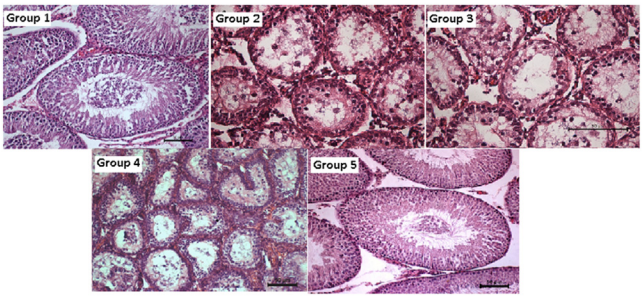
Table 1: Group characteristics

	DM	Oral gavage	Group name
Group 1	-	-	Control
Group 2	+	1 cc isotonic saline/day	Sham
Group 3	+	-	DM
Group 4	+	10 mg/kg/day TMZ	DM+10 mg TMZ
Group 5	+	20 mg/kg/day TMZ	DM+20 mg TMZ

Figure 1: Light microscopy images of testis tissue (groups 1-5)



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MP70-09
INDICATORS OF INFLAMMASOME ACTIVATION IN MEN WITH ABNORMAL SEMEN PARAMETERS

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INTRODUCTION AND OBJECTIVES: Assembly of the inflammasome complex results in caspase-1 activation which promotes the maturation and secretion of the pro-inflammatory cytokines IL-1β and IL-18. Evidence of inflammasome activation and the consequent elevation of semen cytokines has been reported as a possible cause of abnormal semen quality in men with spinal cord injury (SCI) [*Fertil Steril* 2013;99:118-24, *Hum Reprod* 2014;29:2368-73]. This study sought to determine if indicators of inflammasome activation were evident in non-SCI men with abnormal semen parameters.

METHODS: Semen was obtained from men with abnormal semen parameters who were being evaluated in an infertility clinic (n=131). Each of these men was assigned to a group depending on his semen parameters. Oligo: men with low sperm concentration but normal sperm motility (n=29); Astheno: men with low sperm motility (<40%) but normal sperm concentration (≥20 million/cc) (n=14); Oligo+Astheno: men with combined low sperm concentration and low sperm motility (n=70); Terato: men with normal sperm count, normal sperm motility, but low sperm morphology (<4%) (n=8); Azo: men with no sperm in their ejaculate (n=10). Over 80% of the men in the Oligo, Astheno and Oligo+Astheno groups also had low sperm morphology. Semen from healthy normospermic men (n=10) served as control material. Seminal plasma was separated from semen by centrifugation and analyzed for caspase-1, IL-18, and IL-1β concentrations using ELISA (R&D Systems, Minneapolis, MN). Comparisons between controls and patient groups were made by student's t-test. Statistical significance was considered at $p \leq 0.05$.

RESULTS: Caspase-1 concentrations were significantly elevated in all groups compared to controls. IL-1β concentrations were elevated in all groups compared to controls but reached statistical significance only in the Oligo+Astheno, and the Terato groups. IL-18 concentrations were also elevated in all groups compared to controls but reached statistical significance only in the Oligo+Astheno group. (Table 1).

CONCLUSIONS: This study shows elevation of inflammasome activation indicators (caspase-1, IL-1β and IL-18) in semen of patients presenting for an infertility workup. Further investigation of the inflammasome in various etiologies of male infertility may result in therapeutic interventions.

Table 1

	Control (n=10)	Oligo (n=29)	Astheno (n=14)	Oligo+Astheno (n=70)	Terato (n=8)	Azo (n=10)
Caspase-1 (pg/ml)	65 ± 18.0	268 ± 48.0*	716 ± 340.0*	598 ± 184.0*	206 ± 50.0*	246 ± 69.0*
IL-1β (pg/ml)	34 ± 9.6	57 ± 13.0	55 ± 9.3	61 ± 5.9*	52 ± 10.0*	72 ± 20.0
IL-18 (pg/ml)	39 ± 4.7	43 ± 4.9	48 ± 7.2	64 ± 5.2*	62 ± 14.0	64 ± 15.0

Values are means ± standard error of the mean
p-value = comparison to the control group within the same row; *p<0.05

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MP70-10
IMPACTS OF COMMON SEMEN HANDLING METHODS ON SPERM FUNCTION

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INTRODUCTION AND OBJECTIVES: To become fertilization competent, sperm must undergo a maturational process known as capacitation. This process involves plasma membrane changes that occur in response to stimuli within the female tract. Methods currently used in semen analysis, and/or sperm preparation for ART could affect sperm membranes. Here, capacitation was evaluated using localization of the ganglioside G_{M1} (Cap-Score^{TM}Sperm Function Test). In particular we compared methods of liquefaction and washing used to reduce viscosity and determined their effects on capacitation.

METHODS: Three common methods to reduce viscosity were evaluated. Ejaculates were: 1) Incubated for 0.25, 1.25 or 2 hrs, 2) diluted 1:1 with Modified Human Tubal Fluid (Irvine Scientific; Santa Anna, CA) and then passed through a wide orifice transfer pipette (WOTP) or a Pasteur pipette (PP), 3) Enzymatically digested with chymotrypsin (chymo). Pilot studies revealed that passage through a hypodermic needle negatively affected motility and membrane integrity and was not studied further. After liquefaction, samples were washed and incubated under capacitating (CAP) and non-capacitating (NC) conditions. Cap-Score values were obtained via fluorescence microscopy.

RESULTS: Liquefaction time, dilution and pipetting did not alter Cap-Score. Control (incubation only), WOTP and PP treated samples had Cap-Scores of 41±4, 40±5, and 41±6 (n=5; CAP). A decrease in response to capacitating stimuli was observed when samples were liquefied using chymo (P=0.03). Control samples had Cap-Scores of 40±6 (n=5; CAP) whereas samples enzymatically liquefied had Cap-Scores of 31±4 (n=5; CAP). Because chymo is a protease that can cut membrane proteins we checked if the Cap-Score reduction resulted from an alteration in labeling. Samples not exposed to capacitation stimuli were compared and no difference was observed. Control and enzymatically liquefied samples had Cap-Scores of 22±4 and 21±5 (n=5; NC). These data support the view that treating semen with chymo can inhibit the ability of sperm to respond to capacitation stimuli.

CONCLUSIONS: Liquefaction times of up to 2 hours and mechanical liquefaction using WOTP and/or PP did not influence capacitation. In contrast, the use of enzymes such as chymo reduced the ability of sperm to capacitate, as measured by Cap-Score. These results demonstrate the importance of knowing how semen processing methods impact sperm function.

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