Original Article

Effect of PolyVinyl Pyrrolidone on Sperm Membrane Integrity and Chromatin Status

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Abstract

Background: The aim of present study is to evaluate the effect of PolyVinyl Pyrrolidone (PVP) routinly used during ICSI procedure on sperm membrane integrity, and sperm chromatin status.

Materials and Methods: This study was carried out on 21 semen samples from the infertile men referring to Isfahan Fertility and Infertility Center. The processed semen samples were divided into two portions. One portion was added to Ham's F10+ 10% PVP, and the other portion was added to Ham's F10 as a control group. Hypo-osmotic swelling test (HOST), SDS, and SDS+EDTA tests were carried out on the control and PVP groups at 15, 30, and 60min.

Results: The results show that sperm membrane integrity measured by HOST, and sperm chromatin stability measured by SDS test, reduces by increasing the exposure time to PVP. However, the ability of sperm chromatin undergoing decondensation (that has been assessed by SDS+EDTA), does not show any changes by increasing the exposure time to PVP.

Conclusion: The results of current study shows that reducing the exposure time of sperm to PVP may protect sperm membrane, and chromatin integrity.

Keywords: ICSI, PVP, Sperm Chromatin, Membrane Integrity, HOST

Introduction

Intra-cytoplasmic sperm injection (ICSI) has become a well established and accepted method for the treatment of male infertility, and is a commonly method used worldwide. However recent studies suggests that the increase chromosomal abnormalities observed in the children conceived through ICSI is not due to ICSI procedure or the technique itself, it seems to be caused by the materials used during ICSI procedure (1, 2). PolyVinyl Pyrrolidone (PVP) routinely used during ICSI facilitate handling of spermatozoa (3,4) and is also part of density gradient such as Percoll, Sil-Se-lect, and Sperm Grade used for sperm processing or as a cryoprotectant (5-7). PVP, a synthetic polymer, is used for the following items: many medical purposes, spray, cosmetic industry, beverage industry, intravenous injection as a plasma expander, and intramuscularly injection as a vehicle for drug. In vivo PVP, being indigestible by lysosomal enzymes and stored in cells, causes many pathological problems such as skin lesions, abscesses, granulomas (8). Literature studies shows that PVP is toxic when injected to mouse zygotes (9), whereas other studies shows that when PVP is injected to the bovine zygotes, it is neither detrimental to embryonic development nor to embryo quality (10). There are also controversial reports on the effect of PVP on sperm viability, and structure. Therefore, the aim of present study is to evaluate the effect of PVP on sperm membrane integrity and sperm chromatin status.

Materials and Methods

Sperm preparation

Semen samples were collected from 21 infertile patients referred to the andrology unit of Isfahan Fertility and Infertility Center after 3-4 days of sexual abstinence. The samples were processed by Percoll gradients and was showed with Ham`s-F10 medium. Then, equal volume of 10% PVP solution prepared in Ham`s- F10 + HEPES + 10% HAS ,were mixed with equal volume of the processed semen samples. A part of the processed semen sample was considered as control group. Hypo Osmotic Swelling test (HOST) , Sodium dodecyl Sulfate (SDS), and SDS+EDTA tests were carried out on both control and PVP groups at 15, 30 and 60min post incubation of PVP with the sperm.

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Hypo-Osmotic Swelling Test

HOST was carried out according to Liu et al (11). This test is based on the semipermeability of the intact cell membrane, which allows the sperm to swell under hypo-osmotic condition. On exposure of sperm to HOST solution, an influx of water results in swelling of the cytoplasmic space and curling of the tail. In order to obtain a hypo-osmotic solution with approximately 150 mOsmol, 0.09% NaCl was mixed with equal volume of distilled water. A1:2 ratio of the washed semen sample with the hypo-osmotic solution was mixed, and then HOST reaction was observed under a light microscope at 400X magnification after 30min. Different HOST sperm-tail patterns were taken as HOST- positive, and then percentage of the live sperm were recorded. This test was carried out on both control and PVP group at 15, 30, 60min post incubation of PVP sperm.

Sperm chromatin stability (SDS), and nuclear chromatin decondensation (SDS+EDTA) tests, exams the DNA stability of sperm which dependes on the amount and ratio of disulfide bound (S-S), non-covalent bound between Zn with sulfhydryl groups (SH…Zn…SH) and free thiol groups. The ability of nuclear chromatin decondensation (NCD) also depends on the amount of SH…Zn…SH group. Therefore upon removal of spermolemma with SDS as a detergent, nuclear decondensation can take place depending on chromatin stability. Therefore, 50µl of Percoll prepared sperm were mixed with 350µl of 1% SDS in 0.05M borate buffer (88ml NaOH 0.1M +4.2ml Na-tetraborate 0.25M 400µml H_2O, pH 9). After incubation for 60min at 37ºC, the reaction was stopped by the addition of equal volume of 2.5% glutaraldehyde in 0.05M borate buffer. Nuclear swelling was assessed after staining with Gimsa. The percentage of unstable sperm (partially or completely swollen) and stable sperm were recorded. Thus, removal of Zn by EDTA could result NCD, and the ability of NCD was also assessed by SDS+EDTA with addition of 6mM EDTA to 1% SDS solution (12). Stable sperm chromatin, with no change in the size of head, were given a score of zero (S0) sperm with partially swollen head were given a score of 1 (S1), and sperm with completely decondensed head were given a score of 2 (S2). The degree of sperm stability or its ability of decondensation (NCD) was expressed as total score and calculated be the following formulae:

Total score=(number of S1+number of S2 X 2/ Total number of sperm assessed) X 100.

Statistical analysis

Statistical package for social studies (SPSS-10) was used for analyzes .The mean value of each parameters compared through student t-test between the control and PVP groups at different intervals.

Results

Table 1 shows a significant reduction in number of HOST positive sperm (curling tail) between the PVP and control groups at the different interval, suggesting that co-incubation of the sperm with PVP decreases membrane integrity over time.

Table 1: Comparison of HOST between the control and PVP groups after 15, 30 and 60min of exposure to PVP.

Group	Mean±SD	P-value
Control group (without PVP)	92.05±5.21	---
Incubated in PVP	76.10±15.85	0.001
Incubated in PVP	65.43±19.44	0.001
Incubated in PVP	52.76±22.5	0.001

Table 2 shows a significant difference in the mean value of the total scored sperm for SDS test between the control, and PVP groups at the different interval. It is indicated that as the time of incubation in PVP increased, chromatin stability decreased. Whereas in SDS+EDTA test, no significant different was observed in mean value of the total scored sperm between the control and PVP groups. It suggests althought the time of incubation in PVP has been increased the ability of the sperms undergone decondensation in presence of EDTA, has not been affected. Since the mean values of Host, SDS and SDS+EDTA in the control group were similar and not significantly different over 15, and 30 and 60min, the scores for these parameters were pooled and expressed as a single value for each test.

Table 2: Comparison of total score assessed by SDS and SDS+EDTA tests between control and PVP groups after 15, 30 and 60 minutes exposure to PVP.

Group	SDS test		SDS+EDTA test	
	Mean±SD	P-value	Mean±SD	P-value
Control group (without PVP)	79.14±63.12	---	129.62±60.08	---
Incubated in PVP (15 Min)	95.14±47.68	0.001	126.43±64.47	0.762
Incubated in PVP (30 Min)	97.95±41.57	0.002	127.57±51.67	0.867
Incubated in PVP (60 Min)	117.71±40.67	0.005	120.60±45.03	0.626

Discussion

PVP has been suggested to have stabilizing effect on plasma membrane, (13) and this might lead to delayed calcium oscillation in the sperm-penetrated oocytes as well as prevent nuclear decondensation (14, 15). But, Strehler et al found that PVP through submicroscopic alteration causing chromatin damage which might result in chromatin decondensation (16) and DNA lesions (17).

Strehler et al suggested that nuclear deterioration was possibly caused by a general breakdown of sperm membranes and subsequent necrotic processes (16). De leeuw et al also indicated that PVP reduced sperm membrane integrity in bull (7). The results of this study showed that sperm membrane integrity, assessed by HOST, gradually decreased over time in presence of PVP. The result of current study for membrane integrity, which being concordance with the reports of Strehler, and is in contradictory with the reports of Dozortsev, shows that PVP increases sperm membrane stability (13, 16). The difference between the result of our study and Dozortseve could be due to the different methods for assessment of the membrane integrity. They assessed membrane integrity using Eosin-Nigrosin test while in this study, the membrane integrity was assessed through HOST test. Chromatin stability decreased over time when, sperm incubated with PVP. Decreased chromatin Stability observed in PVP groups could be secondary in compare to the effect of PVP on the membrane integrity. Therefore, when the sperm membrane becomes damaged as a result of PVP co-incubation, this effect gives the sperm a greater amount of time to be undergon decondensation. However, when sperm are placed in PVP and then treated with SDS+EDTA, PVP has no significant effect on mean value of total scored sperm between the two groups. This can be due to the fact that both PVP and EDTA may act as chelator of Zn.

Conclusion

During routine ICSI procedure, motile sperm with normal morphology are selected for injection of oocyte from PVP. Since PVP has a damaging effect on membrane it can be resultes in necrotic process. Therefore in order to reduce this damaging effect; either exposure of sperm to PVP solution should be reduced, or alternative viscous agent for oocyte injection should be used.

Acknowledgment

We would like to thank the infertility specialists and embryologists working at the Isfahan fertility and infertility center. We also like to thank Isfahan University of medical sciences for their technical and financial supports (Project Number 81027).

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