PARTHENOGENETIC ACTIVATION AND DEVELOPMENTAL POTENTIAL OF MOUSE OOCYTES AFTER INTRACYTOPLASMIC SPERM INJECTION OF POLYVINYL PYRROLIDONE AND HYALURONIC ACID

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Abstract

Objective: During ICSI, a small amount of PVP is invariably injected into the oocyte cytoplasm raising concerns about its safety and possible adverse impact on embryo development. HA-based products are a physiologic alternative to PVP as they are thought to be safer. However, the literature is not clear in regards to the validity of replacing PVP with HA. The objective of this study was to assess parthenogenetic activation and the developmental competence of mouse oocytes after ICSI of PVP and HA.

Design: In vitro experimental study

Materials and Methods: B6C3F1 frozen metaphase II mouse oocytes (n = 102) were divided into three groups: PVP-treated (n = 36); HA-treated (n = 34) and control (n = 32). In the respective groups, 2-3 picoliters of PVP, HA or culture medium were microinjected into the oocyte cytoplasm. Oocytes were parthenogenetically activated with calcimycin A23187 solution for 15 minutes. Parthenogenetic activation of mouse oocytes was recorded as the number of surviving embryos at the two-cell stage 24 hours after microinjection; embryo development was recorded until the blastocystic stage. Chi-square or Fisher exact tests were used to compare the outcome measures.

Results: The parthenogenetic activation and development potential of mouse oocytes after ICSI of PVP or HA culture medium is presented in Table 1. No significant differences were seen among the groups with regard to these outcome measures. Results are expressed as mean and 95% confidence interval. Differences were not significant among the groups at p<0.05 (Table 1).

Conclusions: In this experimental study in which sperm factors have been controlled, neither PVP nor HA seem to adversely affect developmental competence of parthenogenetically activated mouse oocytes.

Table 1. Parthenogenetic activation and development potential of mouse oocytes after intracytoplasmic injection of PVP and HA (percentage and 95% confidence interval)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PVP (n = 36)</th>
<th>HA (n = 34)</th>
<th>Control (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte degeneration rate (%)</td>
<td>13.33 (18.56-50.97)</td>
<td>26.67 (12.88-44.36)</td>
<td>34.38 (18.57-53.19)</td>
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<tr>
<td>Surviving embryos at 2-cell stage (%)</td>
<td>62.50 (40.59-81.18)</td>
<td>68.0 (46.55-80.05)</td>
<td>61.90 (38.44-81.89)</td>
</tr>
<tr>
<td>Surviving embryos at 4-cell stage (%)</td>
<td>53.53 (25.77-78.73)</td>
<td>52.94 (27.81-77.02)</td>
<td>52.85 (25.14-80.78)</td>
</tr>
<tr>
<td>Surviving embryos at morula stage (%)</td>
<td>53.53 (25.77-78.73)</td>
<td>52.94 (27.81-77.02)</td>
<td>46.15 (19.22-74.86)</td>
</tr>
<tr>
<td>Blastocyst development rate (%)</td>
<td>33.33 (11.82-61.62)</td>
<td>35.29 (14.21-61.67)</td>
<td>38.46 (13.86-68.42)</td>
</tr>
</tbody>
</table>

Introduction

During ICSI, a small amount of the medium containing PVP is usually unavoidably injected into the oocyte cytoplasm. Concerns about the safety of PVP and its possible adverse impact on embryo development have been raised. HA-based products are also commercially available, and a few reports indicated that such medium might represent a good physiologic alternative to PVP. After sperm injections, total fertilization failure occurs in about 1.3% ICSI cycles and the phenomenon can repeat in subsequent cycles. It has been hypothesized that total fertilization failure might be due to oocyte activation deficiency. In this regard, mouse oocytes provide a useful model for the assessment of sperm-associated oocyte activation factor because they are tolerant to Ca2+-oscillation patterns. Parthenogenetic mouse oocyte activation relies on the calcium wave that generates the responses associated with fertilization when calcium release reaches a certain threshold. Calcimycin - known also as A23187 - is a Ca2+-selective ionophore widely used as an oocyte activation agent. This model would be ideal to assess the effects of PVP and HA on embryo development competence because the influence of sperm factors is avoided. Using an ICSI experimental model, we conducted an experiment to investigate the activation and embryo developmental competence of the parthenogenetically activated mouse oocytes after intracytoplasmic injection of PVP and HA.

Materials and Methods

B6C3F1 frozen metaphase II mouse oocytes (n = 116) were used in this experiment. Frozen mouse oocytes were thawed and the surviving oocytes (n = 102) were randomized into three groups for microinjection: PVP-treated (n = 36); HA-treated (n = 34) and control (n = 32). In the respective groups, 2-3 picoliters of PVP, HA or culture medium were microinjected into the oocyte cytoplasm. Of note, no sperm were injected. Immediately after microinjection, the oocytes were parthenogenetically activated by exposure to a ready-to-use calcimycin (Ca2+-selective ionophore) A23187 solution (GMS08 Cult-Active, Gynemed, Germany) for 15 minutes. Parthenogenetic activation of mouse oocytes was recorded as a percentage of surviving embryos at the two-cell stage 24 hours after microinjection. Embryo development was recorded until the blastocystic stage (Figure 1). Chi-square or Fisher exact tests as appropriate were used to compare the outcome measures (expressed as mean ± 95% confidence interval) among the groups using an alpha level of p < 0.05.

Results

The development competence of parthenogenetically activated mouse oocytes after ICSI with PVP or HA culture medium is presented in Figure 2.

1. Oocyte degeneration rate was not different among the groups: 33.33% (12/36) in the PVP group, 26.47% (9/34) in the HA group and 34.37% (11/32) in the control group.
2. Oocyte activation rates were not significantly different among the groups: 62.50% (15/24) in the PVP group, 68.0% (17/25) in the HA group and 61.90% (13/21) in the control group.
3. Of the embryos that reached the two-cell stage in the PVP HA and control groups, respectively, 53.33% (9/17), 52.94% (9/17) and 53.85% (7/13) attained a four-cell stage.
4. Among the embryos that reached the four-cell stage, 53.33% (8/15), 52.94% (9/17) and 46.15% (6/13) achieved the compacted morula stage.
5. On day 5, the blastocyst formation rates were 33.33% (5/15), 35.29% (6/17) and 38.46% (5/13) in the HA, PVP and control groups, respectively.
6. No significant differences were seen among the groups with regard to these outcome measures.

Conclusions

1. Neither PVP nor HA seem to adversely affect developmental competence of parthenogenetically activated mouse oocytes.
2. Our results support the continued clinical use of PVP and HA for selection and manipulation in ICSI processes.

Figure 1. Development competence of parthenogenetically activated mouse oocytes after ICSI of PVP or HA culture medium (control).

Figure 2. Embryo developmental competence of parthenogenetically activated mouse oocytes after ICSI of PVP, HA or culture medium (control). Differences in oocyte degeneration rates, surviving embryos at the two-cell stage, four-cell-stage, compacted morula stage, and blastocyst formation rates were not significant among the groups.