Ind J Hum Genet 5(1): 51-55 (1999)

Time and Temperature Effects on In-Vitro Sperm Chromatin and Stability

Rao KM[1]

1. Special Grade I, CHS, Ministry of Health, New Delhi
Honorary Registrar, Department of Ob. & Gynaec.
Gandhi Medical College, Hyderabad
Gandhi Hospital, Secunderabad 500 003 (A.P) India

Abstract

Semen samples were collected both from men with or without children. Sperm wash preparations were made and the samples were studied at different temperatures and times of incubations for genome packaging efficiency. It is found that sperm chromatin decondensation study could be optimised with SDS reagent if the temperature was 37 degrees and time of incubation was 30 minutes. Similarly, SDS & EDTA, optimum time was 15 minutes at body temperature, thus affecting results of the study. Below, the body temperature the reaction is slow and does not mimick the in-vivo process.

Key words: Sperm chromatin stability; Chromatin decondensation; Temperature.

Introduction

Sperm genome is optimally packaged for transfer to the oocyte. This reversible packaging is optimal when components like zinc (Arver and Eliasson, 1982), albumin (Johnsen O. Eliasson R, 1978) heparin (Delgado NM, *et al.*, 1980), proteins like histones (somatic versus sperm specific) and protamines (Tanphaichitr N, *et al.*, 1978), zinc ligands like citrate (Sillen and Martell, 1971), HMW and IMW protein ligands (Arver and Eliason, 1982), fructose (Mann, 1964), etc., play their predestined roles properly and proportionately. Inadequate stability as well as superstability are obviously not good for proper genome delivery (Calvin H, 1971). Any ejaculated sample has a population of sperms that is heterogenous with respect to stability of its chromatin (Bedford GM, *et al.*, 1973). Of these heterogenously stabilised sperms, only those with adequate and reversible stability are likely to retain the capacity for decondensation on reaching the oocyte (Uehara and Yanagimachi, 1976). In-vitro studies using specific chemicals, temperatures and time intervals for incubation have been conducted. In this study, the in-vitro reactions are carried out at 2 different temperatures and for different incubation times to decide on the optimum time and temperature of the reaction.

Methods and materials

Semen samples were collected from 20 men in reproductive age groups, after 3 days of abstinence. Each sample is divided into 2 portions, the first is kept at 30 degrees and the second is kept at 37 degrees. The samples were thus stored for 1.5 hours and then studied.

From both portions aliquots were taken to wash the sperm in BSS. Washing is done with double the volume BSS. Resuspension is done in equal volume BSS. Reactions are set up in both native and washed samples, in both SDS and SDS with EDTA (1% SDS and 6 mM EDTA), for 0, 5, 15, 45, 120 minute intervals. The reaction is stopped with glutaraldehyde and scoring is done in percentage of stable and decondensed sperm nuclei under phase contrast microscope. Makler chamber is used to count the number of cells under a given field.

Results and discussion

The reaction temperature of 30 degrees is found to produce lesser degree and percentage decondensation, when compared to 37 degrees. Moreover, 37 being the body temperature may be more acceptable for uniformity of the decondensation studies.

SDS, a demulcent destabilises the membranes. In case the chromatin is not adequately stabilised, this produces decondensation. In a heterogenous population of sperms in a given ejaculate, there are bound to be some immature sperms with inadequate stabilisation of chromatin (Sobhon P, 1982). On the other hand, SDS + EDTA reaction destroys the membranes as well as extracts all the extractable Zn from the sperm (which is 70% of total Zn-Huret JL 1986). So, the proportion of cells decondensing in SDS + EDTA is always higher and includes the Zn stabilised cells + membrane stabilised cells. The remaining stable cells are the so called superstabilised cells, whose stability is not merely because of membrane integrity and Zn stabilisation but also involves excess disulfide bridges (ageing / oxidation?) (Huret JL, 1982). But, in addition to the above, there is involvement of free thiol groups, which can break disulfide bridges and bring in decondensation, if enough temperature / time interval is given (Kvist U, 1984). So, to test the mere membrane dependent stability or Zn dependent stability and not to involve the factor of thiols/other factors, optimum time / temperatures for any given chemicals and reaction need to be determined. In this study, 1% SDS and 6mM of

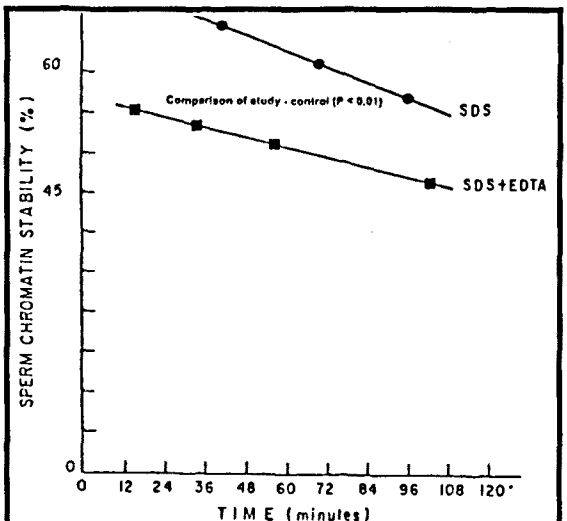

Figure 1: Comparison of sperm chromatin stability in different culture media

EDTA were used and the reaction results were analysed.

It is seen by most observers that the reaction at room temperatures is very slow. So, the temperature used for the reaction is always body temperature, that is, 37 degrees celsius. SDS is used in 1% conc. routinely. SDS + EDTA is found to be satisfactory at 6 mM. Next, the reaction times are set up for both the native samples and washed preparations in SDS alone and SDS + EDTA.

There is time dependent increase in the stability in SDS and also SDS + EDTA for both native and washed sperms (Rosenborg L, Rao KM, 1990; Bjorndahl and Kvist, 1985; Kvist and Bjorndahl, 1985; Kvist, *et al.*, 1987). On the other hand, when the reaction time (when compared to the storage time of sperms of above) is increased, there is bound to be more loss of stability. Figure 1 shows the loss in stability is reaction time dependent and more in SDS as compared to SDS + EDTA. This may look illegical, as always the loss of stability is more in SDS + EDTA for any given reaction time. This discrepancy is explained in further illustrations.

Figure 2, a multiple regression comparison of SDS and SDS + EDTA reactions for time (0, 5,

15, 45, 120minutes) versus stability, shows very clearly that after 15 minutes of reaction time, there is very perceptible and clear increase in stability of SDS + EDTA reacted sperms. This is contrary to known mechanisms of the reaction. Such increase is not seen in SDS reaction, thus showing that even at 120 minutes, the system gives good and accurate results for SDS, but not for SDS + EDTA, for which 15 minutes seem to be the upper limit for good results.

Figures 3 and 4 are regression and multiple regression graphs of the above 2 tests, of time versus number of sperms that are counted in the field / fields. It is seen in Figure 3 that the drop in number of sperms is significantly more in SDS + EDTA reaction than in SDS reaction. This shows clearly that beyond a time limit for the reaction, there is gross decondensation in SDS + EDTA, leading to the disappearance of the cells and giving false readings of the stability and biasing the scoring. This is also observed by Huret JI, 1983.

Now, what is the optimum reaction for SDS + EDTA, given that for SDS reaction, there is not much time dependent bias in scoring? Figure 4 shows that after the reaction time of 15 minutes, the decrease in cell numbers stabilises in SDS, but not in SDS + EDTA. Also, the significantly higher drop in numbers could be noted in SDS + EDTA (70%) as against SDS (40%).

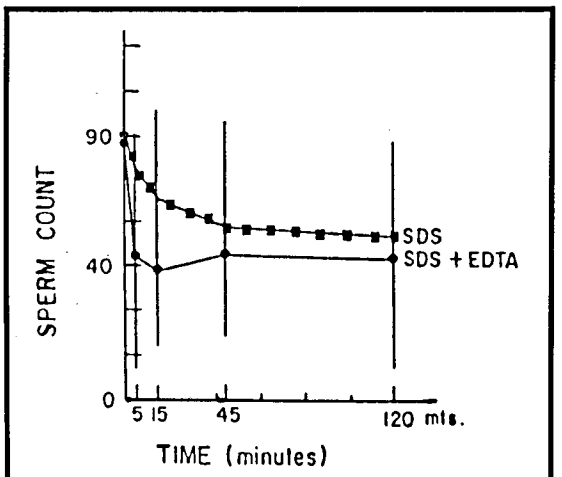

Figure 2: Multiple regression curves of time period versus sperm chromatin stability

Figure 5 shows a 3-dimensional view of stability and cell numbers in SDS + EDTA reaction at 0, 5, 15, 45, and 120 minutes. There is drop in stability up to a reaction time of 15 minutes beyond which there is a biased rise in stability. At the same time, the cell numbers continue to drop, though there is a stabilisation after 45 minutes.

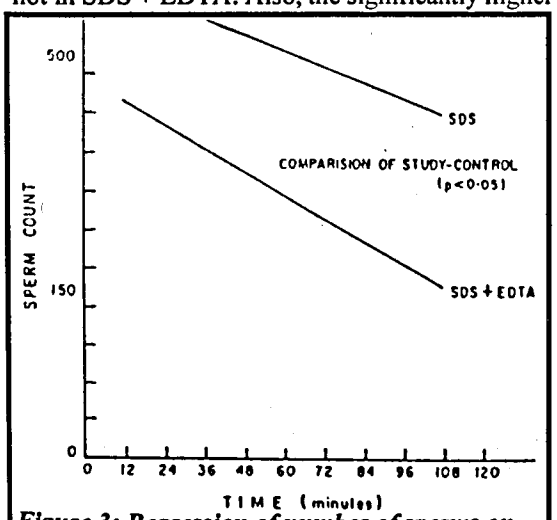

Figure 3: Regression of number of sperms on time

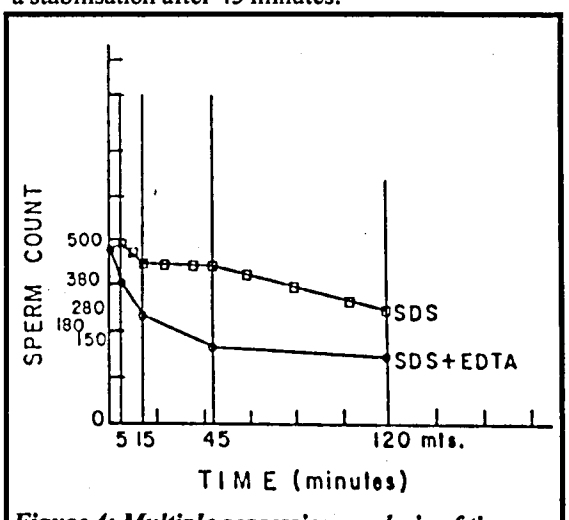

Figure 4: Multiple regression analysis of time versus number of sperms

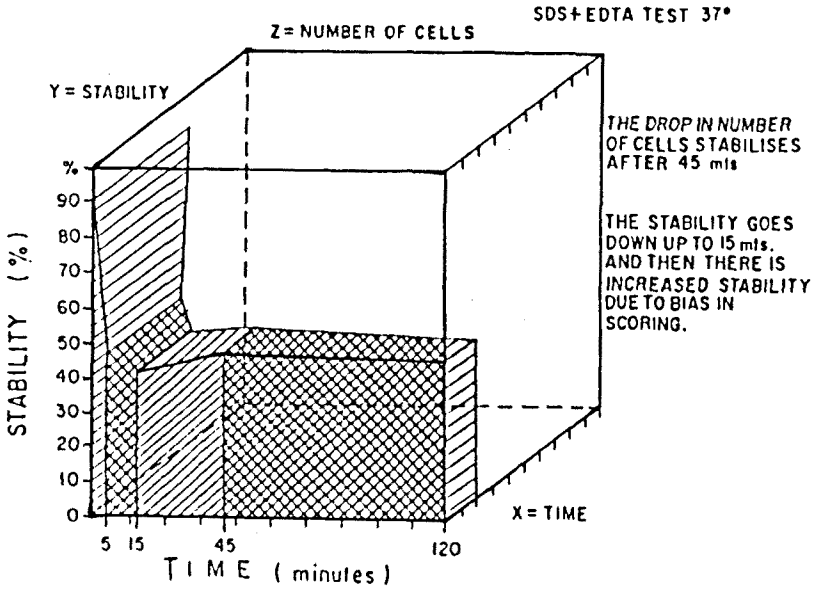

Figure 5: Three dimensional view of sperm chromatin stability

Huret JL, 1986, felt that the optimum concentrations were 1% for SDS and 6 mM for EDTA. According to him the optimum reaction times were 60 minutes for SDS and 5 minutes for SDS + EDTA. The results of the present study clearly show a continuing drop in cell numbers even in SDS and so, to avoid any possibility in biased scoring, the optimum reaction time for SDS alone should be 30 minutes. And for SDS + EDTA reaction 15 minutes, since there is shift in the stability scoring at 15 minutes, thus avoiding the possible inaccuracies associated with too short a reaction time of 5 minutes, suggested by Huret JL.

References

Arver S and Eliasson R (1982). Zinc and zinc ligands in human seminal plasma. II Contribution by ligands of different origin to the zinc binding properties of human seminal plasma. *Acta Physiol Scand.* 115: 217-224.

Bedford JM, Bent MJ and Calvin H (1973). Variations in the structural character and stability of the nuclear chromatin in morphologically normal human spermatozoa. *J Reprod Ferti.* 33: 19-29.

Bjorndahl L and Kvist U (1985). Loss of an intrinsic mechanism for human sperm chromatin decondensation. *Acta Physiol Scand.* 124: 189-194.

Calvin H and Bedford JM (1971). Formation of disulfide bonds in the nucleus and accessory structure of mammalian spermatozoa during maturation in the epidydimis. *J Reprod Ferti.* 13: 1041-1049

Delgado NM, Huacuja L, Merchant H, *et al.* (1980). Species specific decondensation of human spermatozoa nuclei by heparin. *Arch Androl.* 4: 305-313.

Huret JL (1983). Variability of the chromatin decondensation ability test on human sperm. *Arch Androl.* 11: 1-7.

Huret JL and Groyer A (1985). Nuclear chromatin decondensation of human sperm assessed by DNA measurement. *Arch Androl.* 14: 7-13.

Kvist U and Bjorndahl L (1984). Chromatin decondensation in mammalian spermatozoa. *Acta Physiol Scand.* 124 (Supp. 542): 389.

Kvist U, Kjellberg S, Bjorndahl L, *et al.* (1988). Zinc in sperm chromatin and chromatin stability in fertile men and men in barren unions. *Scand J of Uro and Nephro.* 22: 1-6.

Mann T (1964). The biochemistry of semen and of the male reproductive tract. Metheun & Co. Ltd., London. 1-493.

Rosenborg L, Rao KM, Bjorndahl L, *et al.* (1990). Changes in human sperm chromatin stability during preparation for in-vitro fertilization. *Int J of Andro.* 13: 287-296.

Sillen LG and Martell AE (1971). Stability constants of metal ion complexes. Special publication No. 25. The Chemical Society, London.

Sobhon P, Tanpaichitr N, Chutatape C, *et al.* (1982). Electronic microscopic and biochemical analyses of the organisation of human sperm chromatin decondensed with Sarkosyl and dithiothrotol. *J Exp Zool.* 223: 277-290.

Tanphaichitr N, Sobhon P, Taluppet N, *et al.* (1978). Basic nuclear proteins in testicular cells and ejaculated spermatozoa in man. *Exp Cell Res.* 117: 347-356.

Uehara T and Yanagimachi R (1976). Behaviour of nuclei of testicular, caput and cauda epidydimal spermatozoa injected into hamster eggs. *Biol Reprod.* 16: 315-321.