

Appendix 5

Essential requirements for proper washing of mouse and rat embryos as modified for rodents.

Only embryos from **donors having the same health status** should be washed together (confirm before washing).

Twenty-five or fewer embryos should be washed at one time (count before washing).

Only zona pellucida-intact embryos **should be** washed (confirmed both before and after washing)*

Only embryos free of adherent material should be washed (clean when necessary before washing)*

Minimum of ten washes in **sterile medium** (allow sufficient time for thorough, gentle mixing in each wash).

Use a new sterile micropipette each time embryos are moved from one wash to the next.

Regulate volumes so that each wash is at least 100-fold dilution of previous wash.

*To confirm intactness of the zona pellucida and freedom from adherent material, embryos must be observed on all surfaces at a minimum magnification of 60X.