

**MICROSCOPIA ELECTRÓNICA DE EMBRIONES PRODUCIDOS *IN VITRO***

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**Microscopía electrónica de barrido (MEB)**

La técnica de MEB permite observar estructuras de la superficie de los complejos ovocito-cumulus y de embriones producidos *in vitro* con alto grado de resolución. La topografía y morfología de estructuras características se hacen visibles en forma imponente.

Para preparar a los espermatozoides, ovocitos y embriones para la evaluación con un microscopio de barrido, deben ser fijados, deshidratados, secados, colocados sobre una base y cubiertos con oro-paladio.

La **fijación** se lleva a cabo con 1% glutaraldehído en *buffer* Soerensen pH 7,4 (1:5 mezcla de 0,07 M  $\text{KH}_2\text{PO}_4$  y 0,07 M  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ) o en 2,5% Glutaraldehído en 0,1 M *buffer* fosfato pH 7,2. Alternativamente se puede emplear también 2,5% glutaraldehído en 0,1 *buffer* cacodilato pH 7,2 (Kölle y col., 2004, Haenisch-Woehl y col., 2003). La fijación se lleva a cabo durante 1h, 24h ó 48h a 4C° o a temperatura ambiente. Antes de la fijación los espermatozoides, ovocitos o embriones deben ser lavados cuidadosamente 2 a 3 veces en *buffer*. La **deshidratación** se lleva a cabo con una secuencia incrementada de acetona (10%, 20%, 30%, 40%, 50%, 60%; 2x5 minutos cada paso, 70%, 80% y 90% 60 minutos y 100% 12 horas aproximadamente), (Kölle y col., 2004). Alternativamente es posible también emplear una serie de concentraciones incrementadas de etanol para la deshidratación. El **punto crítico de secado** ocurre con  $\text{CO}_2$  en varios pasos con diferentes temperaturas y relaciones de presión. La **fijación mecánica** se lleva a cabo colocando la muestra en un soporte de aluminio. Luego, se cubre la muestra con una capa de 12nm de una mezcla de oro y paladio con ayuda de un "recubridor" *Sputter* (Kölle y col., 2000). Los ovocitos o embriones pueden ahora ser observados en el microscopio electrónico de barrido.

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