Utilization of ZyMōt with Backup Extended Sperm Search and Microfreeze (ESSM) in Severe Oligospermia:

Reliably and Quickly Finds Adequate, Progressively Motile Sperm

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Introduction

Severe oligozoospermia and cryptozoospermia are characterized by sperm count fluctuations that may result in the inability to detect viable sperm for intracytoplasmic sperm injection (ICSI) on day of oocyte retrieval. The ability to reliably find sperm in these specimens, including freezing in advance, would prevent the need for egg freezing or cycle cancellation. It would also dramatically decrease the time spent searching for sperm on the day of retrieval.

We evaluated whether using ZyMōt in place of density gradient centrifugation for specimen preparation would consistently yield sufficient high-quality sperm to proceed with ICSI without the need for extended search.

Background

Microfluidic sperm separation (MFSS) using ZyMōt has been effectively used as a method of sample processing for ICSI that reduces the percent DNA fragmentation[1,2,3] in the final preparation, and can potentially improve outcomes for male factor infertility IVF cases. However, studies have excluded severe oligozoospermia and asthenozoospermia from analysis. The purpose of this study was to evaluate the effectiveness of the ZyMōt MFSS device in isolating progressively motile sperm in severe oligo- and astheno- zoospermic specimens.

Methods

Any patient that presented with a sperm count that was detectable by standard semen analysis was considered for inclusion. Samples with motility >0 (the observation of at least one motile sperm in the neat specimen) but with a total sperm concentration below 1 M/mL (0.0002M/mL - 0.7M/mL, n=20) or motility below 1% (n=2) were processed by ZyMōt Multi 850µL using the standard protocol. The remainder of the ejaculate was processed by density gradient. If the sperm concentration after ZyMōt was <1 sperm per µL, the sample was centrifuged at 300g for 5 minutes and resuspended in 100µL. If the final concentration was still <1 sperm per µL, the sample was subjected to ESSM (plating the pellet in microdroplets and systematically searching.) The majority of these samples were vitrified on SpermVD to be available in the event that the fresh sample on day of retrieval did not yield sufficient sperm. A total of 22 samples were analyzed.

Results

Every sample yielded progressive sperm through the ZyMōt. 17 samples had observable progressively motile sperm in the first 5µL droplet evaluated and were vitrified as suspensions (200-600 sperm). The remaining 5 samples yielded 6-65 progressively motile sperm after extended search.

Patient Demographics

Case #	Sperm Concentration (M/mL)	Sperm Motility	Female Partner Age	*^: same patient
1	0.016	85%	31	
2	0.013	5%	38	
3	0.23	55%	24	
4	0.001	60%	30	
5	0.008	5%	39	
6	0.004	30%	38	
7*	0.0004	*	29	*2 sperm observed, both motile
8	0.5	32%	35	
9*	0.005	86%	29	
10	0.0004	*	41	*2 sperm observed, both motile
11	0.0002	*	32	*1 sperm observed, motile
12	0.0004	50%*	32	*2 sperm observed
13	0.006	100%*	33	*1 sperm observed
14	3.4	<1%	34	
15	20	*	31	*1:80,000
16	0.5	14%	*oocyte donor	
17	0.0014	14%	32	
18^	0.022	82%	40	
19	0.7	12%	44	
20	0.012	20%	24	
21^	0.46	55%	40	
22	0.09	44%	24	

IVF Outcomes

	# MII ICSI/ retrieved	Fresh Sperm ICSI	Fresh Sperm Fertilization	Vitrified Sperm ICSI	Vitrified Sperm Fertilization	Blastocyst Formation	PGT Euploidy	Transfer Outcome	
5	20/24			20	9	0	-	-	
7/9	8/12	8	5	-	-	3	1	biochemical	
7/9	5/9	-	-	5	2	1	aneuploid	-	*3rd cycle without ZyMōt: ongoing pregnancy
8	11	-	-	11	5	2	aneuploid	-	
10	18/20	-	-	18	6	2	0	-	*3rd cycle without ZyMōt: no fertilization
10	12/17	-	-	12	1	2	0	-	
12	10/12	-	-	10	6	6	5	ongoing pregnancy	
14	27/48	-	-	27	7	4	1 + 1 mosaic	no transfer	
14	27/49	-	-	27	4	3	1 + 1 mosaic	ongoing pregnancy	
15	3/6	-	-	3	2	0	-	-	
15	7	-	-	7	2	1	1	no transfer	
15	9/13	-	-	9	4	1	1	ongoing pregnancy	
16	16	-	-	16	5	3	2 + 1 mosaic	no transfer	
18/21	7	-	-	7	0	-	-	-	
18/21	5/9	5	4	-	-	3	-	ongoing pregnancy	

Conclusion

The use of ZyMōt allowed for more samples with severely limited sperm concentrations to be usable for ICSI without extensive searching. It also significantly reduced the time spent per sample in isolating individual sperm from sample debris and immotile sperm. When combined with ESSM, motile sperm were found in all cases despite extremely low initial concentrations. In our experience this protocol dramatically increases the availability of sperm in these challenging cases, while simultaneously significantly decreasing the time spent per specimen.

References

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