



## Effect of microfluidic sperm sorting on equine ICSI blastocyst rate

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When performing equine intracytoplasmic sperm injection (ICSI) it is important to select the best possible sperm to be injected, to increase blastocyst and live foal production. Different techniques are used to prepare equine spermatozoa for ICSI, such as washing, swim up, density gradient or, more recently, microfluidic chip sorting (DxNow ZyMöt™ Multi chip). The ZyMöt™ chip selects a population of sperm with high viability, motility, normal morphology and very low DNA fragmentation (Gonzalez-Castro et al. JEVs. 2018; 66) (Quinn et al. HUMREP. 2018; 33: 1388-1393). The aim of this study was to evaluate the use of the ZyMöt™ Multi chip during equine ICSI of oocytes from subfertile mares (n=7) that had previously failed to produce embryos after one to three OPU-ICSI sessions using semen prepared by a standard washing. The mares were 11 to 22yo (median 18yo). Oocytes were collected by transvaginal ovum pick up, matured for 30 hours and frozen semen was prepared for ICSI by either i) a standard washing method including two centrifugation steps (3 min, 300 g) in G-MOPS™ PLUS (Vitrolife) or ii) with the ZyMöt™ Multi chip by adding

850 µl semen suspension to the inlet port and 750 µl G-MOPS™ PLUS to the collection chamber followed by 30 minutes incubation at 38 °C. Conventional ICSI was performed and the presumptive zygotes were cultured and evaluated for blastocyst formation from days 7 to 10. All blastocysts were vitrified for later transfer. Blastocyst rates were defined as the number of blastocysts per number of injected oocytes. All 7 mare-stallion combinations were able to produce embryos by ICSI, after sorting the sperm on the ZyMöt™ Multi chip. The blastocyst rates ranged from 16.7% (2/12) to 50% (3/6) for each mare with an average blastocyst rate of 26.5% (18/68) across all mare-stallion combinations. Our results demonstrate that in previously unsuccessful mare-stallion ICSI combinations, the ZyMöt™ chip process resulted in significantly higher blastocyst rates (26.5% vs. 0%,  $p < 0.0001$ , Fisher's exact test, two-sided) with an average of 2 embryos per OPU session (ZyMöt™ chip) vs. 0 embryos per OPU session (wash). Further studies are warranted to investigate the relationship between DNA fragmentation and ICSI and the role of microfluidic sorting of spermatozoa.

**Table 1**

Blastocyst rates from ICSI sessions with semen prepared by either simple washing or the ZyMöt™ chip. Statistics: Fisher's exact test.

Mare-stallion combination	Sperm preparation	# Blastocysts (%)	P value
MU-DE	Wash	0/15 (0%)	0.028
	ZyMöt™ Chip	4/12 (33.3%)	
AP-ZO	Wash	0/3 (0%)	>0.99
	ZyMöt™ Chip	4/15 (26.7%)	
CA-CO	Wash	0/14 (0%)	0.263
	ZyMöt™ Chip	1/5 (20%)	
MA-ZX	Wash	0/6 (0%)	0.500
	ZyMöt™ Chip	2/10 (20%)	
CA-MA	Wash	0/3 (0%)	0.464
	ZyMöt™ Chip	3/6 (50%)	
GG-CT	Wash	0/16 (0%)	0.101
	ZyMöt™ Chip	2/8 (25%)	
UDF-DO	Wash	0/6 (0%)	0.529
	ZyMöt™ Chip	2/12 (16.7%)	
<b>All pooled</b>	<b>Wash</b>	<b>0/63 (0%)</b>	<b>&lt; 0.0001</b> <b>****</b>
	<b>ZyMöt™ Chip</b>	<b>18/68 (26.5%)</b>	