Addition of kallikrein and/or human serum to a discontinuous Percoll gradient

Dear Sir,

In their paper, Yding Andersen and Jørgensen (1995) report the effect of the addition of progesterone to media used in Percoll gradient sperm preparation. Recent studies have reported that sperm preparation using a discontinuous Percoll gradient may yield a higher pregnancy rate in intrauterine insemination (IUI) than when the technique of centrifugation-resuspension is used (Depypere et al., 1995), and this has been attributed to the lower reactive oxygen species (Clarkson, 1988) and lower superoxide dismutase activity (Zalata et al., 1995) when Percoll gradient selection is used. The success rate of IUI depends largely on the proportion and motility of selected spermatozoa. Kallikrein is an enzyme which converts kininogen into kinin and this substance has been suggested to increase sperm motility (Schill and Miska, 1992), whereas serum is known to improve sperm function during in-vitro fertilization (IVF).

Freshly ejaculated semen samples from eight men consulting because of infertility and of two fertile semen donors were used. After initial analysis of the native ejaculate (WHO, 1987), each sample was divided into four equal parts. One aliquot was processed over a discontinuous Percoll gradient, a second aliquot over a similar gradient to which kallikrein (Sigma, St. Louis, MO, USA, 5 kIU/ml) and/or human serum (20 μl of heat-inactivated serum) was added, the third aliquot on a Percoll gradient with 20 μl/ml kallikrein and 20 μl/ml of serum. The aliquots were processed simultaneously by centrifugation for 10 min at 500 g and the Percoll layers were aspirated with the remaining pellet being resuspended in a constant amount of the washing medium. The samples were analysed for sperm concentration and motility characteristics using a computer-assisted autosome sperm system (FertiPro, Lotenhulle, Belgium) (Hinting et al., 1988). The results were analysed using the Wilcoxon rank sum test for paired replicates (MedCalc Software, Mariakerke, Belgium).

The results shown in Table I do not reveal any significant differences (P > 0.05) in sperm concentration or total progressive motility (grade a plus grade b) between any of the treatments. Neither Percoll preparation per se, nor the addition of kallikrein, nor kallikrein plus serum had any demonstrable effect on the characteristics studied. The only significant difference was between spermatozoa prepared on a Percoll gradient with or without serum. The former presented a significantly higher curvilinear and linear velocity, which was increased in all 10 samples. Also, the proportion of spermatozoa with grade a motility was significantly higher in samples prepared over Percoll plus serum, and it was increased in all eight the asthenozoospermic cases with initial value below the normal limit of 25%.

In view of this result it may be of interest to study the conception rates when IUI or IVF is performed in cases with asthenozoospermia after sperm preparation on discontinuous Percoll layers to which serum is added.

References


Table I. Results, mean and range

<table>
<thead>
<tr>
<th>Sperm concentrationa (X 10⁹/ml)</th>
<th>Grade a motility (%)</th>
<th>Grade a + b motility (%)</th>
<th>Velocity (μm/sec)</th>
<th>Linear velocity (μm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>35.8</td>
<td>13.0</td>
<td>32.9</td>
<td>24.2</td>
</tr>
<tr>
<td></td>
<td>(5.7-121)</td>
<td>(0-34)</td>
<td>(7-53)</td>
<td>(15.9-45.9)</td>
</tr>
<tr>
<td>Percoll</td>
<td>20.2</td>
<td>13.1</td>
<td>26.7</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>(3.8-117)</td>
<td>(0-64)</td>
<td>(4-85)</td>
<td>(8.9-43.0)</td>
</tr>
<tr>
<td>Percoll + kallikrein</td>
<td>25.3</td>
<td>17.0</td>
<td>27.5</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td>(2.7-196)</td>
<td>(0-53)</td>
<td>(4-68)</td>
<td>(13.2-44.7)</td>
</tr>
<tr>
<td>Percoll + serum</td>
<td>22.1</td>
<td>25.4</td>
<td>36.1</td>
<td>32.7</td>
</tr>
<tr>
<td></td>
<td>(4.5-200)</td>
<td>(4-55)</td>
<td>(4-67)</td>
<td>(21.8-49.9)</td>
</tr>
<tr>
<td>Percoll + kallikrein + serum</td>
<td>36.4</td>
<td>21.4</td>
<td>34.5</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>(9-142)</td>
<td>(0-63)</td>
<td>(12-83)</td>
<td>(15.7-47.2)</td>
</tr>
</tbody>
</table>

aBack transformed after square root transformation.
bp = 0.040.
cp = 0.019.
dp = 0.014.


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Dear Sir,

We thank Dr. Taranissi and his colleagues for their interest in our articles on embryo transfer techniques and wish to comment on their letter.

Their opinion that immediate mock embryo transfer is detrimental to the success rate by causing trauma is not supported by objective evidence. Careful reading of the results presented in our article (Sharif et al., 1995a) shows that the only embryo transfer technique parameter which led to a statistically significant reduction in pregnancy rate was the ease or difficulty with which it was performed. Neither blood staining of the catheter, nor the number of mock embryo transfer steps performed before the real embryo transfer had a significant effect on pregnancy rate. This strongly suggests that immediate mock embryo transfer is not detrimental.

In their letter they also recommended cervical dilatation or re-sterilized one. In our practice, if a difficult transfer is anticipated we would plan to use a stiffer outer sleeve catheter. We have found the use of a Labotech embryo transfer catheter (Rimmer Brothers, London, UK) to be remarkablyatraumatic, leading to no decline in pregnancy rate in difficult transfers. We feel this is due to the design of the catheter and more importantly the softness and narrow diameter of the inner catheter, which ensure the culture medium transferred with the embryos is kept to a minimum (5-10 µL), thereby maximizing the chance for the embryos to be retained in the uterus.

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In their letter they also recommended cervical dilatation or the use of alternative catheters in cases of difficult mock embryo transfer. Our experience with cervical dilatation is similar to that of other groups in that it offers no help, whether performed prior to the in-vitro fertilization (IVF) cycle or at oocyte retrieval (Visser et al., 1993; Barrett et al., 1995); hence we no longer recommend it. The use of stiffer catheters is helpful, as clearly illustrated by our results (Sharif et al., 1995a). Nevertheless, there are a few transfers that are very difficult, or even impossible to perform whatever catheter is used. To address this problem we have recently reported a modified step-wise embryo transfer protocol (Sharif et al., 1995b). It starts by an immediate transcervical mock embryo transfer with a soft catheter and proceeds in a step-wise fashion to stiffer catheters and, if the transcervical route is not easy,