

# An improved PureSperm® Wash

- Three applications in one with improved formulation -

## Introduction

PureSperm® Wash is a balanced salt solution designed specifically for washing the sperm pellet after centrifugation on a PureSperm® density gradient. Since Nidacon continuously strives to further develop the products, PureSperm® Wash has now been reformulated.

The purpose of this study was to compare the improved PureSperm® Wash, both with the previous version and with ReadySwimÎ (for swim-up) as controls.

Furthermore, the results were extrapolated to assess the suitability for the new PureSperm® Wash as a replacement for SpermAssistÎ (for preparation of sperm for IUI).

The results have led to the decision to replace the current PureSperm® Wash, ReadySwimÎ and SpermAssistÎ with the improved formula. The new product will retain the name PureSperm® Wash, and can be used in all three applications.

## Experiments and results

### 1. Performance of sperm after density centrifugation, comparing control and the new improved PureSperm® Wash

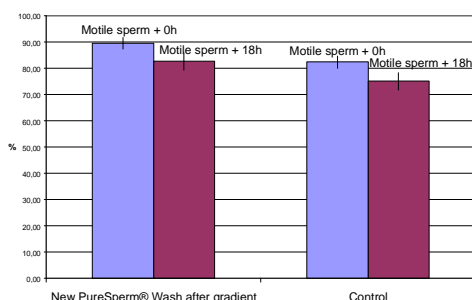
Ejaculates (n=12) were split in two parts and both aliquots (1.5 mL) were prepared on gradients of **PureSperm® 40** and **PureSperm® 80**, 2 + 2 mL, by centrifugation at 300 x g for 20 minutes. The sperm pellets were transferred to the control and the new **PureSperm® Wash**, respectively, (5 mL) for washing by centrifugation at 500 x g for 10 minutes.

The motility was assessed at two time points, both by subjective motility analysis and by computerized analysis using IVOS by Hamilton Thorne (Figure 1).

In addition, the viability was assessed by using Sperm VitalStainÎ. The sperm preparations were kept at room temperature (~ 22°C) and reanalyzed after 18 hrs. The yields obtained after these preparations were the same in both washes.

Furthermore, the viability was better in the new **PureSperm® Wash** compared with control directly after preparation, and 8 % higher after 18 hrs.

Figure 1: Motile sperm after density gradient centrifugation and washing in the old and the new PureSperm® Wash (n = 12).

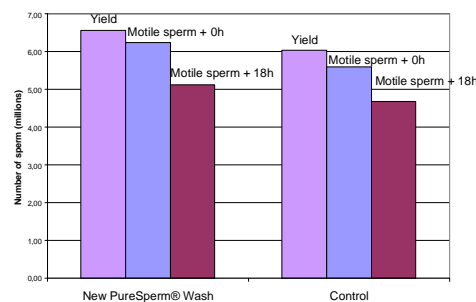


### 2. Performance after swim-up, comparing ReadySwimÎ and the new improved PureSperm® Wash

Ejaculates (n = 10) were split in two parts and both aliquots (1 mL) were placed in round bottomed tubes, the new **PureSperm® Wash** (1.5 mL) was layered on top of one aliquot of the ejaculate and ReadySwimÎ (1.5 mL) on the other. After incubation for 1 hr (37°C, 5% CO<sub>2</sub>), the top layer (1 mL) was transferred to conical centrifuge tubes, and re-suspended in new **PureSperm® Wash** and **ReadySwimÎ** (control), respectively, for washing by centrifugation 500 g for 10 minutes. The samples were analyzed in the same manner as in exp 1.

It was found that, the viability was 3.4 % higher in the new **PureSperm® Wash** compared with control directly after preparation, and 7 % better after 18 hrs. The results regarding motility are illustrated in Figure 2.

Figure 2: Number of motile sperm after preparation with ReadySwimÎ and the new PureSperm® Wash using the swim-up technique (n = 10).



## Conclusions

- The new PureSperm® Wash shows better results regarding motility and viability compared with the old after density centrifugation and in particular after 18 hrs
- The new PureSperm® Wash also shows better results than ReadySwimÎ regarding motility and viability using the swim-up technique
- Based on the results above, we can also conclude that the new PureSperm® Wash is suitable for extending the sperm pellet prior to IUI