A comparison of the swim-up procedure at body and testis temperatures

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A range of different sperm preparation methods have been developed during the last two decades following a rapid increase of clinics using assisted reproductive technologies (ART). Among these methods, swim-up procedures and density gradient centrifugation are widely used techniques for sperm preparation [1–3].

The swim-up from a washed sperm pellet is a commonly used method, originally described by Mahadevan and Baker [4]. The conventional swim-up is based on the active movement of spermatozoa from a pre-washed cell pellet into an overlaying medium. The swim-up is usually performed for 0.5–2 h within a CO₂ incubator at 37°C in many laboratories. The procedure results in the collection of a very high percentage of motile sperm, enrichment of morphologically normal spermatozoa, and the exclusion of other cells and debris.

However, it is well known that the temperature of testis is 2–3°C lower than body temperature. The cooler temperature of the testis is explained by its location in the scrotum outside of the body [5]. It has been reported that an increase in the temperature of the mammalian testis causes a decrease in sperm production and is associated with infertility [6]. A similar condition is found in other mammals such as the mouse, in which the testis temperature is usually 30°C, this being 7°C lower than the body temperature [5]. Furthermore, it has been reported that spermatogenesis in the mouse is affected by a temperature-dependent regulatory process that is testis specific [7, 8].

Despite the reports showing harmful effects on sperm maintained at body temperature [6], the swim-up procedure in ART is usually performed at 37°C. Therefore our aim was to compare the influence of 37°C and 34.5°C on sperm and ART outcome.

We compared the sperm motility and velocity using a computer associated sperm analyzer (CASA) when swim-up was performed at 37°C and 34.5°C. Sperm samples from 28 patients who had more than 120×10^6 motile spermatozoa in the total volume of the ejaculated semen were used in this study.

A liquefied semen sample was placed on top of the density gradient (90% SpermCare; InVirtroCare, USA) and the tube was centrifuged for 20 min at $300 \times g$. The supernatant was removed without disturbing the pellet. The sperm pellet was washed twice with 3 ml of HTF medium containing 10% of 100 mg/ml HSA solution (InVirtroCare) by centrifugation at $300 \times g$ for 5 min. The washed sperm were collected from the bottom of the tube using a clean pipette and the contents were divided between two new, clean tubes containing HTF medium with 10% of a 100 mg/ml HSA solution. Then each tube was placed at a 45° angle into separate incubators, one at 34.5°C and the other at 37°C, each containing 5% O₂. In both incubators the medium was maintained at pH 7.2–7.3 using a CO₂ concentration of 5.4–5.6% at 34.5°C and 6.2–6.4% at 37°C. After 2, 6, 24 h of the incubation, sperm motility and velocity were analyzed using the computer associated sperm analyzer (CASA).

The results showed that a time dependent significant decrease in motility and velocity were seen both at 34.5°C and 37°C (Fig. 1). Also, the overall sperm motility was significantly lower at 37°C compared with 34.5°C (Fig. 1). Furthermore time dependent changes observed in each patient revealed that a severe decline in sperm motility was

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Fig. 1 Time dependent motility and velocity changes in sperm collected at two temperatures

Fig. 2 The time dependent sperm motility changes in each of the 28 patients studied at 34.5°C (left) and 37°C (right) are shown. In patient A (bold pink line) and patient B (bold yellow line), a large decline of the sperm motility was observed 6 h after swim-up started at 37°C

Table 1 A comparison of fertilization and pregnancy rates when sperm were collected at two temperatures

	37.0°C (3 month before the change)	34.5°C (3 month after the change)	P value
Number of ART cycles	61	48	
Average patients age	36.3	35.2	NS
Number of oocytes inseminated	289	209	
Number of oocytes performed ICSI	83	82	
Total number of oocytes	372	291	
Fertilization rate by ICSI (%)	86.8	95.1	NS
Fertilization rate by IVF (%)	67.5	70.3	NS
Average number of ET	1.70	1.83	NS
Pregnant rate (GS+)/ET (%)	40.4	43.9	NS
Implantation rate/ET (%)	26.3	29.3	NS

occurred in two of the 28 patients when their sperm were incubated at 37°C (Fig. 2).

Since better sperm parameters were obtained at 34.5°C, we changed the temperature of the CO₂ incubator for swim-up procedures from 37°C to 34.5°C. This resulted in an improvement of fertilization and pregnancy rates when the 3 months before and after the change of temperature were compared. However, during these early stages of the study the improved fertilization and pregnancy rates, following swim-up at 34.5°C, were not statistically significant (Table 1). This early stage data analysis was performed using the statistical package StatView Ver. 5.0 (SAS Institute Inc.). Clinical characteristics were analyzed using the unpaired Student's *t* test or χ^2 test taking a *P* value of <0.05 to be statistically significant.

Our results suggest that insemination should be performed as soon as the swim-up procedure is finished. We have also shown that sperm incubation should not to exceed 6 h at 37°C. Since in certain patients we observed a large decrease of the sperm motility even by 6 h after incubation at 37°C, it would appear that the sperm swim-up procedure is more reliable at 34.5°C rather than 37°C. Further study will be required to determine whether sperm DNA is more prone to damage at body temperature compared with testis temperature. Also the optimal temperature for sperm preparation for ART needs to be evaluated.

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