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Experimental setup of somatic cell nuclear transfer: procedural optimization

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INTRODUCTION:
Somatic cell nuclear transfer (SCNT) can be described as the removal of the chromosomes from a metaphase II oocyte, followed by the transfer and fusion of a donor somatic cell to the enucleated oocyte. The manipulated oocyte is then activated to induce subsequent embryonic development. After the SCNT procedure, a maximum blastulation rate of 12-15% and 10% has been described for mouse and for human oocytes respectively. Although the general accepted cause for the relatively low success rate is probably due to epigenetic reprogramming failure of the donor somatic cell nucleus by the oocyte, the standard SCNT protocol can be optimized for procedural efficiency.

AIM: The aim of this overview is to identify the bottlenecks in the SCNT procedure in order to plan for optimized experimentation in a mouse model.

MATERIALS AND METHODS:
Female B6D2F1 mice are the strain of choice as oocyte and embryo fibroblast (somatic cell) donors. Approximately 50 mice (each producing at least 20 oocytes) will be scheduled for experimentation, specifically for technique development. Two sections will be discussed, the initial enucleation step followed by nuclear transfer and fusion together with activation and culture procedures.

SECTION 1: Enucleating the mouse oocytes
In mice, non-invasive spindle imaging by PlasDIC/Hoffman modulation contrast microscopy should be used to identify the spindle as a smooth clump with no surface granularity. Once located, the spindle should be aspirated and the resulting enucleated test oocytes incubated (5% CO_2) in pre-equilibrated culture media. Successful enucleation of a portion of the oocytes should be confirmed, i.e. through (i) artificial activation using Ca^{2+} ionophores and kinase or protein synthesis inhibitors, which should result in fragmentation and (ii) staining with a DNA stain to confirm microscopically the absence of chromosomes. As a control for the activation step, a portion of the non-enucleated oocytes should be artificially activated resulting in parthenogenetic development.

SECTION 2: Nuclear transfer and fusion into enucleated mouse oocytes, followed by activation and culture
The oocytes (80-90%) should survive nuclear transfer; with 70-80% activation survival, pseudo-pronuclear formation should be visible in 60-70%; cleavage to the 2-cell stage after 24 hours of nuclear transfer should occur in 50-60%; and finally 72h post nuclear transfer 30-50% should develop to the morula/blastocyst stage. Following enucleation, a single somatic cell exposed to a membrane fusogen is transferred into close contact with the oolemma of the enucleated oocyte. Post fusion, the oocytes are artificially activated, with the addition of a histone deacetylase inhibitor which aids epigenetic reprogramming; the oocytes are subsequently cultured to the blastocyst stage (72-96h post activation). To confirm successful activation, non-enucleated control oocytes are activated and cultured, resulting in development due to parthenogenesis.

DISCUSSION:
Despite the many technical bottlenecks associated with SCNT, this procedure can be optimized and adapted for appropriate experimentation. It will be possible to further improve the SCNT procedure as can be evidenced by a greater number of embryos progressing to the blastocyst stage. SCNT is a cutting-edge technique that can offer innovative clinical applications in assisted reproduction such as preventing the transmission of mitochondrial DNA diseases from mother to child, as well as the treatment of ooplasm pathologies. Studies should be performed to optimise protocol efficiency, understand the molecular mechanisms involved in reprogramming, and ultimately improve the technique for clinical applications.