FERTILITY AND STERILITY
Vol. 61, No. 6, June 1994
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## Sex Selection—On Trial Again?

*To the Editor:*

We read with interest the article by Han et al. (1) on the identification of X and Y sperm. As mentioned in their article we have reported a high percentage of male births using our modified swim-up procedure.

We do agree that the differences in the swim-up procedure may be the reason for the contrast in results. We recently presented at the American Society of Andrology meeting in Tampa, 1993 (Check JH, Kwirenk D, Press M, Breen E, Baker A, abstract) similar results using the same swim-up method as the authors. We reported a 51.2% male birth rate out of 113 births after IVF. However, an 86% male delivery rate occurred after separation using our modified swim-up. The swim-up for IVF requires the sperm to be washed free of the seminal plasma before swim-up. This involves slow speed centrifugation followed by removal of the supernatant, which contains the seminal plasma and some sperm that have not been pelleted. We believe that those sperm lost are predominantly Y-bearing sperm. In the modified swim-up the semen is diluted with media and centrifuged, and the pelleted sperm are allowed to swim-up into the supernatant. After the modified swim-up there is a majority of X-bearing sperm in the pellet and a small amount of X-bearing sperm in the uppermost layer. The majority of Y-bearing sperm usually swim up and remain just above the pellet; that is the part of the specimen used for insemination (2).

The quinacrine staining method has been criticized because it may nonspecifically stain autosomal chromosomes. If this is true, this would not explain why a specimen with a baseline of 50% Y-bearing sperm based on quinacrine staining increases to 85% after modified swim-up (3). Han et al. cannot state that they were unable to confirm our data using DNA probes if they did not evaluate the modified swim-up preparation. Indeed our data with quinacrine confirms that the swim-up methodology used by Han et al. does indeed result in approximately 50% Y-bearing sperm. We would hope that Han et al. might continue their studies and evaluate the modified swim-up by DNA probe methodology to determine if the reason for a high percentage of male births is related to increased concentrations of Y-sperm or is some other mechanisms operational.

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*Reply of the Authors:*

We appreciate the comments of Check et al. concerning our recent report on the unchanged 1:1 ratio of X- to Y-bearing sperm after swim-up (1), and we acknowledge their explanation of why they believe the "modified" swim-up enriches Y-bearing sperm. We did not, however, state that we were unable to confirm their data; we merely stated that our results using swim-up contrasted their results using the modified swim-up. We appreciate that there are some differences between the two swim-up procedures, but we nevertheless find it difficult to comprehend how such slight variations in centrifugal force and number of centrifugation steps would be sufficient to give rise to such divergent X:Y ratios.

Permit us to make some general points about sexing sperm. First, since there is evidence that quinacrine staining can be unreliable (2), it behoves all researchers to employ accurate and well-charac-

terised methods for identifying X- and Y-bearing sperm. In this era of molecular biology, the double-label fluorescence in situ hybridization (FISH) technique using X and Y chromosome-specific DNA probes (3) is the gold standard and provides unequivocal and simultaneous identification of X- and Y-bearing sperm. Thus, one can determine with certainty the X:Y ratio, and one is not left assuming that the unstained sperm carry the other sex chromosome as is the case if single label FISH or quinacrine staining is used. There is potential for substantial error when only one sex chromosome is assessed, especially if the labelling efficiency is variable or <95%.

Second, like Check et al., we believe that prime attention must be focussed on the sex ratio in the semen samples, rather than on the results of clinical outcome studies in which there may be other variables. With precise and reliable methods now available, it is opportune to address critically the key issue of whether or not any of these sperm separation procedures actually enrich X- or Y-bearing sperm, because it is these laboratory procedures that make possible preconceptual sex selection. To this end, we recently completed a series of studies to evaluate some of these procedures and our results to date demonstrate that neither the standard swim-up procedure (1) nor albumin gradients enrich Y-bearing sperm, whereas 12-step Percoll gradients do produce a slight, but clinically insignificant, enrichment of X-bearing sperm. We thank Check et al. for their encouragement in this regard and we would be interested in evaluating their modified swim-up procedure using double-label FISH.

Finally, we believe that to obtain accurate and objective assessment of these sperm separation procedures, it is crucial that collaborative studies are performed in which laboratories that routinely perform the procedures send samples to independent laboratories for assessment using techniques such as double-label FISH. Furthermore, we believe that such studies should be performed blinded and that a third party should collate the coded data and analyze it with strict impartiality. Thus, proper controlled laboratory trials can be undertaken without undue bias. If these simple sperm separation procedures are shown not to significantly influence the ratio of X- to Y-bearing sperm, then the relatively limited clinical evidence that suggests a shift in the sex ratio at birth will need to be extended, with appropriate controls, to confirm or deny the clinical findings.

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## Lactic Dehydrogenase-C4 Activity—Marker of Germinal Activity?

*To the Editor:*

Noguera Velasco et al. (1) recently described the assay of lactic dehydrogenase-C4 (LDH-C4) in seminal plasma. They refer to two (2, 3) of our three articles (2–4) and was thus aware of them. Our message in 1985 was that LDH-C4 activity in the seminal plasma was a good marker of the germinal epithelium provided the activity was expressed in relation to the number of spermatozoa, e.g., nanokatal* per $100 \times 10^6$ spermatozoa.

Velasco et al. claim that ". . . until now, there is no report on any biochemical marker of germinal activity suitable for measure in routine analysis." They assert that their observations would lead them to suggest the seminiferous epithelium to be the major source of the enzyme in the seminal plasma rather than the spermatozoa. Reference 20 is to one of our articles (3). We clearly proved that there was no leakage of LDH-C4 from the spermatozoa after ejaculation (2) but not in reference 3.

Did Velasco et al. read our publications without understanding their message, and have the authors consciously ignored our reports to give themselves credit for a "new discovery?"

* Katal is the generally accepted unit for enzymatic activity. One katal corresponds to the amount of enzyme that catalyzes the transformation of substrate at the rate of 1 mol/s (usually at 37°C). It has replaced the old enzyme unit (U or IU) and the conversion factor is as follows: 1 U = 1 IU = 1 μmol/min = 16.67 nmol/s = 16.67 nanokatal.