HEB CASS

SEX-SORTED HUMAN SPERMATIOZOA AND DNA DAMAGE:1 STATISTICS FEATURES

AJAY KUMAR

Dept of zoology, Research Scholar, J.P University

Email ID- serviceheb@gmail.com

Access this Article Online	Quick Response Code:
Website: http://heb-nic.in/cass-studies	
Received on 11/02/2019	
Accepted on 17/02/2019 © HEB All rights reserved	

INTRODUCTION

Sex-sorting via flow cytometry is currently the only reliable technique the has been used successfully for the cost when compared to the use of conventional semen samples for assisted reproductive technologies (ART). However, the reason for the lower fertility that has been associated with sex-sorted spermatozoa is still subject to debate Due to the limited production efficiency of sex sorting spermatozoa, a normal commercial dose of sexed human semen contains only 8.4 x 10^6 spermatozoa per dose in 0.25 cc straws. An insemination dose of only 2.1 x 10^6 is a low dose for AI. The usual insemination dose for human is around 20 x 10^6 or more cryopreserved sperm in 0.5 cc straws sex-sorting are handled under Para-biological environments such as the interaction of the DNA with flurophores, laser exposure, spermatozoa separation in micro-droplets, acceleration of spermatozoa through geometrically-pressured fluid channels and centrifugation. All of these Para-biological spermatozoa-media or mechanical interactions would theoretically have the potential to produce changes in cell structures, including the DNA molecule. While taking these stressors into account, the decrease in fertility rates could also be related to the amount of spermatozoa containing a certain level of DNA damage generated by the individual. Thus, while spermatozoa concentration could be one of the factors, other external factors may also be affecting the ability of these samples to produce viable offspring.

The main objective of this investigation was to study the level of DNA damage that could be putatively produced after sex selection in the different sub-sorting, the level of DNA damage decrease in the spermatozoa fraction to be used for insemination because of a step included in the sorting methodology that removes nonviable and non-flow orientated spermatozoa. Non-flow orientated sperm may result if abonormal morphological issues prevent them from aligning properly in the flow stream as including increased morphological abnormalities have been shown to have a higher degree of DNA fragmentation associated with them.

MATERIAL AND METHODS

All human included in this analysis for material and methods. For this particular investigation human ranged from 27 to 32 years of age.

Preparation of the semen samples for sex-sorting was similar to the discussed in seidel and Garner[12]. In the this case, X and Y chromosome bearing, sorted spermatozoa samples were selected based on difference in fluorescence singnals using 16.2 mM Hoechst 33342(Molecular Probes, Eugene Oregon, USA), diluted 1:1(Hoechst:Nanopure water,v/v), and Red Food Dye (FD & C#40; Sensient Technologies corporation, St. Louis, Missouri, USA). Although the DNA content of spermatozoa for individual mammalian species is highly conserved. Therefore, the Xchromosome-bearing sperm have about 4% more dye bound to their DNA than Y sperm and a greater fluorescence signal can be observed as a result.

Live spermatozoa that have intact cell membrances exclude Red Food. Dye from entering and quenching the Hoechst fluorescence signal thus exhibiting a higher fluorescence signal than the nonviable spermatozoa. Sorting of X- or Y- chromosome bearing spermatozoa was conducted similar to the procedures previously described by Seidel and Garner, using a MoFlo^R SX (Beckman Coulter, Inc., Miami FL) spermatozoa sorter and Summit v4.0 software.

Each sex-forted sample was produced according to the standards for semen production dose in Sexing Technologies (Navasora, TX, USA). All extenders used in the experiments were of the same formulation having a pH of 6.8 and an osmolarity balanced at 300 MOSM for the TRIS extender. For cryopreservation, sorted and conventional serm samples were processed using a two step extension with glycerol. All frozen-thawed sex-sorted samples used in the experiments contained ~$2.1x10^6$ spermatozoa/ straw (0.25 cc) while conventional samples has ~25 to $30x10^6$ spermatozoa/straw (0.5 cc).

The first experiment was conducted to analyze possible difference in the level of spermatozoa DNA fragmentation (SDF) before and after X and Y choromosome sex selection. Five human, one ejaculated each (n=1 ejaculate/human), were included into two aliquots, one aliquot was sex sorted and thereafter the permatozoa were frozen using an automated freezing device, IMV Digitcool® (IMV, Cedex, France) and stored in liquid nitrogen. The second aliquot was directly cryopreserved for subsequent analysis of spermatozoa DNA fragmentation after thawing. The SDF analysis was performed for the different subpopulations, after X and Y chromosome sex selection, while comparing both aliquots for each respective sex.

SPERMATOZOA DNA FRAGMENTATION ASSESSMENT

To determine the degree of DNA damage in each sample, the human sperm-Halomax® kit (Halotech DNA, Madrid, Spain) was used. This technique is an adapted version of the sperm chromatin dispersion test(SCD;[17]). Details of this technique have been previously reported for human and other mammalian species.

STATISTICAL ANALYSIS

The results for purpose of statistical comparison are reported as mean and standard deviation, while the distributions of the different values are presented as box and whisker plots using Statgraphic plus 5.1 softeware (Academic Enterprise, StatPoint Inc., Herndom, Virginia, USA). The non-parameteric Wilcoxon-Mann-Whitney test was used to test the hypothesis that two samples being compared do not show significant difference.

RESULTS

High resolution images were obtained when SYBR® Green I or GelRed staining was combined with the SCD protocol (Fig.2). Spermatozoa heads presenting compact haloes of dispersed chromatin are indicative of spermatozoa without fragmented DNA, while those presenting large andexpanded haloes are identified as containing fragmented DNA.

Table 1

Whole distribution of sperm DNA fragmentation (percent) observed pre and post X/Y sex sorting.

Reference	Pre-sort	XY
H1	7.00	1.10
H2	7.50	1.10
H3	11.00	4.00
H4	9.00	5.00
H5	5.30	4.60
Averavge ± SD	7.96 ± 2.15	3.16 ± 1.91

It is interesting to note that in all of the samples analyzed herein, the haloes of chromatin dispersion after sex-sorting are smaller that those obtained in conventional spermatozoa samples. In Figure 2a, a sex-sorted spermatozoa sample showing a regular small sized halo of chromatin dispersion is shown. in general the halo size of a spermatozoon identified as containing fragmented DNA after sorting is half the size of one identified in a conventional spermatozoa sample.

The baseline level of DNA damage in the five presorted human semen samples ranged from 5.3 % to 11 % with an average 7.9% ± 2.1% (Mean ± SD; Table 1). The level of spermatozoa DNA fragmentation obtained in X and Y chromosome sorted samples was much lower (3.1± 1.9) than that obtained in pre-sorted samples. Statistical difference were obtained after comparing both groups ($P < 0.01$). On average, the efficiency for SDF reduction was about 63 %, but in some cases the reduction could be as high as 85%. The level of SDF recovered after X and Y chromosome sex sorting was notably reduced in the X and Y subpopulations when analyzed separately. The tendency observed in all of the human was to accumulate spermatozoa containing a fragmented DNA molecule in the sorted non-viable sub population (Fig. 3; Mean ± SD: 12% ± 4.4%). This general tendency of SDF accumulation for each sub population was observed for each individual.

To study the intra-individual consistency of this reduction in SDF, X chromosome sorted straws obtained from 100 X and Y chromosome sex sorted samples were assessed and the results are shown in Table 2. On average the level of SDF obtained after X and Y choromosome sex sorting is low and similar to the level obtained in experiment 1 with values for SDF ranging from 0.03% to 2%. The level of spermatozoa DNA damage obtained after X and Y chromosome sex sorting within each individual is quite similar when straws belonging to different dates and belonging to the same individual are compared interestingly, difference among individual human, regarding the efficiency in decreasing the SDF after X and Y chromosome sex sorting, exist ($F; 3.27; P<0.05$). The descriptive statistic for values of SDF after sex sorting are represented in Figure 4. As observed in Figure4. it is apparent that some human harbour more DNA damaged spermatozoa than others. In 6 out of the 10 human analyed outliers which double the mean for SDF are detected.

DISCUSSION

The results presented in this investigation show that spermatozoa sex-sorting, using a MoFlo® SX and dividing the net sample into three different sub populations is a process that not only increase the percentage (i.e purity) of X or Y chromosome bearing spermatozoa in a sample, but also considerably reduces the level of DNA damage in the sample, but also considerably reduces the level of DNA damage in the samples being used for artificial insemination (AI) or in virto fertilization (IVF). On average, a 69% in the reduction of SDF should produce beneficial effects on the findal spermatozoa quality. However, in terms of sample efficiency for ART, levels of SDG lower than 2% should not have a major impact on off spring when the sample is used for insemination purpose. In fact, althou8gh the impact on fertility of SDF in human her not been thoroughly assessed, existing data indicate that changes in chromation packing may play a major role in infertility SDF may have a negative impact of about 10% pregnancy when the DNA damage is higher than 20%.

At present, variations in the level of SDF after spermatozoa sex- sorting have not been conclusive mainly because of the scarce information available. Persistence in the level of SDF after flow cytometry, without a dramatic increase in the baseline level, has been reported in mammalian species. However, damage to sex sorted spermatozoa has been shown to occur during sorting. Since interaction of these cells with non-orthodox biological conditions is especially high. For example, under certain condition the chemical and mechanical stress of staining spermatozoa combined with centrifugation increased the percentage of dead and damaged spermatozoa by 18.6% but a simple unstained sorted sample increased the level of DNA damage by only Variations in the level of spermatozoa stressing resulting in spermatozoa damage have also been reported using high or low pressure with a MoFlo® SX sorting system. In general, for human lowering the pressure for spermatozoa sorting from 50 psi, considered as the standard pressure, to 40 psi improved spermatozoa quality without a significant decrease in the sorting efficiency.

In the present study, the methodology for spermatozoa sex sorting includes a step which is conducted to eliminate the dead spermatozoa sub population. This particular methodological step demonstrated that the level of SDF does not increase but could be notably reduced in the sample of interest. This strategy is commonly used in human X and Y chromosome sex sorting and appears to be effective in reducing the level of spermatozoa DNA damage indirectly, as previously suggested by BOe-Hansen et al. These authors found a significant difference between the conventional level of SDG established for human and the SDF found in commercial sex-sorted semen when DNA integrity was measured using the sperm Chromatin Structure Assay (SCSA) and neutral comet assay. The authors suggested that this effect was linked to the sorting process by excluding nonviable spermatozoa. In this work we demonstrate that the damaged spermatozoa are effectively accumulated in the sorted dead sub population and this is the reason why an increase of SDF is always observed in this fraction after X and Y chromosome sex-sorting. Although, the previous finding could be assumed as obvious, the reality is not so simple because we have found that not all non-viable spermatozoa contain fragmented DNA and not all spermatozoa selected as being viable are absolutely free of DNA damage. Although a negative correlation with viability and SDF is usually found correlation is not always easy to establish since both parameters may behave

in an independent manner. In this experiment we have additional and clear informantion about this aspect of spermatozoa quality since not all dead spermatozoa account for the level of spermatozoa contaminating a fragment DNA molecule in the sorted dead sub population. This also points to the fact that in human a large proportion of spermatozoa containing fragmented DNA in injured presenting major alternations in the spermatozoa membranes.

REFERENCE

1. Amann RP (1989): Treatment of sperm to predetermine sex. Theriogenology 31:49-60.
2. Beernik EJ, Dmowski WP, Ericsson RJ (1993) : Sex preselection through albumin separation of sperm. Fertil Steril 59:382-386.
3. Johnson LA, Wech GR. Sex preselection: high-speed flow cytometric sorting of X and Y sperm for maximum efficiency.
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.