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Size differences between human X and Y spermatozoa and prefertilization diagnosis*

Ke-hui Cui

Department of Obstetrics and Gynaecology, The University of Adelaide, The Queen Elizabeth Hospital, Woodville, Adelaide, SA 5011, Australia

Present address: The Center for Human Reproduction, 750 North Orleans Street, Chicago, IL 60610, USA

Normal human spermatozoa carry either the X or the Y chromosome. The differences between X and Y spermatozoa (X and Y haploid cells) may exist in two areas: the different chromosomes (i.e. different kinds and numbers of genes) and the different sperm structures and functions (i.e. different genetic expression). The aim of this study was to determine whether there are any size differences between X and Y spermatozoa and whether sperm size and shape varies between men. Identification of the Y (and X inferred) status of individual spermatozoa was carried out by polymerase chain reaction (PCR), amplifying the putative testis-determining gene (*SRY*) together with a control gene (ZP_3). PCR amplification of 871 out of 895 (97.3%) single motile spermatozoa showed that 444 (51.0%) were Y and 427 (49.0%) were X-bearing spermatozoa. Of 233 normally-shaped but immobilized spermatozoa, 217 (93.1%) were photographed and measured. Statistically, the length, perimeter and area of the sperm heads, and the length of the sperm necks and tails of X-bearing spermatozoa were significantly larger and longer than those of Y-bearing spermatozoa. Some peculiarities (or variations) in the X and Y sperm shape and size in individual donors were found. The pre-screening by micro-measurement of these specific haploid characteristics of individual spermatozoa in different donors, which may be closely related to their different genetic conditions (or diseases), may be important in human medicine and animal husbandry, especially in sperm prefertilization diagnosis.

Key words: genetic disease/IVF/preimplantation diagnosis/sex/spermatozoa

Introduction

The existence of X and Y spermatozoa was first proposed in the 1920s (Painter, 1923). Since then, several methods have been utilized for the sex determination in spermatozoa. The traditional method of sex determination using X sex chromatin (Barr's body in the female cells; Barr and Bertram, 1949) was not effective in the condensed DNA of X spermatozoa. However, a fluorescence method enabled sex determination to be feasible in Y spermatozoa using the Y body (Barlow and Vosa, 1970; Sumner *et al.*, 1971). Karyotypic analysis of pronuclei following zona-free hamster oocyte fusion (Rudak *et al.*, 1978), has clearly shown X and Y chromosomes separately in the X and Y spermatozoa. To avoid the problem of non-specificity (i.e. two 'Y' or 'F' bodies) and to improve the precision of sex determination techniques in the spermatozoa, different DNA sequences have been used as fluorescent markers (or probes) to identify X or Y (or both) spermatozoa (Joseph *et al.*, 1984; Han *et al.*, 1993). These methods have allowed some insight into the ratio of X- to Y-bearing spermatozoa in one sample on a glass slide, but they are not convenient for individual sperm analyses. Polymerase chain reaction (PCR) has also been later used for amplification of some genes of single spermatozoa (Li *et al.*, 1988).

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When we started our research in preimplantation diagnosis in 1989, sex determination was obviously the easiest and most essential starting point because the diagnosis can be easily confirmed, and many sex-linked diseases are existing. With PCR amplification of the mouse testis-specific gene on the Y chromosome for sex determination in single biopsied embryo cells, we achieved precision of diagnosis with the birth of 39 baby mice (Cui *et al.*, 1991). Comparing the results from PCR amplification of the repeated sequences in human (DYZ1 sequences) with those in the mouse (pY353B sequences), we found that the testis-specific gene on the Y chromosome was superior to the repeated sequences because of its higher sex specificity and stable results (Cui *et al.*, 1993, 1994). This is because >50% of the human Y chromosome is composed of a variety of repeated sequences, each containing a different Y-specificity (Cui, 1993). Thus identification of absolute chromosomal specificity in the Y-repeated sequences is rather difficult. What is known about the evolution of repeated sequences suggests that they could be represented on the X chromosome (Cooke, 1976). It has been suggested that the Y chromosome has acquired genetic material from both the X chromosome and autosomes at various times during primate evolution (Koenig *et al.*, 1985). It is also known that some sequences which exist on both X and Y chromosomes will easily result in misdiagnosis if used for sex determination (A.H.Sinclair, personal communication). In early 1992, we used the testis-determining gene on the Y chromosome (*SRY*)

for sex determination in human preimplantation diagnosis. PCR amplification was easier and the results were brighter than for the testis-specific gene (Cui *et al.*, 1992). Thus the testis-determining gene on the Y chromosome is superior to the testis-specific gene in sex determination. The *SRY* gene was therefore used for the sex determination of single human spermatozoa in this study. In contrast to the testis-specific gene, one of the important ovary-specific genes (ZP_3) was successfully used as the control gene in sex determination in mouse and human preimplantation diagnosis (Cui *et al.*, 1991, 1992) as well as in this study. The aims of this study were firstly, to use the PCR methodology to identify the gender of individual spermatozoa, and secondly to relate the PCR results to the sizes of these individual haploid cells, which may allow us to determine whether any size differences exist between X and Y spermatozoa.

After publication of the news entitled 'X larger than Y' (Cui and Matthews, 1993), a detailed report on our methods and results was required by other workers to test our finding (i.e. the differing shape or size of the X and Y spermatozoa) so that it might be used directly or indirectly in practice. Spermatozoa from mice can be distinguished easily from human spermatozoa (ovoid head shape) by their sickle head shape (Eddy, 1988; Cui *et al.*, 1993). The obvious differences between mouse and human sperm heads originate from their genetic material; both the chromosomes (i.e. different kinds and numbers of genes) and the genetic expression (i.e. the different sperm structures and functions) differ. On the other hand, it seems that spermatozoa from a marmoset monkey are difficult to differentiate from human spermatozoa because the shape of both sperm heads is ovoid and their sperm speeds overlap (Cui, 1996). Furthermore, the genetic material (chromosome number and gene sequences) and genetic expression in human spermatozoa compared with marmoset monkey spermatozoa show more similarity than in human compared with mouse (Cui *et al.*, 1994). However, the genetic material in human and marmoset monkey is not 100% similar (Cui *et al.*, 1994). Different genetic material and expression may determine different sperm characteristics (including morphology, speed and other physiological characteristics). Can we differentiate motile marmoset spermatozoa from human spermatozoa in a mixture of the two? If a specific speed threshold was set up for measurement (such as 90 μm/s), most spermatozoa above that speed would be of marmoset origin, because the average speed of marmoset spermatozoa is double that of human spermatozoa (Cui, 1996). In the study reported here, some specific thresholds were also set up to detect variations in sperm size and shape between individual men. If such variations exist, the finding may be both interesting and practical, since further refinements may offer important prospects for avoiding some genetic diseases in man, as well as advantages for animal husbandry in the future.

Materials and methods

High specificity of the primers selected in previous study

Primers (Table I) were selected (Cui *et al.*, 1994) from the conserved motif of the human testis-determining gene on the Y chromosome (*SRY*) (Sinclair *et al.*, 1990) and the control primers from the human ZP_3 gene (on chromosome 7) (Chamberlin and Dean, 1990). Blood DNA extracted from 57 men and 67 women accurately confirmed the correct origins of the substrate DNA and, therefore, the primer selection. The *SRY* primers also showed high specificity to human rather than other animals (marmoset monkey, horse, cattle, alpaca, rabbit and mouse) (Cui *et al.*, 1994).

Double lysis method for single lymphocytes

Ten single male lymphocytes and 10 single female lymphocytes were individually aspirated under microscopic observation and individually transferred to PCR tubes which contained 5 μl sperm lysis medium [4 mg dithiothreitol (DTT), 1.7 μM sodium dodecyl sulphate (SDS), 1 mg proteinase K in 1 ml PCR buffer (50 mM KCl, 10 mM Tris–HCl, 2.5 mM $MgCl_2$ at pH 8.3]. After 1 h at 56°C, samples were heated at 94°C for 12 min. After cooling, 2 μl of an alkaline solution (5 mg DTT/1 ml 0.2 M KOH) was added to each sample and heated at 65°C for 10 min. After addition of 2 μl of 0.3 M KCl/0.9 M Tris (pH 8.3), the samples were brought to 20 μl with PCR reagents together with the flank *SRY* gene primers (1F and 1R, Table I) and the flank ZP_3 gene primers (3F and 3R) (but without the addition of 10× PCR buffer) for 30 cycles of DNA amplification. First PCR product (1 μl) was added to the prepared 20 μl PCR mixture containing the nested *SRY* and ZP_3 gene primers (2F and 2R; 4F and 4R) for a further 30 cycle amplification. Agarose gel electrophoresis was performed to show the results.

Sexing motile spermatozoa

Freshly ejaculated human semen was collected from eight different donors. After several dilutions, a single motile spermatozoon was then aspirated under microscopic observation (×100). A total of 895 single motile spermatozoa from eight donors (111 in one sample and 112 in seven samples) was randomly aspirated and placed in individual PCR tubes, and subjected to the double lysis method for sperm DNA decondensation prior to amplification. After 2×30 cycles of nested PCR amplification, those spermatozoa which demonstrated the presence of both *SRY* and ZP_3 gene bands (and ~1% spermatozoa demonstrating only a single SRY band) were designated as 'Y' spermatozoa, while spermatozoa which showed only the presence of the ZP_3 gene band were designated as 'X' spermatozoa.

Sexing non-motile spermatozoa following photography

For photography, 1–2 μl Giemsa stock solution (25 g Giemsa/1600 ml glycerol + 1600 ml methanol) was added to 250 μl of the diluted sperm medium to inhibit sperm movement totally. Individual ovoid-shaped and horizontal-lying spermatozoa were photographed (×400) and then individually aspirated into separate PCR tubes for DNA decondensation and 2×30 cycles of DNA amplification. Under blind conditions, photographs (photo slides) of individual spermatozoa (×400) were further magnified (×30) by a projector and direct measurements of the spermatozoa on a wall (with a pen, paper, thread and ruler) were made. Measurements included the length, width, perimeter and area of the single sperm heads together with the length of the sperm neck and tail (error limits for measurements, ~1–3%). In addition, individual sperm heads were outlined in pencil on a piece of paper placed on the wall over the projected image. Digitized measurements enabled the calculation of perimeter and area of single sperm heads (using a software program, Flinders Imaging; Flinders University, Adelaide, South Australia). After measurement, the individual PCR results (X or Y) were related to the corresponding measured indices of each spermatozoon.

Table I. Oligonucleotide primers used in human sex determination

Gene	Number	Sequences (5′–3′)	Product size (base pairs)
SRY	1F (outer)	CAG TGT GAA ACG GGA GAA AAC AGT	351 (1F and 1R)
	1R	GTT GTC CAG TTG CAC TTC GCT GCA	
	2F (inner)	CAT GAA CGC ATT CAT CGT GTG GTC	254 (2F and 2R)
	2R	CTG CGG GAA GCA AAC TGC AAT TCT T	
ZP_3	3F (outer)	GGA GCT GAG CTA TAG GCT CTT CAT	295 (3F and 3R)
	3R	ACA CTC GTG GAG TCC AAC CTC AAA	
	4F (inner)	AGC CAT CCT GAG ACG TCC GTA CA	177 (4F and 4R)
	4R	CCT GAC CAC ATC TTC TGT GTC CAT	

F = forward and R = reverse relative to the coding sequence.

Individual sperm sample analyses and setting of size thresholds

The size data of the spermatozoa from every donor were further analysed individually. After setting of size threshold (or standards) according to each donor's data, the X and Y sperm peculiarities could be detected. For example, if the spermatozoa from donor no. 8 were suspected to have longer heads than those from other donors, then the threshold head length to distinguish X and Y spermatozoa would have to be correspondingly longer for that sperm sample (detailed in the results).

Statistical analysis

The data were considered to be normally distributed and were analysed by one-sided and two-sided Student's *t*-test, paired and non-paired (Snedecor and Cochran, 1980). The mean values of the sperm measurements from each donor were treated as independent observations.

Results

Sexing single lymphocytes

SRY and ZP_3 genes were confirmed to be present in nine out of 10 single lymphocytes of male origin while nine out of 10 female-derived single lymphocytes contained only the ZP_3 gene (Figure 1a) with the double lysis method. The remaining two lymphocytes (one male and one female) did not amplify. In a previous study, 58 male and female lymphocytes showed 100% PCR amplification and correct diagnosis with the lymphocyte lysis method (Cui *et al.*, 1994).

Sexing motile spermatozoa

Of a total of 895 single motile spermatozoa examined, 871 (97.3%) spermatozoa showed satisfactory PCR amplification, 444 (51.0%) were 'Y' spermatozoa, and 427 (49.0%) were 'X' spermatozoa. Figure 1b shows the results for 112 spermatozoa from donor no. 5. The ratio of 'X' to 'Y' spermatozoa was not significantly different from 1:1 in 871 spermatozoa.

Sexing non-motile spermatozoa and size comparison

A group of 19–25 single non-motile spermatozoa from the semen samples of 11 normal donors was analysed (total, 233 spermatozoa). These 11 healthy donors were confirmed to be fertile with normal sperm counts, motility and morphology. Their consecutive ejaculates were analysed and showed consistency over the course of this study. DNA was amplified in 217/233 (93.1%) photographed spermatozoa. Of these, 106 (48.8%) were designated 'Y' and 111 (51.2%) were 'X' as described above. The sperm measurements were performed under blind conditions. Statistical analysis showed that the length, perimeter and area of the 'X' sperm heads and the length of the 'X' sperm neck and tail were significantly larger and longer than those of the 'Y' spermatozoa (Table II). The mean area of 'X' sperm heads was 6% larger than that of the 'Y' sperm heads. In the analyses of the data from the different donors, the majority ($>$50%) of the 'X' sperm heads in donor nos. 4 and 10 were longer than the 'Y' sperm heads. Additionally the majority of the 'X' spermatozoa in donors nos. 2 and 4 had greater head perimeters. In donors nos. 2, 4 and 8, the majority of 'X' spermatozoa had a larger head area and in donor no. 7 longer tails than the corresponding 'Y' spermatozoa.

Special size thresholds for individual sperm samples

In order to find out the high 'X' and 'Y' purity from these donors, some special size standards (Table III) were further set up for the individual donors according to their respective results, (i.e. different donors with different size characteristics standards). Thus, six of the 11 donors (donors nos. 2, 3, 4, 7, 8 and 9) demonstrated shapes and sizes of 'X' and 'Y' spermatozoa peculiar to themselves (Table III). It was not possible to set up special 'X' and 'Y' size standards for the other five donors due to a greater size overlap. In the six donors included in Table III, some parameters showed no overlap for the thresholds adopted, or only a small overlap. For example, in donor no. 7, nine out of 19 (47.4%) spermatozoa contained longer tails ($\geqslant$45.0 μm), and eight (88.9%) of these were 'X' spermatozoa. In donor no. 2, six out of 14 (42.9%) spermatozoa contained larger head area ($\geqslant$15.0 μm^2), and all of these six spermatozoa (100%) were 'X' spermatozoa. Interestingly, the individual size thresholds helped to increase the value of the analyses, so that in five donors the head area measurement allowed for a complete separation of X- and Y-bearing spermatozoa (Table III). In donor no. 8, simple selection of head length ($\geqslant$5.75 μm) achieved 71.4% (10/14) 'X' purity, while selection of a larger head area ($\geqslant$16.2 μm^2) achieved 100% (five out of five) 'X' purity, albeit with a lower sperm number. On the other hand, the results from donors nos. 3 and 9 showed the reverse situation, i.e. spermatozoa with narrower heads in donor no. 3 showed higher 'X' purity, whilst

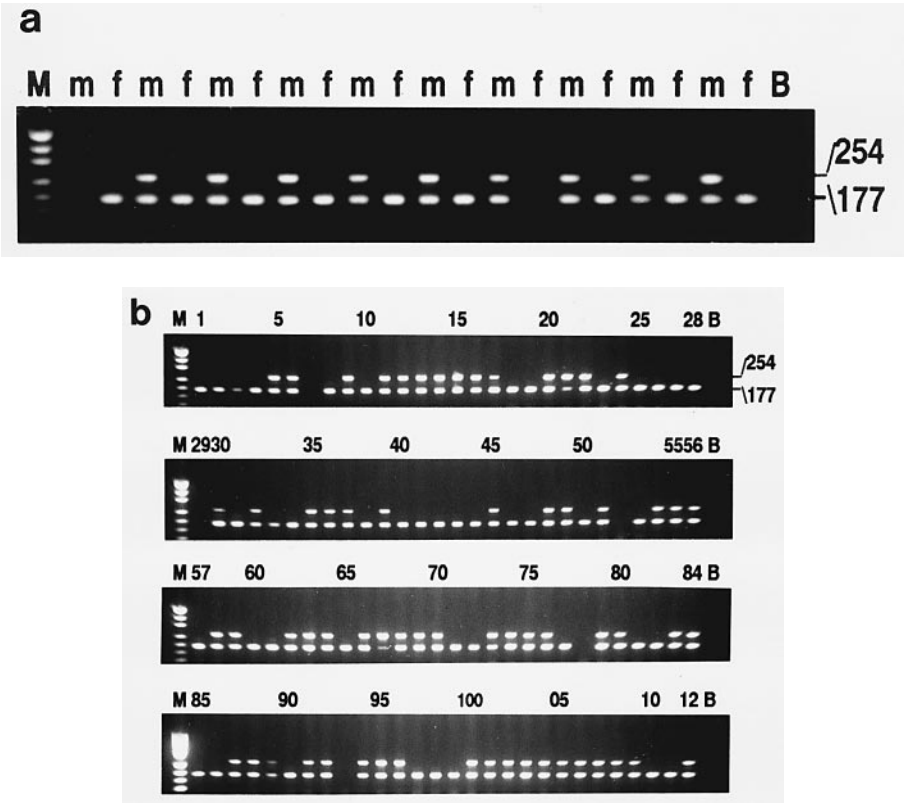

Figure 1. Polymerase chain reaction (PCR) amplification of fragments of *SRY* (254 bp) and ZP_3 (177 bp) genes. M was marker pUC19. B = blank; m = male; f = female; bp = base pairs. (**a**) Amplification of single human lymphocytes of known sex derivation by PCR (with double lysis method). Apart from one male and one female lymphocyte which did not amplify, all male-derived lymphocytes showed both *SRY* and ZP_3 gene bands to be present while females were only positive for the ZP3 gene band. (**b**) Amplification of 112 single motile spermatozoa of donor no. 5 with the double lysis method. The spermatozoa which were both *SRY* (254 bp) and ZP_3 (177 bp) gene positive were designated 'Y' spermatozoa, and the spermatozoa which were ZP_3 gene positive only were designated 'X' spermatozoa.

Table II. Parameters of measurement of X and Y bearing spermatozoa (mean ± SD)

	Y	X	*P* value
Sperm head length (μm)	5.23 ± 0.40	5.38 ± 0.43	<0.01
Sperm head width (μm)	3.53 ± 0.30	3.53 ± 0.33	NS
Sperm neck and tail length (μm)	41.18 ± 3.47	42.22 ± 4.37	<0.05
Sperm head perimeter (μm)	14.73 ± 1.07	15.26 ± 1.17	<0.001
Sperm head area (μm^2)	13.93 ± 1.79	14.74 ± 2.09	<0.001

NS = not significant.

the spermatozoa with larger head area in donor no. 9 showed higher 'Y' (rather than 'X') purity.

Discussion

Shettles (1960), using direct microscopic observation, claimed an absolute bimodal nuclear size distribution (all X spermatozoa being larger than Y spermatozoa); however, subsequent investigators were unable to substantiate these claims without a method to determine the gender status of individual spermatozoa. Sumner *et al.* (1971) examined DNA content using fluorescence intensity but without morphological measurements, and inferred that there were no size differences between X and Y spermatozoa. The current findings, based on direct morphological measurements of individual X and Y spermatozoa provide strong evidence that X spermatozoa are statistically larger and longer than Y spermatozoa, but the threshold for this size difference varies between individual sperm samples. Human X spermatozoa contain, on average, 3 or 4% more DNA than Y spermatozoa (Sumner *et al.*, 1971). However, size differences are unlikely to relate solely to chromosomal (or DNA) content but may also relate to cytoplasmic content and structure, which are sperm phenotypes resulting from genetic expression during meiosis (Hiyoshi *et al.*, 1991) and further spermatogenesis (Shannon and Handel, 1993; Lankenau *et al.*, 1994).

The methodology chosen in this study provided satisfactory rates (>97%) of PCR amplification and confident 'Y' identification using a Y-linked testis-determining gene in the presence of a control gene. The PCR conditions were refined to ensure that the *SRY* gene was amplified efficiently, so that the X status of individual spermatozoa could be confidently inferred

Table III. Measurement of individual 'X' and 'Y' spermatozoa in relation to specific size thresholds set up for specific donors

	Donor no.	Sperm size threshold	No. spermatozoa (total spermatozoa)	No. of 'X' spermatozoa (%)	No. of 'Y' spermatozoa (%)
Head length (μm)	2	⩾5.25	7 (14)	5 (71.4)	2 (28.6)
	4	⩾5.50	6 (17)	6 (100)	0 (0)
	8	⩾5.75	14 (22)	10 (71.4)	4 (28.6)
Head width (μm)	2	⩾3.75	2 (14)	2 (100)	0 (0)
	3	⩽3.0*	3 (20)	3 (100)	0 (0)
	7	⩾3.75	3 (19)	3 (100)	0 (0)
	9	⩾3.75	7 (22)	1 (14.3)	6 (85.7)*
Tail length (μm)	7	⩾45.0	9 (19)	8 (88.9)	1 (11.1)
Head perimeter (μm)	2	⩾15.4	6 (14)	6 (100)	0 (0)
	4	⩾14.3	6 (17)	6 (100)	0 (0)
Head area (μm^2)	2	⩾15.0	6 (14)	6 (100)	0 (0)
	4	⩾13.9	4 (17)	4 (100)	0 (0)
	7	⩾16.3	3 (19)	3 (100)	0 (0)
	8	⩾16.2	5 (22)	5 (100)	0 (0)
	9	⩾15.2	4 (22)	0 (0)	4 (100)*

* These values are a reversal of the general condition

by the lack of *SRY* gene amplification and the presence of a control gene. The error-free results of blood DNA ($n = 124$) and single lymphocytes (in this and previous experiments, $n = 76$) (Cui *et al.*, 1994) confirmed that the methodology utilized was appropriate and further results showed a 1:1 ratio of motile X to Y spermatozoa.

Interestingly, the PCR conditions which achieved 100% amplification for the single lymphocytes and single embryo cells (Cui *et al.*, 1994), only achieved ~34% (10/29) amplification for single spermatozoa in the preliminary experiment of this study (unpublished data), which indicated that the double lysis method should be used for sperm PCR amplification. This is probably due to the nature of the DNA complement of spermatozoa being highly condensed, protamine-rich and haploid (Balhorn, 1982; Ward and Coffey, 1991). The double lysis method was modified from two different methods for sperm lysis (Li *et al.*, 1988; Cui *et al.*, 1989). This new modified method was crucial for suitable sperm decondensation and the achievement of a 97.3% rate of PCR amplification in single spermatozoa. Conversely, a lower (90%) amplification rate was obtained in single lymphocytes due to their DNA instability with this double lysis method, thus demonstrating the need for different PCR conditions for different substrate amplifications. More extensive testing of single lymphocytes in this experiment was not considered necessary since the lymphocyte DNA complement was less stable than the sperm DNA complement by the double lysis method. Giemsa showed minimal effects on PCR amplification of single spermatozoa, and was found to be preferable to the use of formalin or alcohol.

The peculiarity of shape and size of 'X' and 'Y' spermatozoa from different donors is very interesting. All spermatozoa with much longer heads in donor no. 4 were 'X' spermatozoa, which were easily recognized. The head area was the simplest parameter to differentiate 'X' and 'Y' spermatozoa in five out of 11 donors. The characteristics of head area of 'X' spermatozoa from donor no. 9 were reversed from those of donors nos. 2, 4, 7 and 8. The reason that the different donors contained different sperm characteristics is perhaps mainly related to the genetic origins of the donors' fathers (different Y chromosomes and gene expression) and mothers (different X chromosomes and gene expression). The recombination of the 22 autosomes during meiosis also produces the various genetic characteristics of shapes and sizes of 'X' and 'Y' spermatozoa in the same donor. So the head sizes of 'X' and 'Y' spermatozoa will be expected to overlap under general conditions and cannot be divided into two distinct groups for the population as a whole. Specific size thesholds (rather than a general, overall size threshold) should, therefore, be set up individually for each donor analysis. Pre-screening of sperm size of individual donors will be extremely important in the determination of which donors may be suitable candidates for sperm sex separation.

The precise determination of the sex of a pregnancy by prefertilization sperm selection has been a long standing quest (Lindahl, 1958; Glass, 1977). A variety of procedures are currently in clinical use to obtain populations of human spermatozoa enriched with either X- or Y-bearing individuals (Gledhill, 1988; Windsor *et al.*, 1993). However, the validity of these procedures is debatable given the lack of methods and theory precisely to identify X and Y spermatozoa. To date, techniques have been based on purported physical differences in X and Y spermatozoa. Our study showed that the area of 'X' sperm heads overall was only about 6% larger than that of 'Y' sperm heads, with individual variation between donors. It has also been suggested that only small numbers of spermatozoa should be collected for sex separation (to avoid the 'X' and 'Y' spermatozoa overlapping) with gradient methods (Ericsson *et al.*, 1973; Ericsson, 1994). In this study, the spermatozoa from donors nos. 3 and 9 showed some reverse characteristics in head width and area respectively, compared with some of the other donors (Table III). Our results further suggest that it is important to select different parameters and methods for the different donors in the separation of X and Y spermatozoa, in which the pre-screening of X and Y spermatozoa of individual donors will be a basic step.

Flow cytometry is proving to be a useful method to markedly enrich 'X' and 'Y' spermatozoa in some animal species (Johnson *et al.*, 1989) and in the human (75–82% enriched)

(Johnson *et al.*, 1993) with corresponding birth report data (Levinson *et al.*, 1995). However, the flow cytometry technique needs to be proven safe for use (i.e. that it will not produce any new kind of molecular genetic and chromosomal disease) in the human, given the need for laser (or ultraviolet) excitement of the spermatozoa which contain the genetic material (DNA) for the offspring.

The ability to identify individual human X and Y spermatozoa, and the size differentation between X and Y spermatozoa specific to certain donors may be valuable for newer approaches to the enriched selection of X and Y spermatozoa in both human and domestic animal husbandry. Microinjection of a single spermatozoon (intracytoplasmic sperm injection; ICSI) followed by preimplantation diagnosis is now another technique used for human in-vitro fertilization (IVF) (Palermo *et al.*, 1992). In sex-linked diseases, the choice of a single spermatozoon with specific morphological characteristics of gender (following pre-screening such as in Table III) to inject into the oocyte may offer an important approach for future prefertilization diagnosis, producing enriched male or female embryos. The size and morphology of individual spermatozoa after immobilization can be easily recognized under the microscope. It has also been reported that some sperm morphology relates to genetic condition (Wyrobek, 1979) or diseases. Globozoospermia (round-headed spermatozoa) is due to a genetic defect resulting from the absence of acrosomal structures (Check *et al.*, 1993). The acrosomal vesicle in the Golgi region does not develop in close contact with the nucleus and results in the formation of globe-shaped heads (Check *et al.*, 1993). In animals, free-living koalas normally produce spermatozoa with a high incidence of structural heterogeneity almost solely confined to the head region (Wildt *et al.*, 1991). The selection of individual spermatozoa with specific gene expression (phenotype) for fertilization will be a good technique for animal phenotypic improvement.

The size and shape differences between X- and Y-bearing spermatozoa are only two of the most basic characteristics related to varied genetic and chromosomal expression. The association of other physical, chemical and physiological differences in individual spermatozoa with different genetic and chromosomal conditions remains to be studied. The recognition of this basic theory may improve the practice in, and understanding of, the field of sperm biology. Pre-screening individual spermatozoa in some patients will help determine important new relationships between the morphological structure of spermatozoa and the genetic (or chromosomal) constitution in certain genetic diseases. Thus it may be possible to decrease the transmission of undesirable genes by enriched selection of healthy spermatozoa and thus to complement existing preimplantation and prenatal diagnostic techniques. Currently the knowledge from animal investigations with respect to whether there are differences in morphology between spermatozoa of different gender (or other genetic conditions) is limited but such investigation may be fruitful.

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