ANDROLOGY

The Correlation of Sperm Chromatin Decondensation Following In Vitro Exposure to Heparin and Sperm Penetration Rates

DOUGLAS T. CARRELL,^{1,3} BENJAMIN R. EMERY,¹ and C. MATTHEW PETERSON²

Submitted: February 5, 1998

Accepted: May 7, 1998

Purpose: The aim of this study was to evaluate the possible correlation of low-dose heparin-induced decondensation of sperm chromatin with sperm concentration, motility, morphology, membrane hypoosmotic response, ejaculate volume, and the ability of sperm to penetrate zona-free hamster oocytes.

Methods: Twenty-two donors of known fertility and 105 patients undergoing evaluation at an andrology laboratory were evaluated by standard World Health Organization semen analysis techniques and a modified sperm penetration assay (SPA). An aliquot was also incubated for 60 min and Ham's F10 medium containing 50 USP/ml heparin. The percentage of sperm undergoing chromatin decondensation was evaluated and correlated to SPA rates and semen quality parameters.

Results: No significant correlation was observed between semen parameters and decondensation rates. A nonsignificant ($P = 0.11$) inverse correlation ($P = -0.21$) was observed between SPA rates and chromatin decondensation. Significant ($P < 0.001$) differences were observed in the decondensation rate of donors (3.7 ± 0.6), patients with normal SPA rates (7.8 ± 1.5), and patients with decreased SPA rates (21.7 ± 1.8). The decondensation rates were significantly different ($P < 0.01$) between patients with a normal SPA rate and patients with a decreased SPA rate.

Conclusions: These data indicate a significant inverse relationship between the SPA rate, which has previously been

shown to correlate highly with fertilization ability, and heparin-induced sperm chromatin decondensation.

KEY WORDS: protamine; chromatin; decondensation; capacitation.

INTRODUCTION

The final stages of spermiogenesis include a higher degree of chromatin packing than that observed in other cells. This is partially accomplished by the replacement of nuclear histones with a class of low-molecular weight proteins termed protamines (1,2). Protamines are characterized by high levels of arginine and cysteine, and the cysteine residues form numerous disulfide bridges. Following penetration of the oocyte, formation of the male pronucleus is initiated by the breaking of the disulfide bridges and the gradual decondensation of the sperm chromatin. Abnormal chromatin packing, or conversely abnormal chromatin decondensation, may affect fertility (3).

Specific binding sites for the glycosaminoglycan heparin have been identified on the sperm plasma membrane (4). These binding sites have been characterized, and are believed to originate from the seminal plasma. Incubation of sperm with heparin has been shown to induce sperm capacitation and the acrosome reaction (5,6). In addition, high concentrations of heparin have been shown to induce sperm chromatin decondensation. Several studies have attempted to correlate the decondensation of sperm chromatin following incubation with heparin or detergent with semen quality parameters such as sperm concentration, motility,

¹ Division of Urology, University of Utah School of Medicine, 50 North Medical Drive, Salt Lake City, Utah 84132.

² Department of Obstetrics and Gynecology, University of Utah School of Medicine, 50 North Medical Drive, Salt Lake City, Utah 84132.

³ To whom correspondence should be addressed.

and morphology (7,14). These studies have generally shown no correlation between semen parameters and chromatin decondensation induced by incubation with detergent or heparin.

In this study we have evaluated the induction of sperm chromatin decondensation by incubation with a low concentration of heparin. Standard semen quality parameters were correlated with the rate of chromatin decondensation. In addition, the rate of chromatin decondensation was compared among semen donors of known fertility, infertile patients with normal sperm penetration assay (SPA) rates, and infertile patients with decreased SPA rates.

MATERIALS AND METHODS

Semen Analysis and Sperm Penetration Assay

Patients and known fertile donors were initially evaluated by standard semen analysis following 2–5 days of sexual abstinence. Sperm concentration, motility, differential morphology, ejaculate volume, and viability were analyzed using standard World Health Organization (WHO) criteria (15).

A portion of the ejaculate was evaluated by the SPA. This assay was performed as described previously (16, 17). Briefly, two aliquots of the semen sample were separately prepared. The first aliquot was prepared by standard Percoll gradient centrifugation of the fresh sample. The second aliquot was prepared by Percoll gradient centrifugation following refrigeration in TEST-yolk buffer (TYB) for 2–8 hr. Twenty to thirty-five zona free hamster oocytes were incubated with each aliquot of the prepared samples, which were previously adjusted to a motile sperm density of 5×10^6 . The oocytes were incubated with the sperm for 3–5 hr, then analyzed by phase-contrast microscopy for the presence of decondensed sperm heads. Results are reported as the percentage of oocytes penetrated by at least one sperm.

Evaluation of Chromatin Decondensation

Preliminary studies were performed to evaluate the optimal concentration for sperm chromatin decondensation. Semen samples were divided into 11 equal aliquots, then diluted with Ham's F10 culture medium and centrifuged at 400g for 10 min. Following centrifugation, the supernatant was decanted and the sperm pellet was resuspended in Ham's F10 culture medium containing heparin in concentrations ranging from 0

to 5000 USP/ml. The samples were incubated at 37°C for 1 hr, then diluted with Hams F10 culture medium and centrifuged at 400g for 10 min. The sperm pellet was resuspended in medium not containing heparin, and sperm motility was again evaluated. Additionally, a smear of resuspended sperm was prepared on a clean microscope slide and dried at 37°C. After drying, the slide was stained using a standard eosin/hematoxylin staining procedure.

To evaluate decondensation a minimum of 200 sperm cells was evaluated under bright-field microscopy at a magnification of at least $\times 400$. The sperm were scored as condensed, slightly to moderately decondensed, or extremely decondensed. The percentage of sperm undergoing decondensation was calculated by adding the percentages of slightly–moderately and extremely decondensed sperm.

All subsequent studies evaluating decondensation in patients and known fertile donors were performed using the technique described above. The concentration of heparin used for all studies was 50 USP/ml. A minimum of 200 sperm was counted for each sample.

Statistical Analysis

The relationship of the sperm chromatin decondensation with standard semen parameters was evaluated using standard regression analysis. Differences in the decondensation rates for donors and patient categories was evaluated by analysis of variance. All data are reported as mean $\pm$ standard error. The Macintosh version of Statview was used for all analyses.

RESULTS

Chromatin decondensation was increased at all concentrations of heparin analyzed and significantly ($P < 0.05$) increased at the concentration of 50 USP/ml (Fig. 1). No significant correlation was observed between the decondensation rate and the HOS value, sperm motility, sperm concentration, or percentage of morphologically normal sperm heads.

A nonsignificant inverse correlation ($r = -0.21$, $P = 0.11$) was observed between the decondensation rate and the SPA rate of all samples analyzed (Fig. 2). However, when the decondensation rates were compared among donors of known fertility, infertile patients with normal SPA results, and infertile patients with decreased SPA rates, significant differences were observed in the mean decondensation rates (Fig. 3). The mean decondensation rate was 3.7 ± 0.6 for

Fig. 1. Dose-response curve of the effect of increasing concentrations of heparin on chromatin decondensation.

donors of known fertility, compared to 7.85 ± 1.5 ($P < 0.01$) for infertile patients with a normal SPA value. The mean decondensation rate for patients with a decreased SPA value was 21.7 ± 1.8 ($P < 0.001$), significantly lower than the other two groups.

DISCUSSION

Defects in sperm chromatin decondensation may be significant in two respects. First, chromatin decondensation is an essential event in pronuclear formation and the fertilization process, therefore, defects in chromatin decondensation may directly affect fertility. Second, defects of decondensation may reflect general abnormalities of spermiogenesis which may involve other important aspects of sperm function. In this study we

have not observed any correlation between abnormal sperm decondensation following exposure to heparin and sperm motility, concentration, or morphology. However, we have observed that patients with a measurably decreased penetration ability exhibited an increased propensity of chromatin decondensation following exposure to heparin.

The sperm penetration assay evaluates the functional ability of sperm to undergo the capacitation process and certain steps requisite in normal human fertilization. Decreased SPA values have been shown to be indicative of a decreased ability to undergo fertilization with human oocytes both in vitro and in vivo (16–20). Therefore, the SPA is used by some in vitro fertilization (IVF) laboratories as a screening mechanism prior to patient therapy. We have previously reported a high correlation between SPA rates and IVF results. There-

Fig. 2. Regression analysis of SPA rates vs. decondensation rates.

Fig. 3. The mean decondensation rate of donors, patients with a decreased SPA rate, and patients with a normal SPA rate. * $P < 0.01$ compared with other groups.

fore, the data reported in this study, that the patients identified by the SPA as having a poor capacitation ability exhibited a significantly higher degree of chromatin decondensation than patients with normal sperm penetration ability.

Previous studies have used higher concentrations of heparin than used in this study. Additionally, detergents have been used in place of heparin to induce decondensation. Those studies have reported no consistent correlation between sperm motility, concentration, or morphology and decondensation rates. It has generally been assumed that a higher decondensation rate would be indicative of normal fertility. In our studies, the opposite result was observed. Samples exhibiting a higher rate of chromatin decondensation under the test conditions used were correlated with a reduced sperm penetration ability. It is possible that the differences in protocol, including the decreased concentration of heparin used in our study, may be responsible for this effect.

It is known that avian sperm contain a protamine with fewer cysteine amino acids, therefore, fewer disulfide bonds are formed. In studies recently reported, transgenic mice were formed with an avian gene for protamine. Sperm from the transgenic mice exhibited a higher degree of induced chromatin decondensation than normal mice, indicating a link between the decreased number of disulfide bridges and the increased induction of chromatin decondensation (21). It is possible that our studies indicate an altered protamine structure with decreased disulfide

bond formation in certain patients, resulting in an increased susceptibility to decondensation when exposed to heparin. Current studies at our laboratory are comparing levels of protamine in sperm from donors of known fertility and patients with poor SPA values. In addition, we are attempting to evaluate possible differences in the protamine proteins and genes. The data presented here did not indicate a significant correlation between SPA rates and decondensation rates, but indicate that the sperm decondensation rate may be useful in assessing penetration ability. Future studies will also evaluate the possible use of this assay in screening infertile patients.

REFERENCES

1. Pongsawasdi P, Svasti J: The heterogeneity of the protamines from human spermatozoa. *Biochim Biophys Acta* 1976; 434:462-463
2. Bench GS, Friz AM, Corzett MG, Morse DH, Balhorn R: DNA and total protamine masses in individual sperm from fertile mammalian subjects. *Cytometry* 1996;23:263-271
3. Huret JL: Nuclear chromatin decondensation of human sperm: A review. *Arch Androl* 1986;16:97-109
4. Delgado NM, Reyes R, Huacuja L, Merchant H, Rosado A: Heparin binding sites in the human spermatozoa membrane. *Arch Androl* 1982;8:87-95
5. Valencia A, Wens MA, Merchant H, Reyes R, Delgado NM: Capacitation of human spermatozoa by heparin. *Arch Androl* 1984;12 (Suppl):109-113
6. Carrell DT, Bradshaw WS, Jones KP, Middleton RG, Peterson CM, Urry RL: An evaluation of various treatments to increase sperm penetration capacity for potential use in an in vitro fertilization program. *Fertil Steril* 1992;57:134
7. Carranco A, Reyes R, Magdaleno VM, Huacuja L, Hernandez O, Rosado A, Merchant H, Delgado NM: Heparin-induced nuclei decondensation of mammalian epididymal spermatozoa. *Arch Androl* 1983;10:213-218
8. Bedford JM, Bent MJ, Calvin H: Variations in the structural character and stability of the nuclear chromatin in morphologically normal human spermatozoa. *J Reprod Fertil* 1973;33:19-29
9. Lassalle B, Testart J: Relationship between fertilizing ability of frozen human spermatozoa and capacity for heparin binding and nuclear decondensation. *J Reprod Fertil* 1992;95:313-324
10. Le Lannou D, Blanchard Y: Nuclear maturity and morphology of human spermatozoa selected by Percoll density gradient centrifugation or swim-up procedure. *J Reprod Fertil* 1988; 84:551-556
11. Reyes R, Magdaleno VM, Hernandez O, Rosado A, Delgado NM: Effect of zinc on decondensation of human spermatozoa nuclei by heparin. *Arch Androl* 1983;10:155-160
12. Reyes R, Sanchez-Vasquez ML, Merchant-Larios H, Rosado A, Delgado NM: Effect of heparin-reduced glutathione on hamster sperm DNA unpacking and nuclear swelling. *Arch Androl* 1996;37:33-45
13. Sanchez-Vasquez ML, Reyes R, Delgado NM, Merchant-Larios H, Rosado A: Differential decondensation of class I (rat) and class II (mouse) Spermatozoa nuclei by physiological concen-

- trations of heparin and glutathione. Arch Androl 1996; 36:161-176
14. Reyes R, Carranco A, Huacuja L, Delgado NM: Male pronuclei formation release of phosphorylation of histone H-3 during decondensation of human sperm nuclei activated in vitro by heparin. Arch Androl 1991;26(2):53-60
 15. World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. New York, Cambridge University Press, 1992
 16. Carrell DT, Urry RL: A review of the use of modified sperm penetration assay to predict fertilization ability. Assist Reprod Rev 1996;6:170-174
 17. Carrell DT, Jones KP, Peterson CM, Urry RL: The predictive ability and variability of the sperm penetration assay (SPA): An analysis of 1033 patients undergoing SPA(s) and in vitro fertilization (IVF). Arch Androl (in press)
 18. Wolf DP, Sokoloski JE, Quigley MM: Correlation of human in vitro fertilization with the hamster egg bioassay. Fertil Steril 1983;40:53-59
 19. Margalioth EJ, Feinmesser M, Navot D, Mordel N, Bronson RA: The long-term predictive value of the zona-free hamster ova sperm penetration assay. Fertil Steril 1989;52:490-494
 20. Karp LE, Williamson RA, Moore DE, Shy KK, Plymate R, Smith WD: Sperm penetration assay: useful test in evaluation of male fertility. Obstet Gynecol 1981;57:620-623
 21. Maleszewski M, Kuretake S, Evenson D, Yanagimachi H, Bjordahl J, Yanagimachi R: Behavior of transgenic mouse spermatozoa with galline protamine. Biol Reprod 1998; 58:8-14