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Tetraploidy Induced by Electrofusion - A Review

Jitendra Kumar Agrawal[1*], Suresh Dinkar Kharche[2], Atul Saxena[1], Akhil Patel[1], Juhi Pathak[2], Anuj Kumar Singh Sikarwar[2]

[1]Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Science and Animal Husbandry, U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura-281001; [2]Physiology, Reproduction and Shelter Management Division, Central Institute of Research on Goats, Makhdoom., Farah-281122, India.

Abstract | *In vitro* tetraploid embryo production has turn into an inestimable means in various research studies for researchers since last few years. The natural tetraploidy stage in embryo is exceptionally reported; hence different techniques have been engaged to bring the tetraploidy experimentally in mammals. There are three fundamental techniques to induce tetraploidy *in vitro*. First technique is that a nucleus (2n) is fused to a fertilized ovum surgically. Second technique is to double the genome, without cell division; by inhibiting cytokinesis Third technique is to stimulate fusion of 2-cell stage diploid embryos. Electrofusion is one of the most accurate, measurable, repeatable, less toxic and well defined procedure which can be performed with the embryos having the zona pellucida. The tetraploidy status can be assessed after culture the embryo *in vitro* or shifting the embryo into surrogate mothers. Tetraploidy status of embryos can be determined through different procedures like cytogenetic study, karyotyping, microdensitometry and fuelgen staining. Although, various experiments have been made in laboratories during last few years to induce tetraploid embryonic development but to the limited extent, success rate has been achieved.

Keywords | Tetraploid, Electrofusion, Embryo, Voltage, Duration

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***Correspondence** | Jitendra Kumar Agrawal, College of Veterinary Science and Animal Husbandry, U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura-281001; **Email:** jituvet11@gmail.com
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Tetraploid embryos have been extensively applied in the field of animal biotechnology since last few years. The tetraploid embryos, having double complement of chromosomes instead of the usual diploid one, are useful model for generating valuable information on embryonic development. The application of tetraploid embryos developed an investigation area in which each blastomere of the cultured embryo had tetraploidy stage instead of diploid (Tarkowski et al., 2005).

In an experiment in mouse, tetraploid embryos developed upto midgestation stage, after that spontaneous abortion occurred (Kaufman and Webb, 1990; Kaufman, 1992) as polyploidy (except the extra-embryonic tissues) is generally not suited with the development of the majority of mammalian tissues (James et al., 1995). Tetraploid embryos are commonly employed to generate mice directly from embryonic stem cells and to avoid embryonic lethality which may arise due to defective extra embryonic phenotypes (Nagy et al., 1990).

TETRAPLOID EMBRYO PRODUCTION METHODS

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. The third technique was to induce fusion of two cell stage embryos. 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.

Presently, the most common technique to produce tetraploid embryos is fusion of 2-cell stage embryos when induced by electric current. Fusion of embryos induced by electrical pulses was used for production of tetraploid embryos in different species including mouse (Sekirina et al., 1997), rabbit (Ozil and Modlinski, 1986), pig (He et al., 2013), bovine (Darabi et al., 2008) and rat (Krivokharchenko et al., 2002).

ELECTROFUSION METHOD

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 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). Non-electrolyte solution (mannitol) and electrolyte solution (PBS) were used as a fusion media for electrofusion of embryos (Kubiak and Tarkowski, 1985).

Brief History of Electrofusion Method

Senda et al. (1979) reported first time the successful electrofusion of two cells of plant protoplast. Later on, Richter et al. (1981) reported the electrical induced fusion between two eggs of sea urchin. After electrofusion, cytoplasmic mixing occurred in sea urchin eggs that were also able of being fertilized although cleavage did not occurred in fertilized eggs. Electrofusion of 2-cell stage embryos was first documented in mouse and fusion was performed using 1 to 4 kV/cm DC (direct current) pulses for 1 to 5 minutes duration (Berg, 1982). Kubiak and Tarkowski (1985) reported maximum fusion rate with 1 kV/cm DC pulses and 100-250 µs duration for electrofusion of mouse embryos and observed that fused embryos had similar viability when compared the non-electrolyte (mannitol) and electrolyte (PBS) solution as a fusion media.

Fusion of Embryos with different Field Strength and Durations

Various researchers have produced the tetraploid embryos using different aspects (field strength, exposure time, number of pulses, AC voltages, and fusion medium). Nagy et al. (1990) reported 98% fusion rate with a single, 100 µs exposure time and 1 kV/cm DC pulse. Cheong et al. (1991) observed in mouse that fusion and subsequent development of embryo was not influenced by alternating current voltages (6 and 12 V/mm) and also reported a higher fusion and embryo developmental rate, when 1.0 - 2.5 kV/cm field strength, 30 to 90 µ sec pulse duration and 1 - 6 number of pulses were used having a wire fusion chamber. Sekirina et al. (1997) applied 30 µs duration and two 4 kV/cm DC pulses for fusion of mouse embryos and observed that the fusion was influenced by the stage of cell cycle, where 98% fusion was achieved by mid-stage embryos, while low fusion rate was observed at the early and late stage 2-cell embryos (24 and 31%, respectively).

Prochazka et al. (2004) evidenced in pigs that two 100 kV/cm DC pulses and 10 to 25 µs duration was most suitable treatment while, Xiangyung et al. (2005) reported that two 100 kV/cm DC pulses and 50 µs duration was most appropriate for tetraploid embryo production in mice. Stekelenburg-Hamers et al. (1993) reported in bovines that a higher fusion rate (80%) was observed at 1.75 kV/cm DC pulse and 40 µs exposure time. May be these difference in electrofusion parameter was related to kind of fusion buffer, electrofusion machine and species of animal.

Darabi et al. (2008) documented that when applied 1.5kV/cm for 100 µs, fusion rate was 88% and observed that the fusion rate was voltage dependent. With increases of voltage intensity from 0.5 to 1.5kV/cm, fusion rate increased but increase in duration of electrical pulse to 100 µs did not affect the fusion rate in different voltage. Curnow et al. (2000) evidenced that when applied 1.4 kV/cm for 100 µs duration, fusion rate was 76% in bovine. Lan Li et al. (2008) also reported that fusion rate increased upto 77% in goat with increases of voltage intensity.

Park et al. (2011) reported that most favourable electrofusion voltages were 100 volts DC pulse and 20 volts alternating current in mouse. Tetraploid embryos were further developed and 2-cell stage tetraploid embryos (93%) were achieved after 16 hours of electrofusion, whereas 4-cell stage tetraploid embryos (80%) were achieved after 24 hours. However, embryos reached the morula stage (95%) after 32 hours of electrofusion. At last, blastocyst stage of tetraploid embryos (93%) was achieved after 48 hours of electrofusion.

He et al. (2013) analysed 2DC electro pulses of different electric field intensities, including 0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 kV/cm for 30 µs for production of porcine 4n embryos and recorded that the optimum condition for electrofusion of 2-cell embryos to produce porcine 4n embryos was 2DC pulses at 0.9 kV/cm for 30 µs.

Iwasaki et al. (2000) documented a higher fusion rate (95%) and viability when used 1 kV/cm DC pulses and 10 to 25 µs duration in bovines. Krivokharchenko et al. (2002) observed 96% fusion rate after electrofusion of 2-cell stage embryos in rat when used 0.6 kV/cm DC pulses for 20 µs duration.

Other Experimental Studies at different Fusion Conditions

Cheong et al. (1991) compared electrode geometrics which showed that when using chambers of various electrode geometries, fusion conditions also changed and documented that embryo development was significantly lowered in a rectangular fusion chamber when applied against the wire fusion chamber. The author also reported in a rectangular fusion chamber that when field strength amplified from 1.0 - 2.5 kV/cm, the embryo developmental rate was decreased but when used a wire fusion chamber, it was easier to apply a relatively broad range of field strength, exposure time and pulse number, hence, rate of fusion and embryo development rate was increased.

Tatham et al. (1995) studied that by fusing enucleated bovine oocytes with blastomeres (with different aged) and showed that increase voltage intensity up to certain threshold level increased the fusion rate, after which fusion rate decreased. However, they also showed that increasing inpulse duration had no fundamental effect on fusion rate up to the threshold level. Zhelev et al. (1988) observed that there was a correlation between pulse intensity and pore formation.

Development of Fused Embryos to Tetraploid Blastocyst

He et al. (2013) observed that the development rate to blastocyst of porcine 4n embryos was 28.5%. The authors (He et al., 2013) observed that development of 2n embryos was faster than 4n embryos at the cleavage stage in porcine embryos under the optimum conditions and also recorded that the timing of blastocyst formation of 2n and 4n was very similar but rates of blastocyst formation between 2n and 4n were not significantly different suggesting that the blastocyst forming capacity in 4n embryos was similar to those in 2n embryos.

Curnow et al. (2000) observed that embryos when fused at 2.4 kv/cm DC pulse and a single pulse for 30 µs duration showed a significantly lower cleavage and blastocyst rate. Curnow et al. (2000) recorded that blastocyst formation rate was 72.5% in bovine while Iwasaki et al. (2000) documented 18.8% mean blastocyst formation rate in bovine. In Rhesus monkey, two 1.5 kV/cm DC pulses and 50 µs duration were used to produce tetraploid embryos and 82% fusion rate was documented after 45 minutes and blastocyst stage of embryos was achieved by 51% of the electrofused tetraploid embryos (Schramm and Paprocki, 2004).

Darabi et al. (2008) recorded maximum cleavage rate at 0.75 kV/cm in 80 µs duration while maximum formation of blastocyst stage (35%) was recorded at 0.75 kV/cm field strength and 60 microsecond duration. The author (Darabi et al., 2008) also observed that there was a negative correlation between voltage and cleavage rate in all durations. This was possibly due to large pore formation (and leakage of cytoplasm) over the two-blastomere membranes. Therefore the author suggested that for optimal cleavage rate, exposure of 2-cell bovine embryo to higher than 1kV/cm should be avoided while Curnow et al. (2000) documented that when applied 1.4 kV/cm for 100 µs, cleavage rate was higher (72.5%) in bovine embryos.

Comparative Study of Developmental Potential of Diploid and Tetraploid Embryos

Snow (1973) observed in mouse that the cell cycle length of tetraploid and diploid embryos was similar. Whereas, two hour increase was recorded in the cell cycle length of fused tetraploid embryos then diploid embryos and it was reported that in tetraploid and diploid embryos, compaction and formation of blastocoel occurred at the same moment, although cell numbers differed between tetraploid and diploid embryos (Koizumi and Fukuta, 1995).

In mouse, compaction occurred at the same time between electro-fused embryos and control embryos, although electro-fused embryos had lesser number of cells per embryo (Kubiak and Tarkowski, 1985). Later on, the cell doubling time was reported similar for tetraploid and control embryos by Henery and Kaufman (1991). A significantly longer cell doubling times was recorded in both groups (kept *in vitro* and which were transferred to recipient females) than control embryos that stayed in vivo during the investigational phase and also observed that rate of formation of

tetraploid embryos was not slow than diploid embryos at appropriate and similar experimental environment.

The lower developmental capacity of the fused embryo may be due to alteration in distribution and behaviour of microtubules and microfilaments affecting normal formation of mitotic spindle and the contractile ring, respectively (Suzuki et al., 2001) and also possibly could be due to electrical stimulation, exposure to non-electrolyte medium or due to chromosomal construction of these embryos (Darabi et al., 2008). The lower developmental rate in high intensity may be likely due to large pore formation and leakage of cytoplasmic material needed for development. The reasons for discrepancy among species were not quite clear but it was proposed that the functional centrosomes exist during the early stages of cleavage in fused porcine and bovine embryos, which may render the embryos more prone to forming a disorganized bipolar or tri-polar spindles. However, lack of centrioles in mouse embryos, up until the blastocyst stage, may cause the 4n embryos to develop after fusing normally. Therefore, due to failing of combining of 2 sets of diploid chromosomes after fusion, 2n embryos occurred at higher rate in fused porcine and bovine embryos (He et al., 2013).

Characterization of the Tetraploid Status

Karyotyping analysis showed that the electrofused 2-cell embryos exhibited significant species variances in their ploidy. At the blastocyst stage of the electrofused embryos of rats and mice, the uniform tetraploidy was documented (Krivokharchenko et al., 2002).

However, at the morula and blastocyst stage, only some embryos displayed uniform tetraploidy in cattle while most of the embryos were diploid or their mosaics (Curnow et al., 2000). In pigs, about 50% of tetraploid blastocysts were of 4n status (Prochazka et al., 2004). When tetraploid embryos were examined by in situ hybridization in mouse, these embryos were found uniformly tetraploid (James et al., 1992). On cytogenetic analysis of embryos, Darabi et al. (2008) showed that over 76% of fused embryos were true tetraploid while Iwasaki et al. (2000) reported 78% tetraploid embryos in bovines. Prochazka et al. (2004) evidenced that 50% embryos were tetraploid in swine while Curnow et al. (2000) recorded very low percent (12.5%) of tetraploid embryos in bovines. He et al. (2013) recorded a high percentage of tetraploid embryos (68.18%) in porcine when evaluated their ploidy at the blastocyst stage by FISH.

During normal embryo development, tetraploid embryos may provide useful information about regulation of cell size and cell cleavage rate in early fetuses. Tetraploid embryos have been used in ancestry studies and for alteration of the balance of parental genomes (Eakin and Behringer, 2003). In chimeras, the observable fact by which diploid and tetraploid cells are separated out has been exploited in various examples; for sorting of genetically dissimilar tissues, to avoid extraembryonic defects and to increase the rate to produce transgenic mice and to study of their phenotypes. Gene balancing is required for successful tetraploidy and gene balance is a significant outcome of genomic imprinting in all mammals (Eakin and Behringer, 2003). Although, various experiments have been made in laboratories during last few years to induce tetraploidy *in vitro* but there is still a necessity of more investigations to induce tetraploidy i*n vitro*.

CONFLICT OF INTERESTS

There is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have equal contribution in providing necessary resources.

REFERENCES

- Berg H (1982). Biological implications of electric field effects. Part V. Fusion of blastomeres and blastocysts of mouse embryos. Bioelectrochem. Bioenerg. 9: 223-228. http://dx.doi.org/10.1016/0302-4598(82)80178-5
- Cheong HT, Taniguchi T, Hishinuma M, Takahashi Y, Kanagawa H (1991). Effects of various electric fields on the fusion and in vitro development of mouse two-cell embryos. Theriogenol. 36 (5): 875–885. http://dx.doi.org/10.1016/0093-691X(91)90353-F
- Chernomerdik LV, Sowers AE (1991). Physical and ultrastructural evidence that integrin of the spectrin network controls the macroscopic fusion produce morphology following the electrofusion of erythrocyte ghosts. Biophysical J. 60: 1026-1037. http://dx.doi.org/10.1016/S0006-3495(91)82140-3
- Curnow EC, Gunn IM, Trounson AO (2000). Electrofusion of two-cell bovine embryos for the production of tetraploid blastocysts *in vitro*. Mol. Reprod. Dev. 56: 372–379. http://dx.doi.org/10.1002/1098-2795(200007)56:3<372::AID-MRD7>3.3.CO;2-N
- Darabi MR, Nasr-Esfahani MH, Baharvand H, Mardani M, Karimi-Jashni H (2008). Fusion and development of 2-cell bovine embryos to tetraploid blastocyst with different voltages and durations. Iranian J. Reprod. Med. 6(4): 181-186.
- Eakin GS, Behringer RR (2003). Tetraploid development in the mouse. Dev. Dyn. 228:751-766. http://dx.doi.org/10.1002/dvdy.10363
- Graham CF (1971). Virus assisted fusion of embryonic cells. Acta Endocrinol. Suppl. (Copenhagen). 153: 154-167. http://dx.doi.org/10.1530/acta.0.068s154
- He W, Kong Q, Shi Y, Xie B, Jiao M, Huang T, Guo S, Hu K, Liu Z (2013). Generation and Developmental Characteristics of Porcine Tetraploid Embryos and Tetraploid/diploid Chimeric Embryos. Genomics Proteomics Bioinformatics. 11: 327–333. http://dx.doi.org/10.1016/j.gpb.2013.09.007

- Henery CC, Kaufman MH (1991). Cleavage rates of diploid and tetraploid mouse embryos during the preimplantation period. J. Exp. Zool. 259:371-378. http://dx.doi.org/10.1002/jez.1402590312
- Iwasaki Sl, Campbell KHS, Galli C, Akiyama K, Iwasaki S (2000). Production of live calves derived from embryonic stem-like cells aggregated with tetraploid embryos. Biol. Reprod. 62: 470-475. http://dx.doi.org/10.1095/biolreprod62.2.470
- James RM, Kaufman MH, Webb S, West JD (1992). Electrofusion of mouse embryos results in uniform tetraploidy and not tetraploid/diploid mosaicism. Genetic Res. 60:185-194. http://dx.doi.org/10.1017/S0016672300030937
- James RM, Klerkx A, Keighren M, Flockhart JH, West JD (1995). Restricted distribution of tetraploid cells in mouse tetraploid-diploid chimaeras. Develop. Biol. 167: 213-226. http://dx.doi.org/10.1006/dbio.1995.1018
- Kaufman MH (1992). Postcranial morphological features of homozygous tetraploid mouse embryos. J. Anat. 180: 521–534.
- Kaufman MH, Webb S (1990). Postimplantation development of tetraploid mouse embryos produced by electrofusion. Develop. 110:1121–32.
- Koizumi N, FukutaV (1995). Preimplantation development of tetraploid mouse embryo produced by cytochalasin B. Exp. Anim. 44:105-109. http://dx.doi.org/10.1538/expanim.44.105
- Krivokharchenko A, Galat V, Ganten D, Bader M (2002). In vitro formation of tetraploid rat blastocysts after fusion of two-cell embryos. Mol. Reprod. Dev. 61: 460- 465. http://dx.doi.org/10.1002/mrd.90001
- Kubiak JZ, Tarkowski AK (1985). Electrofusion of mouse blastomeres. Exp. Cell. Res. 23: 557-561. http://dx.doi.org/10.1016/0014-4827(85)90143-0
- Lan Li, Wei SHEN, Lingjiang MIN, Yujian SUN, Jixian DENG, Quingjie PAN (2008). Nuclear transfer of goat somatic cells transgenic for human lactoferrin gene. Front. Biol. China. 3: 269-274. http://dx.doi.org/10.1007/s11515-008-0052-8
- McLaughlin MJ (1993). Production of tetraploid embryos by electrofusion. Methods Enzymol. 225:919-930. http://dx.doi.org/10.1016/0076-6879(93)25058-A
- Modlinski JA (1981). The fate of inner cell mass and trophectoderm nuclei transplanted to fertilized mouse eggs. Nature. 292: 342-343. http://dx.doi.org/10.1038/292342a0
- Nagy A, Gocza E, Diaz EM, Prideaux VR, Ivanyi E, Markkula M, Rossant J (1990). Embryonic stem cells alone are able to support fetal development in the mouse. Develop. 110: 815-821.
- Ozil JP, Modlinski JA (1986). Effects of electric field on fusion rate and survival of 2- cell rabbit embryos. J. Embryol. Exp. Morph. 96:211-228.
- Park MR, Lee AR, Bui HT, Park C, Park KK, Cho SG (2011). Chromosome remodeling and differentiation of tetraploid embryos during preimplantation development. Dev. Dyn. 240:1660–1661. http://dx.doi.org/10.1002/dvdy.22653
- Prather RS, Hoffman KE, Schoenbeck RA, Stumpf TT, Li J (1996). Characterization of DNA synthesis during the two-cell stage and the production of tetraploid chimeric pig embryos. Mol. Reprod. Dev. 45:38–42. http://dx.doi.org/10.1002/(SICI)1098-2795(199609)45:1<38::AID-MRD5>3.3.CO;2-E
- Prochazka R, Vodicka P, Zudova D, Rybar R, Motlik J (2004). Development of in vivo derived diploid and tetraploid pig embryos in a modified medium NCSU 37. Theriogenol. 62:155-164. http://dx.doi.org/10.1016/j.theriogenology.2003.08.017
- Richter HP, Scheurich P, Zimmermann U (1981) Electric field-induced fusion of sea urchin eggs. Dev. Growth Diff. 23:479-486. http://dx.doi.org/10.1111/j.1440-169X.1981.00479.x
- Schramm RD, Paprocki AM (2004). *In vitro* development and cell allocation following aggregation of split embryos with tetraploid or developmentally asynchronous blastomeres in rhesus monkeys. Cloning Stem Cells. 6: 302-314.
- Sekirina GG, Bogoliubova NA, Antonova NV, Dyban AP (1997). The behavior of mitochondria and cell integration during somatic hybridisation of sister blastomeres of the 2-cell mouse embryo. Zygote. 5:97-103. http://dx.doi.org/10.1017/S0967199400003762
- Senda, M, Takeda J, Abe S, Nakamura T (1979). Induction of cell fusion of plant protoplasts by electric stimulation. Plant Cell Physiol. 20:1441–1443.
- Snow MHL (1973). Tetraploid mouse embryos produced by cytochalasin B during cleavage. Nature. 244: 513-515. http://dx.doi.org/10.1038/244513a0
- Stekelenburg-Hamers V, AE, Van Inzen WG, Van Achterberg TA, Kruip TA, de Laat SW, Weima SM (1993). Nuclear transfer and electrofusion in bovine *in vitro*-matured/*in vitro*-fertilized embryos: effect of media and electrical fusion parameters. Mol. Reprod. Dev. 36(3): 307-12. http://dx.doi.org/10.1002/mrd.1080360304
- Suzuki H, Ogasavara I, Takahashi H, Imada Y, Toyokava K (2001). Electrofusion of blastomeres of hamster 2-cell embryos and dynamic changes of the cytoskeletal distribution. J. Reprod. Develop. 47: 227-235. http://dx.doi.org/10.1262/jrd.47.227
- Tarkowski AK, Ozdzenski W, Czolowska R (2001). Mouse singletons and twins developed from isolated diploid blastomeres supported with tetraploid blastomeres. Int. J. Develop. Biol. 45: 591–596.
- Tarkowski AK, Ozdzenski W, Czolowska R (2005). Identical triplets and twins developed from isolated blastomeres of 8- and 16-cell mouse embryos supported with tetraploid blastomeres. Int. J. Develop. Biol. 49: 825–32. http://dx.doi.org/10.1387/ijdb.052018at
- Tatham BG, Pushett DA, Giliam KJ, Dowsing AT, Mahavorasilpa TL, Trounson AO (1995). Electrofusion of *in vitro* produced bovine embryonic cells for the production of isofusion contours for cells used in nuclear transfer. J. Reprod. Fetil. Suppl. 49:549-553.
- Xiangyun L, Wei W, Yong J, Jia Q, Yu Y, Di K (2005). The genetic heterozygozygosity and fitness of tetraploid embryos and embryonic stem cells are crucial parameters influencing survival of mice derived from embryonic stem cells by tetraploid embryo aggregation. Reprod. 130: 53-59.
- Zhelev DV, Dimitrov D, Doinov SP (1998). Correlation between physical parameters in electro fusion and electroporation of protoplasts. Bioelectrochemistry Bioenergeti. 20: 155-167. http://dx.doi.org/10.1016/S0302-4598(98)80013-5