Discussion

The results are predominantly in agreement with the findings of Eltes et al., who divided the abnormalities into those separable and those non-separable by the technique. They included acrosomal abnormalities in the category of separable malformations, but in the present trial it appeared as if the acrosomal abnormalities were inseparable. In 8 of the cases there was even a rise in the percentage of sperm with acrosomal abnormalities, but the difference was not statistically significant.

The reason for the different results is not known, but we are of the opinion that primary acrosomal abnormalities (lipped acrosomes, acrosome cysts) are unlikely to influence progressive motility, especially if small and tightly membrane-bound. Thus the sperm should be able to swim up normally, but are unlikely to be fertile owing to possible interference with the normal acrosome reaction, or competition with the normal sperm. We postulate that this may be important in explaining why subjects who have a large percentage of abnormal acrosomes in their sperm have reduced fertility, and yet their swim-up specimens show good quality when evaluated according to motility and count only (K. Wiswedel — unpublished data). Among domestic animals, the significance of acrosomal abnormalities has long been established.

Influence of the swim-up procedure on acrosome damage induced by freezing human semen

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Summary

Twenty frozen semen samples from normal donors were thawed, washed in Earle's medium, and allowed to swim up using standard techniques. The samples were evaluated before and after the swim up for progressive motility and concentration. Morphologically normal sperm were assessed for acrosomal integrity according to the relative percentages of normal, damaged or lost acrosomes. The motility improved by 75.07 ± 90.38%, the count was reduced by 97.1 ± 1.9%, but there was no significant change in the frequencies of the different types of acrosomal state. It was concluded that although the swim-up technique is successful in reducing most major morphological abnormalities, influence over the frequencies of secondary acrosomal abnormalities could not be demonstrated.

The swim-up technique results in a large loss of sperm, but this is compensated for by the selection of approximately the best 10% of the sperm. Sperm numbers needed for IVF are low, and adequate concentrations are obtained using this method. At the IVF centre at Groote Schuur Hospital, the technique is routinely used on all patients for intra-uterine insemination or IVF with much success.

REFERENCES


The swim-up procedure is supposed to select a small population of highly motile and morphologically normal sperm for in vitro fertilisation (IVF) and other purposes. Sperm with primary acrosome abnormalities, however, seem to escape selection by the swim-up procedure, and may be found in varying frequencies in the harvested portion. Freezing of human sperm is known to induce secondary acrosomal damage, but the influence of these damaged sperm, which may be motile although functionally impaired, on the swim-up harvest has not been fully investigated.

A trial was conducted to compare the acrosome integrity of cryopreserved sperm before and after the swim-up procedure and in order to investigate whether or not morphologically normal sperm, whose acrosomes had been damaged by the freezing process, were able to escape selection by the swim-up procedure and be found in the supernatant along with normal, acrosome-intact sperm.

Materials and methods

Twenty semen samples from normal donors were obtained by masturbation, after a period of 3 days' sexual abstinence. Samples were diluted, cooled and frozen in an egg yolk citrate medium, containing 8% glycerol, as previously described. One week after freezing, the samples were defrosted in air at room temperature for 20 minutes. Samples were evaluated for progressive motility and concentration. The volume of each sample was adjusted by removing suitable aliquots to ensure that a total of approximately 40 million progressively motile sperm were present. The samples were diluted with Earle's medium supplemented with 10% inactivated fetal cord serum. This...
post-thaw dilution took place over a period of 10 minutes with a final dilution rate of approximately 1:1. The samples were centrifuged at 800 g for 5 minutes, and the supernatants aspirated and discarded. The sperm pellet was resuspended in 2 ml of Earle’s medium, and thoroughly mixed. Aliquots of 0.2 ml were drawn for the assessment of motility, concentration and acrosome integrity.

Motility was assessed at 37°C under 100× and 400× magnification using phase optics. Sperm concentration was determined using a haemocytometer. Smears were made and stained with Spermac stain for assessment of acrosomal integrity.

Only morphologically normal sperm (that is, without primary abnormalities) were assessed for acrosomal integrity. The confines of light microscopical resolution limits effective differentiation between the subtle degrees of acrosome damage, and thus acrosome integrity was assessed only according to the following parameters: (i) normal acrosomes — the acrosome displays a smooth, regular outline and a uniform deep-green staining characteristic; (ii) damaged acrosomes — here the acrosome displays a decreased or irregular staining affinity or a swollen or ruffled outline; (iii) lost acrosomes — the whole sperm head stains red; the equatorial region of the acrosome may or may not be lost as well.

The samples were then centrifuged again and the supernatant discarded. Sperm pellets were resuspended in a small volume of Earle’s medium (approximately 0.25 ml) and 1 ml of Earle’s medium was carefully overlayed onto the sample, ensuring a distinct interface between the fluids. Samples were maintained in an incubator at 37°C under an atmosphere of 5% carbon dioxide in air, for 1 hour. Aliquots of 0.2 ml were removed from the middle of the overlayered 1 ml and assessed in the same way as before the swim up.

Statistical analysis. The difference between the above parameters before and after the swim up were assessed using Student’s t-test for paired populations.

Results

The results are shown in Table I. After swim up, the motility was improved by 75,07 ± 90,38%. There was a reduction in concentration of 97,1 ± 1,91%. There was no statistical difference between the means of sperm with normal acrosomes (P = 0.37), sperm with altered acrosomes (P = 0.57), and sperm which had lost their acrosomes (P = 0.53).

![Table I. Change in Sperm Motility, Concentration and Acrosome Integrity Before and After the Swim-Up Procedure](https://example.com/table1.png)

**Discussion**

The improvement in motility and reduction in sperm concentration after the swim up concurs with other reports on the technique, although the size and quality of the harvest was lower than might have been expected from fresh semen. However, there was no statistical change in the percentage of normal, damaged or lost acrosomes seen in the normal sperm after the swim up. Thus it appears that secondary acrosome abnormalities induced by cryopreservation are not selected by the swim-up procedure, since they occurred in similar frequencies before and after the swim up.

The acrosome is a sensitive organelle, and freezing of sperm has been shown in most species to induce damage to the acrosome. Acrosome integrity has been strongly correlated with fertility in humans, but is not well correlated with motility. Thus, sperm with damaged or lost acrosomes may be progressively motile, and may therefore be able to swim up effectively. This may be one of the reasons for the finding that frozen semen is often less fertile than fresh semen. Although acrosomal damage is progressive with time, it is unlikely that this will be reflected in the results, considering the short time needed for the swim up.

The results of the above trial emphasise the importance of reducing damage to a minimum by the selection of the most efficient processing techniques. While electron microscopy affords greater details than light microscopy, time and expense limit its use. Since the staining affinity of Spermac stain is not affected by commonly used diluents, the evaluation of correctly prepared smears offers an alternative rapid-screening technique for the sequential monitoring of acrosome state, and facilitates the search for the ideal freezing protocol.

**REFERENCES**