CLEMENTE ASSOCIATES

33 Harkness Drive, Madison, CT 06443
U.S.A.

Magnetic Activated Sperm Sex Selection Client Feedback

Latest Human spermatozoa sorting results

(confirmed by FISH analysis)

FISH Summary of Human Nano Particles Sex Chromosome Sorting Experiments.

1000 sperm assessed/sample with FISH

Table 1. Mean Values for Sex Chromosome Proportions in Neat and Nano Sorted Human Sperm Samples (n=3)

Sample	X Sperm (%)	Y Sperm (%)
Neat	49.5%	50.5%
Sorted	39.9%	60%

Table 2. Raw data for Human Sperm FISH Study

Sample	X	X	Y	Y	XY % Aneuploid
	No.	%	No.	%	
Neat #1	577	54%	480	46%	0.3%
Sorted #1	380	37.8%	625	62%	0.2%
High Neat #2	440	44%	560	56%	0.0%
High Sorted #2	369	37%	630	63%	0.1%
Combo Neat #3	517	50.4%	507	49.4%	0.1%
Combo Sorted #3	450	45%	549	55%	0.1%

Animal Study (Species: Donkey) 2018 by Prof Hernan Ramirez Castex

Title: Magnetic nanoparticles for X sperm separation from donkey semen

1Hernan Ramírez Castex , MV, MSc; 2Esteban Domínguez, MV, PhD(c); 3Ana Flores Bragulat; 4Cristian Ugaz, MV,MSc, PhD; 5Henry Clemente; 2Laura Giojalas, Biol., PhD 3Luis Losinno, MV, PhD.

Abstract

Gender selection before conception has been a goal of scientists and breeders since ancient times. Dairy and beef cattle industries have productive and economic reasons to gender prediction. However, in equids, just a few breeds like Polo Argentino horses have the pressure for one sex on the progeny for sport reasons. The only reproducible and efficient way of separating X- and Y-bearing sperm based on DNA content is by the use of high-speed flow cytometry commercially available mostly for bovine species. The number of sperms that can be effectively sex-sorted per hour has been a limitation for the application and widespread use of this technology in species such as the equids that require large number of sperms for insemination. The main objective of this study was to test a new nanotechnology-based procedure for sorting equine sperm bearing X and Y chromosomes. Three Donkey Stallions of different breeds were used. Each ejaculate was divided into two fractions: control group (without nano-particle exposure) and Sex-sorted group (with nano-particle exposure for chromosome X bearing sperm collection). Both fractions were frozen for further post-thawed evaluation with flow cytometer for sorting efficacy and viability. Sperm parameters were assessed by computerized video analysis. Sperm capacitation, sperm selection and DNA fragmentation tests were performed. **Results showed that viability was not compromised and the average percentage of X-bearing sperm obtained was over 90%.** Sperm motility, velocities and capacitation levels showed no difference between control and sex sorted groups. DNA fragmentation was higher in the sex sorted group.

Table. Percentage of X spermatozoa determined by Flow Cytometry

Donkey	A	B	C	Mean +- SEM
% X Spermatozoa	92	97	80	90 +-5

Corresponding author

Hernán Ramírez Castex , MV, MSc. Flor de Azucenas 112, Dpto. 111, Las Condes, Santiago, Chile, CP 7560923 .
hernanramirez@bioteq.cl

Chile Study Equine 2017

x Sorted semen with Nano-particles

		Mares + Donkey Semen			Jennies + Donkey Semen		
fer							
# Pregnancies	% Total Eficiency	# A.I	# Pregnancies	Fertility rate /cycle	# A.I	# Pregnancies	Fertility rate /cycle
6	54.55%	8	5	62.50%	1	1	100.00%
11	61.11%						
					1	0	0.00%

125 days of gestation and at Foaling

of N.P in Equine and Donkey Semen

In-vivo Fertility rates and numbers using Se

	Mares + Stallion Semen					
	Direct Pregnancies			Embryo Trans		
	# A.I	# Pregnancies	Fertility rate /cycle	# A.I	# Flushings (+)	% Embryo Recovery
FRESH SEMEN	21	17	80.95%	11	8	72.73%
COOLED SEMEN				18	14	77.78%
FROZEN SEMEN	2	1	50.00%			
TOTAL A.I	62	TOTAL FERT. RATE	66.13%			
TOTAL PREGNANCIES	41					

In-vivo results of gender determination at 1

Number of pregnancies	41	
Number of pregnancies gender determinated at 125 days	23	
Positive Filly gender determinated at 125 days	22	96%
Number of foalings until today	11	
Number of fillies born until today	11	100%

In-vitro results for X-sperm sorting Eficacy