The Fundamental Techniques Used to Induce Tetraploidy

There are three fundamental techniques to induce tetraploidy in vitro. First technique is that a nucleus (2n) is fused to a fertilized ovum surgically. The repeatably uniform tetraploid embryos were produced by this method but only 9–15% of the injected blastocysts survived due to surgical trauma (Modlinski, 1981). The second technique was to inhibit the cleavage with the help of chemicals such as cytochalasin B (CB), a microtubule destabilizer. The third technique was the most common technique to produce tetraploid embryos to induce fusion of two cell stage embryos by electric current. Initially inactivated Sendaivirus was used as the fusion agent (Graham, 1971) but main drawback of this method was that 2-cell stage embryos have to be treated individually as a result causing the slow rate of embryo production.

Along with this, due to removal of the zona pellucida (before fusion) and culture of the embryos for two days in vitro, the survivability of embryos was lowered. In order to produce tetraploid embryos, matured oocytes are collected 14 – 16 hr after the hCG injection then they are artivated artifically by using Calcium-free CZB Medium, SrCl2, and Cytochalasin B in Vitro. After that, two-cell stage embryos are electrofused by using the electro cell fusion system in optimal electrofusion solution to produce tetraploid embryos and then cultured in vitro for preimplantation development to blastocyst. Then, the charateristics of preimplantation development of tetraploid embryos are evaluated by assessment of chromosome remodeling and formation of daughter nuclei after electrofusion and karyotyping of tetraploid embryonic cells at the blastocyst stage. The phosphorylation/ dephosphorylation of histone H3 was reported by Bui et al. which is the key event in chromosome condensation and decondensation in oocytes (Bui et al., 2007). For analyzing chromosome condensation and decondensation, histone phosphorylation at serine 10 (p-H3-S10) is used.

Electrofusion is one of the most accurate, measurable, repeatable, less toxic and well defined procedure which can be performed with the embryos (2-cell stage) having zona pellucida. During this procedure, these embryos are placed between two electrodes in fusion buffer, electrical stimulus is provided for very short duration (Darabi et al., 2008). During electrofusion due to applied direct current (DC) electric field, the membranes are polarized and in-stabilized, results in attraction of other membrane (point membrane fusion) and formation of unstable flat membrane diaphragm, through reversible pore formation followed by reversible breakdown of the membrane or diaphragm (Darabi et al., 2008). Under favourable environment, the flat diaphragm becomes weak to allow cell mixing, indicating through cell-to-cell fusion (Chernomerdik and Sowers, 1991). Many factors affect the fusion efficiency, such as fusion medium, alignment of embryos between electrodes, pulse number, exposure time and electric field intensity Alignment of embryos was important factor for successful fusion of the two cell

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stage embryos. The embryos must be aligned in the fusion chamber with their inter-blastomeric axis parallel to the electrodes using AC current and when alignment performed with AC current and mannitol (nonelectrolyte solution) was utilized for fusion of embryos (Kubiak and Tarkowski, 1985; McLaughlin, 1993). An alternating current field cause polarization of the 2-cell stage embryo and cause rotation of embryos in such a manner that appropriate alignment of embryos occurred for electrofusion (McLaughlin, 1993).

In mice, the highest tetraploid blastocyst formation rate (93.0%) of fused embryos was achieved when electrofusion was performed using 20-volt AC and 100-volt DC (Park et al., 2011)

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