HISTOLOGICAL EVALUATION OF THE EFFECTS OF TRANSPORT ON BOVINE CORNEAS FOR OCULAR SAFETY ASSESSMENTS

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Abstract

Examination of stromal thickness, including thickness of Descemet’s Membrane, suggests corneas undergo minimal artifactual changes as a result of refrigerated transport and the BCOP assay procedures. However, the cornea on the whole globe was notably thicker than any of the dissected corneas. The thickness of Descemet’s Membrane was proportional with the increase in thickness of the corneas. Evaluation of the stromal layer immediately below the epithelium revealed consistent collagen organization changes, histopathology has been used in BCOP studies for nearly a decade. Although the state of the negative control corneas at the end of the BCOP assay has been characterized histologically, artifactual changes in the corneas associated with the collection and storage of the enucleated eyes, or the BCOP methodology have not been evaluated.

Materials and Methods

Corneas were excised and fixed at various steps in the assay process: 1. At the time of enucleation (fixed with and without a histology cassette) 2. After refrigerated transport 3. At the end of the BCOP assay (untreated) As a control for control enucleation, whole globe eyes were fixed at the time of enucleation. Sources of differences in stromal thickness (or measured thickness) are needed. Therefore, multiple stromal measurements for evaluating changes in stromal thickness and stromal swelling are needed.

Results

Average Normalized Stromal Thickness

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of Measurements</th>
<th>Average Stromal Thickness ( microns)</th>
<th>Normalized Stromal Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Globe</td>
<td>278 (6)</td>
<td>903.8 ± 123.96 microns</td>
<td>903.8 ± 123.96 microns</td>
</tr>
<tr>
<td>Enucleation</td>
<td>147 (4)</td>
<td>636.3 ± 67.95 microns</td>
<td>1211.9 ± 60.1 microns</td>
</tr>
<tr>
<td>Arrival</td>
<td>113 (4)</td>
<td>598.2 ± 57.36 microns</td>
<td>876.7 ± 84.2 microns</td>
</tr>
<tr>
<td>After Refrigerated Transport</td>
<td>291 (8)</td>
<td>754.8 ± 60.32 microns</td>
<td>829.8 ± 63.4 microns</td>
</tr>
<tr>
<td>End of Assay</td>
<td>152 (4)</td>
<td>802.1 ± 120.51 microns</td>
<td>721.2 ± 17.2 microns</td>
</tr>
</tbody>
</table>

Stromal Organization

Evaluation of the stromas immediately beneath the epithelium showed minimal differences in collagen organization or keratocyte morphology between the different groups evaluated. However, the interlamellar spacing, or clefting, appeared to be more prominent in the whole globe stroma, relative to the excised corneal stroma. The figures below show the stroma immediately beneath the epithelium.

Discussion

Examination of stromal thickness, including thickness of Descemet’s Membrane, suggests corneas undergo minimal artifactual changes as a result of refrigerated transport and the BCOP assay procedures. Evaluation of the stromal layer immediately below the epithelium revealed consistent collagen organization and keratocyte morphology among all groups evaluated, which further suggests that corneas undergo minimal artifactual changes. However, there is a large variability in the measurement of stromal thickness within each group, suggesting that stromal thickness alone is not a definitive measure of corneal swelling and/or damage. Sources of differences in stromal thickness (or measured thickness) include:

- Animal variability
- Fixation and embedding technique
- Microtome angle during sectioning
- Various sectioning angles

These results show that the corneas undergo minimal artifactual changes as a result of refrigerated transport and the BCOP assay procedures.

Acknowledgements

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References