



Effect of Temperature on Membrane Integrity of Human Spermatozoa

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Available online at: www.isca.in, www.isca.me

Received 29th November 2014, revised 11th January 2015, accepted 26th February 2015

Abstract

Temperature plays a very important role in the integrity of human spermatozoa. During *in vitro* fertilization, it is necessary to maintain optimum temperature to maintain the viability of sperms outside the body, especially in cases of male factor infertility where only a few sperms are available for use. A functional membrane is required for the sperm to properly fertilize the oocyte, because it plays a key role in capacitation of the sperm, acrosome reaction and binding of the sperm to the surface of the oocyte. This study was conducted to observe the effect of temperature on human spermatozoa and thus deduce the optimum temperature for good survival of the sperms. Semen samples were collected from normozoospermic patients. Processed semen samples were exposed to two different temperatures, and the membrane integrity of the sperms was assessed by performing hypo-osmotic swelling test and the sperms were checked for tail-curling under 200x microscope.

Keywords: Sperm, temperature, membrane integrity.

Introduction

The fertility rate of humans has been steadily decreasing over the past few years¹. Infertility can be defined as the failure to conceive after 12 months of unprotected sex with the same partner. The prevalence of infertility in India is not yet clear, yet, according to a study by WHO, the incidence of infertility is estimated to be 10 % to 15% and approximately 13 to 19 million couples are infertile (Rowe and Farley, 1988; Sharma et al., 2005). Male factors play an important role in about 50% of infertile couples (Schill, 1981; Sharma et al., 2005). Many external factors are responsible for this steady deterioration. One of these factors is temperature which affects spermatogenesis². Infertility can be managed by Assisted Reproductive Technology. Sperms have to be maintained at an optimum temperature *in-vitro*, so that their fertilizing potential can be maintained. Membrane integrity is one of the factors that play a key role in the fertilization potential of human spermatozoa. This study was conducted to determine the optimum temperature at which the sperms should be maintained *in vitro*.

Material and Methods

Twenty normozoospermic (sperm concentration $>50 \times 10^6/\text{ml}$) patients were selected for this experimental work. Semen samples were collected after 4-5 days of abstinence. They were analyzed according to the methods described by WHO. Samples were processed using the swim-up technique and the final washed sample was maintained at a count of $15 \times 10^6/\text{ml}$, 100% motility. Three aliquots were prepared, 0.1ml each and each was subjected to 20°C, 25°C and 37°C respectively for half an hour each. Hypo-osmotic swelling³ test was performed on each of the aliquots at the end of ½ hour. 0.1 ml of each aliquot was treated with 1 ml of hypo-osmotic solution (7.35 gms sodium

citrate.2H₂O and 13.51 gms fructose in 1L of distilled water). The aliquots were then incubated at 37°C for 30 mins. A drop of this solution was observed under phase contrast microscope at 200x to observe curling of the tail.

Results and Discussion

The aliquots subjected to 20°C showed considerably lesser membrane damage (27.16%) as opposed to the aliquots subjected to 37°C (36.78%) and 25°C (32.61%). T test was applied to the above data. T-test for 20°C and 37°C was 1.387198 which is significant at $p < 0.10$ level of significance. T-test for 37°C and 25°C was 0.631275 which is not significant at $p < 0.10$ level of significance.

Increasing evidence has shown that the male reproductive health has declined¹. The results from this study suggests that an adequate incubation temperature is required for the expression of human sperm fertilizing ability *in vitro*. Incubation temperature affects the percentage of motile sperms after it is retrieved⁴. Sperms with low motility values may be significantly affected by the incubation temperature. It is still a controversy as to what is the optimum temperature for handling sperm in a laboratory. Under normal conditions, the human sperm spends its entire life span in the male and female genital tract which is at 37°C. It is a known that the temperature of the testis is around 2-3°C below body temperature. This temperature difference is necessary for the normal production and maintenance of live sperms. Many articles have been published on the harmful effects of long term incubation of human sperms in the lab at 37°C, yet, most IVF labs store it at 37°C prior before using it in assisted reproductive technologies (ART). Therefore, the current study was conducted to observe the effect of different temperatures on the membrane integrity of human spermatozoa.

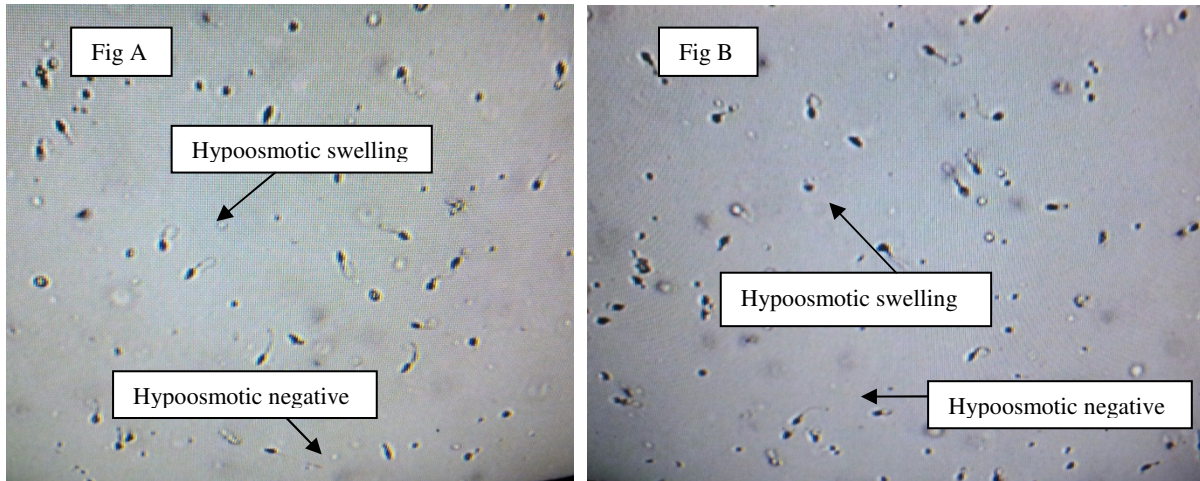


Figure-A-B
Sperms exposed to 20°C with more sperms showing hypoosmotic swelling (tail curling)

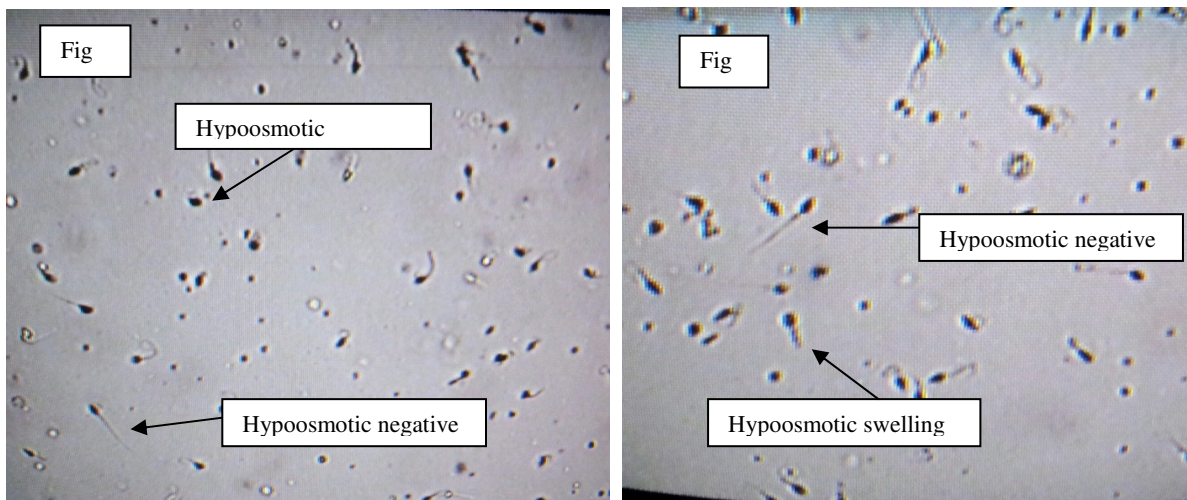


Figure-C-D
Sperms exposed to 25°C showed lesser number of hypoosmotic positives as compared to the ones at 20°C.

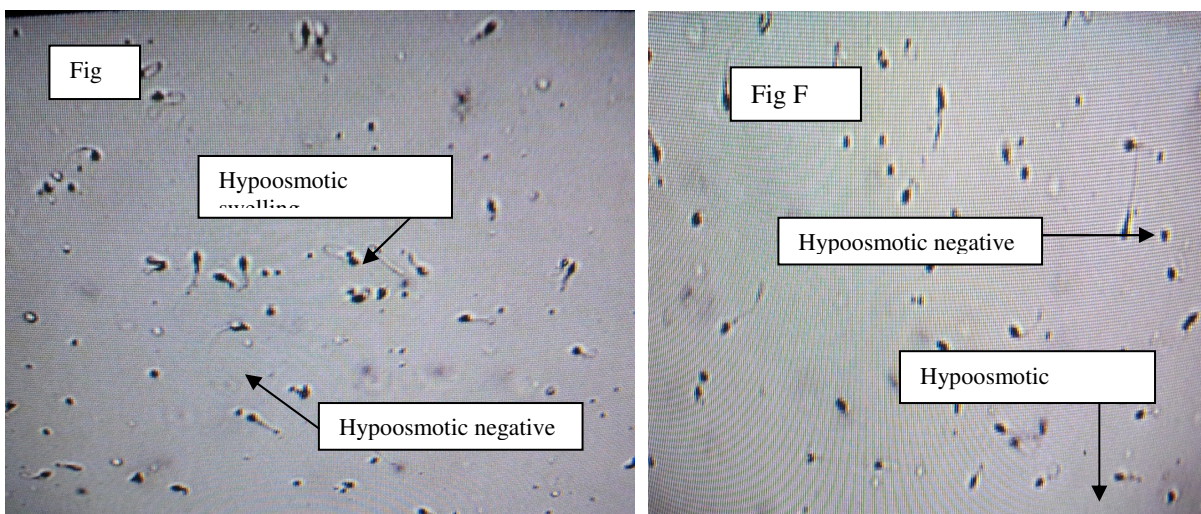


Figure-E-F
Sperms exposed to 37°C showed even lesser number of sperms with hypoosmotic swelling compared to 25 and 20°C.

Table-1

Temperature	% Hypo-osmotic Negative
20°C	27.16%
25°C	32.61%
37°C	36.78%

Conclusion

Membrane has to be intact for effective fertilization. An intact membrane affects motility and hyperactivity of the sperm. Temperature affects the fluidity of the membrane and thus affects its fertilizing capacity.

Our study revealed that a temperature lower than 37°C is preferred while treating and handling sperms in vitro contrary to the current practice of incubating the sperms in vitro at 37°C. Sperm membrane remained intact at 20°C according to the current observation. More work needs to be conducted in order to confirm this study.

References

1. Andersson A.M., Jorgensen N., Main K.M., Toppari J., Rajpert-De Meyts E., Leffers H., Juul A., Jensen T.K. and Skakkebaek N.E., Adverse trends in male reproductive health: we may have reached a crucial 'tipping point', *Int. J. Andrology*, **(31)**, 74–80, **(2008)**
2. Derek Robinson MD, John Rock MD and Miriam F Menkin, Control of Human Spermatogenesis Intrascrotal Temperature, MA, *JAMA*, **204(4)**, 290-297, **(1968)**
3. Muhammad Zubair, Laeeq Akbar Lodhi, Ejaz Ahmad and Ghulam Muhammad., Hypo osmotic swelling test as screening for evaluation of semen of bull, *Journal of Entomology and Zoology Studies*, **1(6)**, 124-128, **(2013)**
4. Watanabe A., The effect of heat on the human spermatogenesis, *Kyushu J Med Sc*, **(10)**, 101-17, **(1959)**
5. Franken D.R, R. van Wyk, Stoumann C. and Avari K., Temperature controlled centrifugation improves sperm retrieval, *Andrologia*, **(43)**, 217-221, **(2011)**