

# Genetic Damage to Sperm Following Treatment with Azathioprine/6-Mercaptopurine in Men with IBD

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# Introduction

- Little is known about the effects of AZA/6MP on fertility & pregnancy outcomes among fathers with IBD
- AZA/6MP, though FDA pregnancy category D, are often continued in pregnant women as the available data suggests low risk
- Data among fathers on AZA/6MP are mixed
  - Since AZA/6MP interferes with nucleic acid synthesis, treatment with these agents can theoretically produce germ cell mutations as well as teratogenic effects.

# Animal Studies

- Mice treated with 6mp:
  - Chromosomal aberrations and SCE in germinal cells
  - Dominant lethal mutations (chromosomal aberrations that may not affect ability of sperm to fertilize ovum, but result in early embryonic death around the time of implantation)
    - Major congenital malformation rate 2% cases/controls
    - % females impregnated decreased in direct relation to escalating doses 6mp (2,5,8 mg/kg IP)
    - 6MP did not impair sperm production and morphology, however, significantly higher rates of nonviable pregnancies in the females indicated occult sperm damage
- Suggests that genetic evaluation of sperm is needed to detect damage from 6mp

# Human Studies

- Retrospective Case Series:
  - 37 men, similar outcomes regardless of 6mp use
  - WAGR (Wilms, aniridia, genital anom, retardation)
  - 4/13 pregnancies 6mp w/i 3 months of conception:
    - Risk vs. 6MP>3 mos ( $p<0.013$ ) no exposure ( $p<0.002$ )
- Danish population cohort study
  - 54 exposed pregnancies vs. 57,195 control
    - Pts: glomerulonephritis, severe skin disease
  - 7.4% (4/54) vs. 4.1% (2334/57195) CA
  - OR= 1.8 (95% CI 0.7-5.0)
  - All male children
  - No CA if conception w/i 3 months of prescription

# Semen Analysis

- Semen quality
  - 23 IBD pts on AZA vs. WHO standards
  - AZA/SAS: markedly reduced semen morphology
  - AZA 1.5-2 mg/kg (n=18):
    - No difference before and after therapy with AZA.
  - However, some decrease in motility and morphology compared to normal suggesting effect of disease itself

# Hypothesis

- Basic semen analysis is inadequate to determine damage to sperm from AZA/6MP
- AZA/6MP may lead to genetic damage that results in infertility rather than congenital malformations
  - Dominant lethal, nonheritable mutations
- Study Aim:
  - Compare rates of genetic damage to sperm among men with IBD exposed and unexposed to AZA/6MP using sperm chromatin structure assay (SCSA)

# SCSA

- Established technique to study male infertility
- Rapid flow cytometry measurements of 5,000 individual sperm per sample
  - SCSA utilizes metachromatic properties of acridine orange to distinguish between native DNA (green fluorescence = double-stranded) and low pH denatured DNA (red fluorescence = single-stranded) at sites of DNA strand breaks.
  - Data are presented as DNA fragmentation index  
 $\%DFI = \text{Red} / (\text{red} + \text{green}) \text{ fluorescence}$
  - **%HDS** = % cells with high green fluorescence
    - Immature sperm with intact DNA

# Methods

- All men in the UCSF IBD Database were contacted by phone or mail, as well as during routine office visits.
- Populations:
  - *Exposed* population: men with IBD on 6MP/AZA for at least 3 months, with a stable dose for at least one month.
  - *Unexposed*: men w/ IBD not on 6MP/AZA for at least 3 mos
  - Biologics, other immunosuppressives, corticosteroids, sulfasalazine, abx not allowed for either group. Mesalamine, budesonide allowed
  - Patients must be in remission. CDAI, MTWSI measured.
- Two semen samples were procured 2 weeks apart
  - Semen analysis w/ strict morphology on both samples
  - SCSA on 200-ul aliquot of raw, liquefied semen from 2nd
- A serum sample was drawn for AZA/6MP metabolites
- Subjects were identified by exposure status and then the prevalence of DNA damage between these populations was contrasted

# Results: Patient Demographics

	Unexposed N=9	Exposed N=16
Mean Age (yrs)	34.2 (28-44)	32.9 (23-45)
Current Tobacco/ETOH	2 tobacco/8 etoh	1tobacco/10 etoh
Married	3 (33%)	4 (25%)
Prior Pregnancies	6: 1 preg/ 5 term	1: miscarriage
Venereal Disease	0	3
IBD Type: UC	6 (3 IPAA)	9
CD	3	7
Mean IBD Duration (yr)	8.7 (6-13)	7.1 (1-22)
Mean CDAI	32.3 (7-61)	93 (57-165)
MTWSI	1.5 (0-5)	2.8 (0-7)
Metabolite Lvl: 6TG		211 (75-400)
6MMP		3048 (245-5011)

# Results

Mean values [WHO normal]	Unexposed N= 9 (range)	Exposed N=16 (range)	P value
Days Abstinent	3.3 (2, 4.5)	3.4 (2.0, 6.5)	0.75
Volume [1.5-5 ml]	2.9 (1.4, 4.8)	3.4 (1.1, 6.3)	0.38
Concentration [>20 mln/ml]	58.9 (25.5, 136)	66.5 (5.5, 140)	0.65
Motility [>50%]	65% (52, 80)	52% (41, 66)	<b>0.0007</b>
Progression [1-4]	2.88 (2.38, 3.5)	2.62 (1.8, 3.3)	0.18
Total Motile Ct [>40million]	101.4 (50, 141)	106.2 (8, 231)	0.83
Normal oval forms [>14%]	7% (1.5, 12.5)	6.4 % (0, 14)	0.73

# SCSA Results

Sample #2 Mean values	Unexposed N= 9 (range)	Exposed N=16 (range)	P value
DFI (%)	9.1% (3.9,14.7)	13.9% (3.7,31.6)	0.071
$\leq$ 15% excellent >15 to <30% good $\geq$ 30% fair to poor	9 excellent	11 excellent 4 good 3 fair-poor	0.123
HDS [<15% = normal]	5.6% (4-8.7)	7.71% (3.1-22.9)	0.17

# Summary

- Confirmed that men with IBD have sperm morphology below normal levels
- Men with IBD in remission exposed to AZA/6MP compared to those not exposed had
  - Significantly lower sperm motility rates
  - A trend towards higher % DNA Fragmentation
- Men with IBD on AZA/6MP having difficulty conceiving should undergo semen evaluation and consider stopping AZA/6MP during the conception period