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Comparative Motility Of X And Y Chromosome Bearing Human Sperm Separated On The Basis Of Dna Content By Flow Sorting

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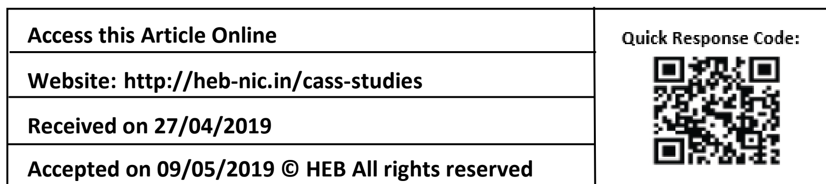

INTRODUCTION

Gender pre-selection has been a desire of humankind for generations. Numerous research studies have been conducted to achieve this goal for livestock production as well as for the human population (Amann 1989). Among the methods devised to separate X and Y sperm by apparent "physical characteristics" is the albumin gradient method described by Ericsson et al. in 1973 and applied by various clinics in human medicine for the past 15 years (Beernink et al.,1993). Supposedly the faster swimming speed of the smaller Y sperm enable those sperm to reach the bottom of the gradient before the X-bearing sperm. No conclusive proof has ever been put forth that this view is indeed true, since no method previously existed by which sperm could be separated into nearly pure X and Y sperm population (Johnson, 1992). This is so despite the fact that the albumin method has been offered to clinicians for many years as an effective methods for preselecting sex.

The X chromosome is larger than the Y chromosome and therefore contains more DNA. It might be expected that difference in DNA mass between X and Y chromosome –bearing sperm (Johnson et al.,1989) would influence swimming velocity.

Separation of X and Y sperm based on a difference in DNA content has been validated by the birth of offspring for the rabbit (Johnson et al., 1989), boar (Johnson, 1991), bull (Cran et al., 1993), and sheep (Cran et al., 1997); it also has been demonstrated that X and Y sperm can be sorted into separated population based on DNA in the human (Johnson et al., 1993). In general spearted populations have been determined to be 80 – 90% pure for populations of X or Y chromosome-bearing sperm. The CASA system used in this study the Hobson sperm Tracker (HST), is able to carry out continual assessment of sperm in real time, allowing a new approach to sperm assessment. The HST has been thoroughly investigated as an effective CASA system with high precision and coefficients of variation below 3% for each motion parameter measured (Holt, 1996). In contrast to other CASA systems that track an individual sperm for approximately 30 frames, the HST can track a sperm for as long as it remains in the area being an individual sperm for approximately 30 frames, the HST can track a sperm for as long as it remain in the area being viewed. Increasing the number of frames over which a sperm is tracked increases the accuracy of the data (Owen and Katz 1993).

The objective of this study was to evaluate the motility of human sperm (a factor of swimming speed) that had been sorted flow cytometrically into separate X and Y chromosome-bearing sperm populations. Sperm were prepared for flow sorting on the basis of a difference in DNA mass, and the sorted collected sperm were measured for the various parameters of motility that can be assessed with CASA.

MATERIALS AND METHODS

Semen Collection and Preparation

Ejaculates from three human were collected three times over 9 days in Experiment 1, and ejaculates from two human were collected four times over 8 days in Experiment 2. The semen was diluted in HEPES buffered medium (130 mM NaCl, 4mMKCI, 14 mM fructose, 1mM CaCl2, 0.05 mM MgCl2, and 1mM HEPES) containing 0.1% human serum albumin (HEPES-BSA)(Johnson et al., 1989) to a concentration of 15 X 106 sperm per milliliter. Samples of diluted semen were prepared as follow: stained with 5 ug/ml of Hoechst 33342 fluorochrome (HO42; calbiochem-Behring corp., La Jolla CA) and incubated for 35 min at 35c (Johnon et al., 1989) and incubated for 35 min 35 c in the absence of HO42 stain control hereafter refered to as *Unstained).* After initial incubation propidium iodide (PI) was added to all samples to give a concentration of 25 ug/ml, and the samples were further incubated for 5 min at 30c in order to stain the dead sperm in each population to increase sorting efficiency (Johnson et al., 1994)

Computer-Assisted Analysis of Sperm Movement

Aliquots of 7ul from each sperm concentration in Experiment 1 and each treatment group in Experiment 2(X-sorted sperm, Y sorted sperm, stained unsorted control and unstained control) were transferred to alcohol-washed, pre warmed (37c) standard microsope slides. The aliquots were covered with 18 X 18 mm coverslips and immediately transferred to the warm stage (37 c) of a Zeiss Axiophot microscope (Carl Zeiss, Thornwood, NY). The microscope was equipped with a 310 phase objective and a high-resolution video camera (Hamamatsu CCD Model) connected to a C2400 camera control unit (Hamamatsu CCD). The slide was left for 30 sec on the stage for the sample to equilibrate and minimize drift. Sperm motion was continuously recorded for 2 min, and care was taken to ensure similar light levels for each sample. Motion parameters (Boyer et al., 1989) were examined using HST with HST version 7 software and a frame rate of 25Hz (Hobson Tracking systems, Ltd, Sheffield UK). Definitions of each motion parameter are as follows:

Curvilinear velocity (VCL): Velocity over the total distance moved i.e including all deviations of sperm head movement.

Straight- line velocity(VSL): Velocity calculated using the straight-line distance between the beginning and end of the sperm track.

Average path velocity(VAP):Velocity over a calculated, smoothed path i.e a shorter distance than that used for calculating VCL.

Mean angular displacement(MAD): the mean angular displacement of the sperm head around the curvilinear path.

*Beat cross-frequency (*BCF): the frequency with which the actual track crosses the smoothed track.

Amplitude of lateral head displacement (ALH) the average value of the extreme side-to-side movement of the sperm head in each beat cycle.

TABLE 1: Mean of Velocity Head Movement and Trajectory Variable for Sperm Concentrations of 1, 5 and 10 x 10^6 sperm/ml

Sperm X10^6/ml (n=9)	No. of Sperm counted	Motion parameter (μm/sec)							
		VCL	VSL	VAP	MAD	DCF	ALH	LN	STR
SE±		9	10	11	2.1	0.3	1.4	4	5
1	645	195[a]	99[a]	138[a]	86[a]	5[a]	13[a]	48[a]	74[a]
5	1793	204[a]	98[a]	143[a]	85[a]	5[a]	14[a]	47[a]	76[a]
10	3364	191[a]	70[b]	110[a]	85[a]	6[a]	13[a]	35[a]	67[a]

[a,b]Column means followed by difference superscripts are significantly different superscripts are significantly different (P 0.05)

Linearity (LIN) ratio of distances (as a percentage) of straight-line track length to actual track length (this value is 100% for a completely linear track).

*Straightness of path (*STR): straight line velocity divided by the average path velocity.

Conditions for HST setup

Filter setting were 1,1,1 and 2 for 1,2,3 and 4, respectively. Predict was off and search

radius was 33.2 um. Minimum track points were 15 frames. Contrast thresholds were +12/-8. Tracking time was 2 min.

Experiment 1:Effect of Sperm Concentration on Motility

Since the process of flow cytometry and cell sorting dilutes sperm samples from their initial concentration, the effects of concentration only on motion parameters were examined initially. Human semen (n=9 total ejaculates) was diluted in HEPES-BSA to concentrations of 1,5, and 10 x 10^6 sperm per milliter and incubated at 37c for the duration of the motility analysis. Three aliquots from each concentration were video recorded for CASA in a 3 x 3 Latin square design.

Statistical Analysis

Weighted means were taken into account for difference in the numbers of sperm analyzed. A mixed model ANOVA(SAS, 1994) was used to avoid underestimating the vaiance of the group means and where human and day were considered random effects. Difference in motility parameter means for X and Y sperm population were compared using the least significant difference test.

RESULTS

Reanalysis of separated X and Y Chromosome-Bearing Sperm

Aliquots of the sorted X and Y chromosome-bearing sperm were reanalyzed for DNA content. Flow cytometric analysis confirmed puritities of > 85% for X and Y sperm, respectively/

EXPERIMENT 1: Effect of Sperm Concentration on Motility

No difference in motion parameters were seen between concentrations of 1 x 106 and 5 x 106 sperm per milliliter (Table 1; P=0.05). Lower velocities (VSL,VAP) and lower measurement of trajectory (LIN P =0.01; STR P=0.05) were recorded for concentration of sperm at 10 x 10^6 sperm per milliliter compared with 1 x 10^6 and 5 x 10^6 sperm milliliter (see Table 1).

Was significantly different between X and Y sperm (See Table 2; P =0.04). it was not significantly difference between Y and unstained unsorted control (See Table 2; P =0.09)

TABLE2: Means of velocity and Head Movement Variables for Unstained, Hoechst Stained Unsorted and X and Y separated sperm

Treatment	No. of Sperm counted	Motion parameter (µm/sec)					
		VCL	VSL	VAP	MAD	BCF	ALH
SE±		8	8	9	0.7	0.1	1.6
Unstained	3713	257[a]	86[a]	118[a]	85[a]	65[a]	16[a]
Stained	4145	268[b]	91[a]	123[a]	87[b]	6.6[a]	16[a]
Unsorted	3191	263[a]	84[a]	118[a]	87[b]	6.8[a]	16[a]
X sorted	4116	256[a]	72[a]	105[a]	85[a]	6.8[a]	16[a]
Y sorted							

[a,b] Column means followed by different letters are significantly different from each other at the *P* = 0.05 level.

DISCUSSION

The results of this study do not support the frequently held view that Y chromosome- bearing sperm swim faster than X chromosome- bearing sperm (P≤0.05). Sperm velocities (VCL, VSL, or VAP) were not stasistically different between X and Y chromosome- bearing sperm. However, significant differences were seen for LIN (P=0.04) and STR (P=0.01) between X and Y-sorted sperm. Although MAD was found to be significantly different between X and Y sperm, this result was unexpected, since MAD is normally highly conserved among sperm populations (W Holt, personal communication).

The effect of sperm concentration on velocity, previously described for human sperm (Yi Lui et al., 1991; Suttiyotin and Thwaites, 1992), also was confirmed for human sperm in

this study. From the results in Experiment 1, the importance of standardization of sample concentration between control and flow-sorted samples was confirmed. Samples collected after flow sorting have a low concentration (<1x 10^6 sperm per milliliter); thus concentrations of all samples were adjusted to 5x106 sperm per milliliter in this study to standardize video recording of sperm. Higher VCL seen in sperm samples from Experiment 2. Sperm samples in Experiment 2, including controls, were diluted as a process of or to mimic flow ctometry/cell sorting, and samples were recorded 1 hr after flow sorting or dilution of control samples were recorded 1 hr after sorting or dilution of control samples. The increase in velocity may have been indicative of the onset of the capacitation process, with sperm exhibiting some hyperactivated motility and thus increased velocities, although no increase in velocity or hyperactivated motility was observed visually.

In this study, CASA was carried out on sperm samples that had been diluted in a buffered medium. In the female reproductive tract, the fluids encountered by the sperm would be significantly more viscous. This increased viscosity would also be true of the albumin procedure for human sperm . Elevated fluid viscosity has been shown to affect the motility of the sperm (Suarez et al., 1991). It is possible that differences in motility between X and Y sperm may be more or less pronounced in a more viscous medium. Differences in swimming pattern have been observed in Ficoll and cumulus matix, possibly due to macromolecular differences in structure (Suarez et al., 1991). Interestingly, serum albumin in medium has been shown to have a "shear-thinning" effect, reducing the effects of viscosity as the shear rate increases and altering the efficiency at which a sperm swims (Suarez et al., 1991).

Reference

1. Amann RP (1989) : Treatment of sperm to predetermine sex. Theriogenology 31:49-60.
2. Beernik FJ, Dmowski WP, Ericsson RJ (1993) : Sex preselection through albumin sparation of sperm. Fertil Steril 59:3825-386.
3. Johnson LA, Welch GR. Sex preselection : high-speed flow cytometric sorting of X and Y sperm for maximum efficiency. Theriogenology 1999; 52:1323-41.
4. Johnson LA. Sex pre-selection in swine : altered sex ratios in offspring following surgical insemination of flow sorted X- and Y- bearing sperm. Reprod Dom Anim 1991; 26:309-14.
5. Johnson LA. Sexing mammalian sperm for production of offspring; the state of the art. Anim Reprod Sci 2000; 60-1:93-107.

Zschokke S, Studer P, Baur B. Past and future breeding of the Mammals in captivity. Int Zoo News 1998;45:261-76