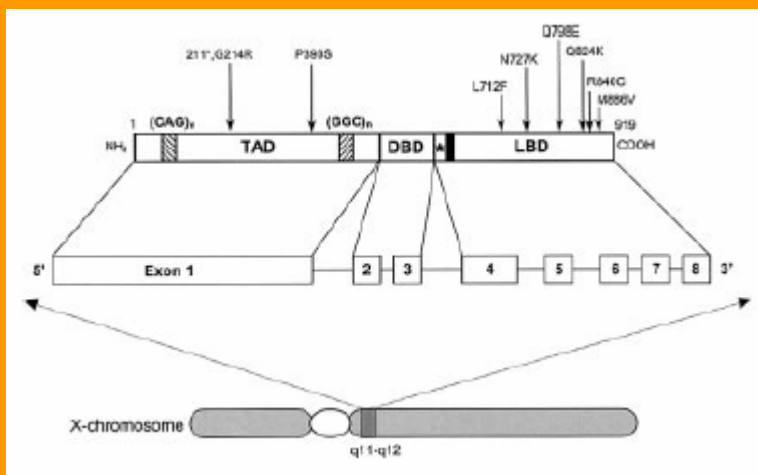
Y haplogroups in Macedonian men with AZF rearrangements



Y haplogroups in Macedonian infertile and fertile men with AZF rearrangements

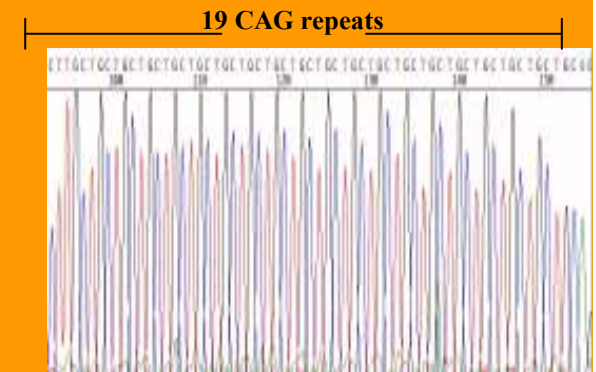
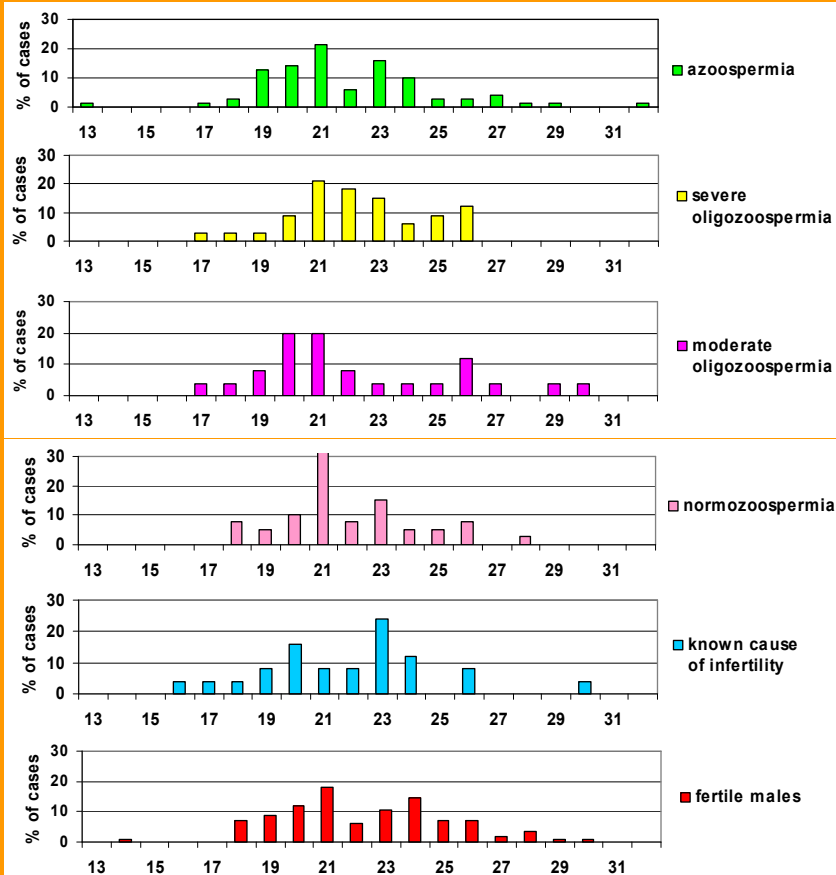


Androgen receptor gene CAG repeat and male infertility



- **Polymorphic CAG repeat in exon 1 (10-36 repeats)**
- **>38 Kennedy disease or SBMA**
- **Longer CAG repeat lengths result in reduced AR transcriptional activity**
- **Link between long CAG repeats and male infertility**
- **Discordant results in the literature**

Androgen receptor CAG repeats among Macedonian infertile men



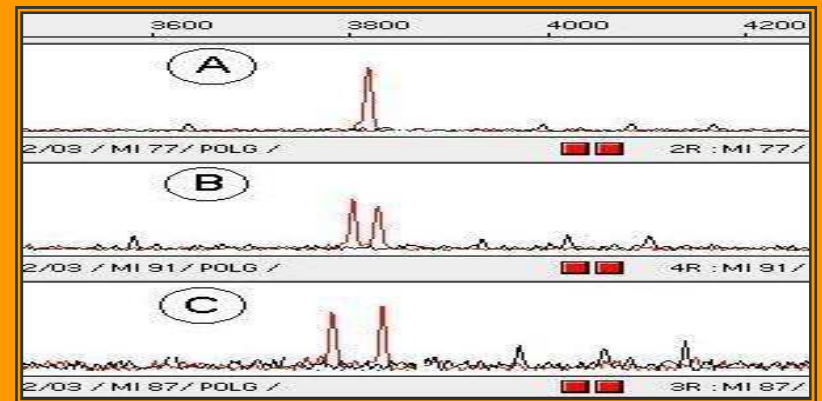
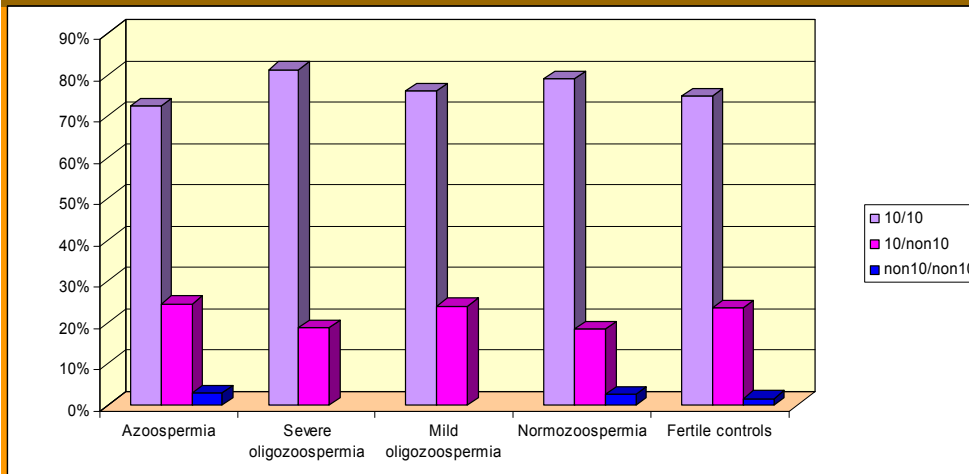
Group (number of chromosomes)	CAG Repeats					
	>28		>27		>26	
	n (%)	p Value	n (%)	p Value	n (%)	p Value
Azoospermia (n = 73)	2 (2.7)	0.449	3 (4.1)	0.866	7 (9.6)	0.424
Severe oligozoospermia (n = 51)	0 (0.0)	0.410	1 (2.0)	0.401	2 (3.9)	0.486
Mild oligozoospermia (n = 31)	3 (9.7)	0.009	5 (16.1)	0.018	6 (19.4)	0.022
Normozoospermia (n = 40)	0 (0.0)	0.266	1 (2.5)	0.553	1 (2.5)	0.323
Known cause of infertility (n = 36)	1 (2.8)	0.529	1 (2.8)	0.625	2 (5.6)	0.821
Fertile controls (n = 152)	2 (1.3)		7 (4.6)		10 (6.6)	

Plaseski et al, BJMG 2007

POLG gene CAG repeat and male infertility

- **POLG gene (15q24) encodes for the catalytic subunit of the human mitochondrial DNA polymerase γ**
- **Polymorphic CAG repeat**
- **Association between the absence of 10 CAG repeat allele and male infertility**
- **POLG CAG polymorphism – possible contributing factor for idiopathic subfertility with normal spermiograms**
- **Several additional studies did not confirm the first observation**

POLG CAG repeats among Macedonian infertile men

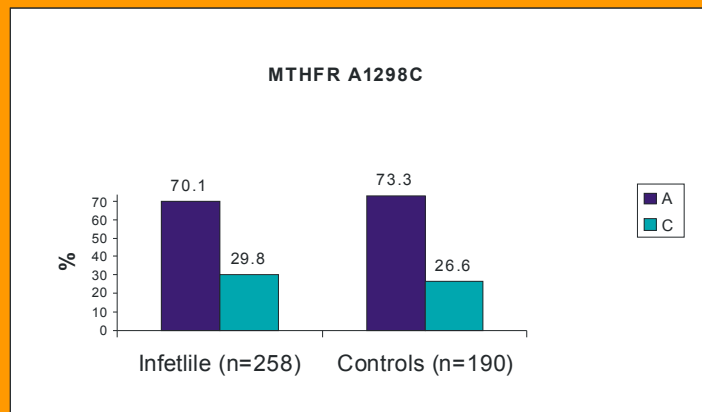
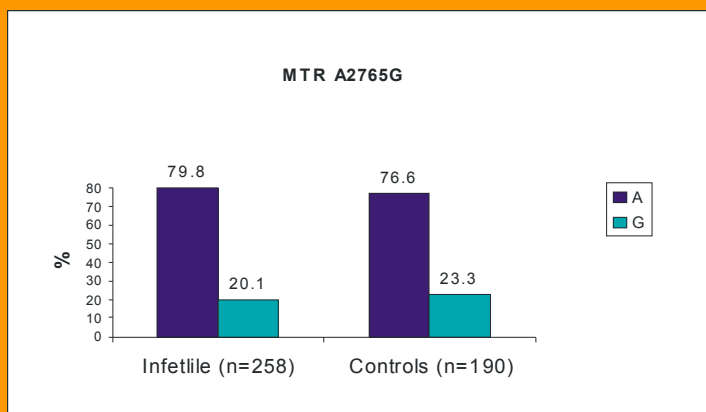
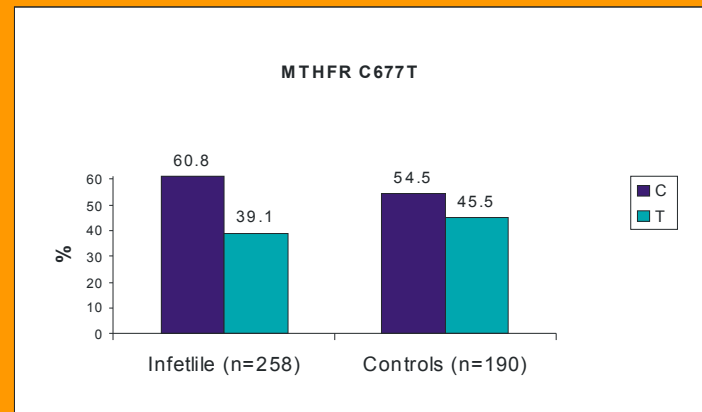
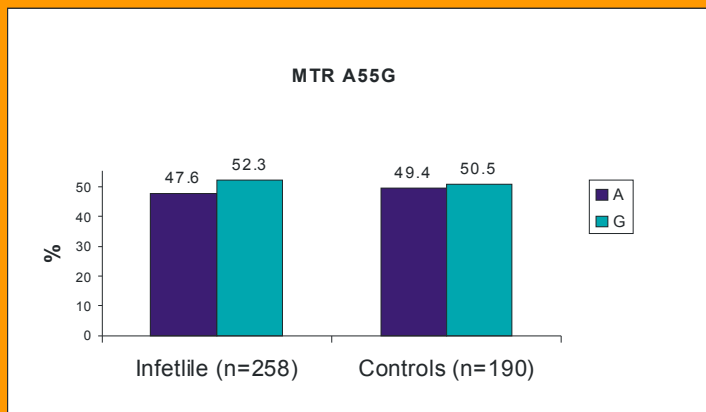


Genotype	10 / 10		7 / 10		8 / 10		9 / 10		10 / 11		10 / 12		9 / 11		11 / 11		Total n
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Infertile/subfertile men (Total)	173	76.9	1	0.4	2	0.9	5	2.3	39	17.4	1	0.4	1	0.4	3	1.3	225
Azoospermia	54	73.0					3	4.0	14	18.8	1	1.4	1	1.4	1	1.4	74
Severe oligozoospermia	45	80.4	1	1.8			1	1.8	9	16.1							56
Mild oligozoospermia >5	21	77.8			1	3.7			5	18.5							27
Normozoospermia	30	76.9			1	2.6			7	17.9				1	2.6		39
Known cause of infertility	23	79.3					1	3.5	4	13.7				1	3.5		29
Fertile controls	92	74.8	1	0.8	2	1.6	1	0.8	22	17.9	3	2.4			2	1.6	123

Folate gene polymorphisms and male infertility

- **Folate deficiency and related hyperhomocystinemia are considered a risk factor for male infertility**
- **Folate related genes MTHFR, MTR, MTRR play a critical role in DNA synthesis and methylation reactions**
- **Possible negative effect- alteration in expression of spermatogenesis genes due to undermethylation**
- **Conflicting results in the literature**

Folate gene polymorphisms in Macedonian infertile men



***Noveski et al
BMHG 2009
Poster No.***

Acknowledgements

➤ Participants:

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