

## FRESH VERSUS FROZEN SEMINAL PLASMA FOR ENHANCING SPERM MOTILITY IN ASTHENOZOOSPERMIC MALES

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Previous data demonstrated improvement in severe asthenozoospermia in men with retrograde ejaculates by adding donor seminal plasma (DSP); interestingly, no such improvement was demonstrated following suspension in Ham's F-10 medium. A study was performed to examine whether DSP would also improve asthenozoospermic ejaculates from men with antegrade semen. In addition, the study further explored whether the ameliorative effect of DSP could be maintained after freeze-thawing. The mean increase in motility following fresh DSP in 37 specimens was 102% and was -1.3% for DSP frozen at -20°C and 16.7% for DSP frozen at -196°C. The only statistical difference (using Student's *t* test) was seen in the comparison of fresh DSP to DSP frozen at -20°C ( $p = .001$ ). Nine of twenty-four men exhibited doubled baseline motility rates following addition of fresh DSP, compared to only 1 of 20 and 1 of 5, respectively, following addition of semen treated with DSP frozen at -20°C and -196°C, respectively. The data suggest that poor motility may improve when DSP is added to the specimen. However, proper quarantine may not be possible because efficacy is lost after freezing.

**Key Words:** Semen cryopreservation; Seminal plasma; Asthenozoospermia sperm.

### INTRODUCTION

Previous reports have been made of improved sperm motility by suspension of sperm in donor seminal plasma (DSP) obtained from males with retrograde ejaculation [1, 2]. Interestingly, these same males failed to demonstrate motility following suspension in supporting medium (Ham's F-10 medium). The samples of DSP used in this study were from fresh ejaculates.

Whether the improvement in motility is specific for men with retrograde specimens only or if it may have a wider applicability for other men with asthenozoospermia, remains to be determined. However, with the ever present risk of sexually transmitted disease (STD), it would be advantageous to freeze DSP for 6 months before use to first ensure negative HIV test results. A study was thus initiated to (1) corroborate previous data of the effectiveness of DSP in improving motility in asthenozoospermia males with antegrade ejaculation and (2) to determine if freezing seminal plasma has any deleterious effects.

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Received October 4, 1990, accepted October 17, 1990.

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**TABLE 1** Comparison of Percentage of Motility Increase in Semen Following Addition of Donor Seminal Plasma

Sample No.	Fresh % Increase	Frozen (-20°C) % Increase	Frozen (-196°C) % Increase
1	133.0	3.7	
2	316.0	3.5	
3	5.0	0.0	
4	700.0	0.0	
5	180.0	-5.0	
6	92.2	-21.4	
7	0.0	-5.0	
8	-5.0	5.0	
9	0.0	-21.4	
10	304.0	23.6	
11	-3.1	-3.1	
12	76.1	-66.6	
13	7.4	-48.1	
14	3.5	0.0	
15	47.0	-11.7	
16	133.0	3.7	
17	56.2		12.5
18	8.6		-29.2
19	129.6		11.1
20	59.3	6.3	-37.5
21	433.3	166.6	166.6
22	32.4	-27.0	-21.6
23	50.0	-28.5	-35.7
24	250.0	66.6	66.6

## MATERIALS AND METHODS

DSP was prepared by centrifuging semen from known fertile donors with at least 70% motile sperm with forward progressive motion. The semen was centrifuged at high speed and the supernatant checked microscopically for the absence of sperm. The semen of 37 asthenozoospermic men experiencing infertility problems were used as the test specimens. Twenty-two samples were used to compare the efficacy of motility enhancement following the addition of fresh DSP and frozen-thawed DSP (-20°C), and eight samples were used to compare fresh DSP and frozen-thawed DSP (-196°C); all three comparisons were made in four specimens. The DSP was frozen for 1 to 4 weeks.

## RESULTS

Comparison of baseline motility following addition of DSP was made by evaluating the percentage of increased improvements as seen in Table 1. The mean increase in motility following fresh DSP in 37 specimens was 102% with an SD of 45; for frozen DSP (-20°C), -1.3% with an SD of 45; and for frozen DSP (-196°C), 17% with an SD of 70. A paired Student's *t* test showed that the difference between fresh and frozen (-20°C) was significant

( $p = 0.001$ ) but no statistical difference was seen in the comparison between fresh and frozen ( $-196^{\circ}\text{C}$ ) or frozen ( $-20^{\circ}\text{C}$ ) and frozen ( $-196^{\circ}\text{C}$ ).

The comparisons were made on samples obtained from 26 patients. Testing samples were split into 2 or 3 aliquots and evaluated after adding fresh DSP and either frozen DSP at  $-20^{\circ}\text{C}$  and/or frozen DSP at  $-196^{\circ}\text{C}$ . Nine of the twenty-four men (37.4%) demonstrated at least a doubling of their baseline motility when fresh DSP was added, compared to only 1 of 20 (5%) when frozen DSP ( $-20^{\circ}\text{C}$ ) was added and 1 of 8 (12.5%) when frozen DSP ( $-196^{\circ}\text{C}$ ) was added. In fact, the same patient responded to both types of frozen DSP, compared to 250% improvement with frozen DSP. Fisher's exact test showed a statistical significance between fresh DSP and frozen DSP ( $-20^{\circ}\text{C}$ ) ( $p = 0.01$ ).

## DISCUSSION

The data collected corroborated that DSP may sometimes improve motility of asthenozoospermic ejaculates. The findings also demonstrated that conventional freezing does suppress the motility-enhancing effect of DSP. DSP is effective for some men whose specimens are entirely antegrade, whereas previously this beneficial effect had only been noted in men with retrograde specimens or the small antegrade fraction of men with the majority of the ejaculate retrograde.

To ensure STD control, DSP should be quarantined for 6 months and should not be used until HIV testing gives repeat negative results. Unfortunately, the efficacy of the DSP was not maintained after freezing either in liquid nitrogen or by conventional freezing methods.

## REFERENCES

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