Techniques for sorting X and Y spermatozoa may adversely affect histone-associated regions in human spermatozoa

Dear Sir,

The recent paper (Fugger et al., 1998) which demonstrates that sorting of X and Y spermatozoa can be used to produce apparently healthy babies of a chosen sex is a milestone in assisted reproduction technology in humans. Previously, this technique has been used successfully only for several other animal species, primarily cattle.

Over the past few years, concerns have been raised concerning the safety of a technique which subjects a sperm cell to a potentially mutagenic agent such as Hoechst 33342 dye (bisbenzimide). Fugger’s group have addressed these concerns in detail. For instance, they argue that although some somatic cells are sensitive to bisbenzimide (Durand and Olive, 1982; Van Zandt and Fry, 1983) this may not be true for sperm cells whose DNA is compacted and stabilized, compared with somatic cells. This argument may indeed hold for cattle whose DNA is compacted and whose sperm cell DNA is relatively loosely packaged with histones in nucleosomes and whose sperm DNA has almost all the histones replaced with protamine molecules (Palmer et al., 1991). However, I consider that the same reasoning does not hold for human spermatozoa, since the displacement of histones by protamines is incomplete in human spermatozoa. In human spermatozoa, about 15% of the DNA remains complexed with histones (Tanphaichitr et al., 1978; Gatewood et al., 1987) and nucleosome-like structures have been observed (Banerjee et al., 1995). This raises the uncomfortable possibility that the bisbenzimide could adversely affect DNA in the histone-associated regions of human spermatozoa in the same way it does in somatic cells.

As discussed by Fugger et al., successful animal trials have been carried out in rabbit, swine, sheep and cattle (Fugger et al., 1998). However, none of these species has been demonstrated to have a human-like form of sperm DNA packaging. Further work needs to be carried out on the sperm chromatin structure in these test species before one can infer from the animal trials that human offspring will never be adversely affected. I consider that it would be unwise to continue with human trials without first finding an animal species with a similar sperm chromatin structure to ourselves and conducting animal trials using this species.

Fugger and coworkers argue that bisbenzimide is safe because it did not cause mutations within the β-globin gene in human spermatozoa (Watkins et al., 1996). Our recent study shows that the β-globin gene in spermatozoa is protamine-associated at the sites tested and that it is probably more compact and protected than other genes (Gardiner-Garden et al., 1998). This makes it an unfortunate choice for mutagenicity studies. In our study, other genes such as the ε- and γ-globin genes, which are expressed earlier in human development, were shown to contain histone-associated regions in spermatozoa (Gardiner-Garden et al., 1998). Such histone-associated genes would form better safety controls. Many more studies of mutagenicity on a variety of genes in human spermatozoa are required to prove that the detrimental affects of bisbenzimide observed in somatic cells will not also occur in important genes in sperm cells.

References


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