measurements of the intracellular potassium concentration in blastomeres determined by the electron-probe analysis. Since the concentration of potassium in KSOM is considerably lower than the measured levels found in mouse oviductal fluid (Borland et al., 1977), it is misleading to refer to KSOM as potassium-enriched SOM.

Fortuitously, KSOM was also found to be beneficial for the development of the mouse blastocyst (Erbach et al., 1994) and has been modified several times which we have called the KSOM family (see Biggers, 2002 for review). One of these was produced by increasing the concentrations of glucose to 5.56 mmol/l and bovine serum albumin (BSA) to 4 mg/ml in order to support in vitro fertilization in the mouse (Summers et al., 1995). This medium was denoted mKSOM. A second modification is KSOMAA, which is KSOM supplemented with 19 natural amino acids (Ho et al., 1995; Biggers et al., 2000). A third modification, mKSOMAA, which contains high glucose, albumin and the 20 natural amino acids has also been used to support in vitro fertilization (Summers et al., 2000, 2005).

We wish to stress that the abbreviations used by Fraser et al. (2007) are not the same as used by us. They have used mKSOM to mean our KSOM, and KSOM to mean KSOMAA. This type of confusion unfortunately arises frequently in the scientific and clinical literature due to the failure of authors to reference exactly the media they use. Since the constituents of media can have significant, sometimes unpredictable, effects, we urge authors and editors to ensure that the media used be uniquely identified and referenced.

References

Bringing together clinical embryology and basic reproduction around human cloning

Sirs,

A recent publication on human cloning reports the development of human blastocysts after somatic cell nuclear transfer using adult fibroblasts (French et al., 2008). French et al. (2008) work gives a big and good support to the human cloning area; first, this research was performed with ‘traditional’ procedures, those used in the creation of Dolly (the most famous clone); second, this comes at a difficult time for this area: where the fraudulent research in human cloning is still fresh in the public’s and academic’s minds (Kennedy, 2006) and also it has been created a new world in stem cell research, the induced pluripotent stem cells in humans, which are against nuclear transfer research for stem cells derivation (Park et al., 2008; Takahashi et al., 2007; Yu et al., 2006); third and finally, USA presidential elections and the possible changes in US government policies to support human stem cells and cloning research are around the corner. Also a big step was made with the derivation of embryonic stem cell lines from rhesus monkey-cloned embryos (Byrne et al., 2007), where now it has been independently confirmed (Cram et al., 2007); a step towards the goal of stem cell derivation from human cloned embryos. In this case, the success was attributed to a new tool: the polarization microscopy for enucleation and this represents a real improvement in more than 10 years of mammalian nuclear transfer technology.

The paper guarantees controversy in all fronts and has already begun to be commented upon (Cervera and Stojkovic, 2008). However, it inspires me to think in an unexplored part of the human cloning puzzle: how has the human cloning done? Who are the best people to continue developing this area? In the majority of attempts, human cloning comes from basic scientists in human reproduction (sometimes people with non traditional academic qualifications in human medicine, e.g. veterinarians) involved in exceptional experiments. But who are the people those work every day with human embryos, who produce and manipulates them with confidence and also care for their development and knows more that anyone about the secrets of preimplantation life of human embryos? They are the clinical embryologists, people working in fertility.
clinics, who perform all laboratory procedures related with human assisted reproduction procedures (ARTs).

French et al’s (2008) paper comes from an ART clinic and private company working together; one of the authors is a renowned clinical reproductive endocrinologist and CEO in Stemagen (a stem cells company), and the other is a senior embryologist of Reproductive Science Center in La Jolla, California (again a triumph for California world’s leadership in stem cells development). It is a necessity, holding together these two fields (clinical and basic reproduction) and there are many reasons, one of them is fundamental: it may exist around the world more ART clinics than reproduction research laboratories and even animal cloning laboratories, for example, the Center for Disease Control reports more than 400 clinics in USA, ESHRE more than 780 clinics in EU and there also exist clinics in developing countries where animal cloning is too far to be a reality.

The problem of developing human cloning and converting this into a clinical reality can be covered in one necessity. More people are needed to work is this area; as many as possible. With the accomplishment of this goal, the answers and procedures in human cloning will soon follow. Like in French et al’s (2008) work, the success in cloning is not simply due to polarization microscopes, it is the people involved in it. I am sure that the best way to do it is attracting embryologists to human cloning research and fusing the best of both worlds.

References


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Co-existence of a diploid hydatidiform mole and a normal fetus: mysterious events at the pronuclear stage

Sir,

Using polymorphic DNA markers, Niemann et al. (2008) have investigated the origin of twin pregnancies that comprised an androgenetic diploid mole and a normal fetus. In particular, they found a homozygous mole in which the paternal alleles were identical to those of the normal fetus in 13 informative loci, indicating the participation of one spermatozoon and one oocyte. In Figure 1:1 of their publication, the authors present an interesting so-called ‘one-oocyte-model’ that depicts the possible events at fertilization. Although this figure unfortunately lacks an explanatory legend, it can be deduced from the text that the suggested model involves a duplication of the paternal chromosomes before pronuclear fusion and development of a triploid pronuclear stage with one haploid maternal pronucleus and two haploid paternal pronuclei.

At first sight, this concept bears analogy to a recent review on the origin of triploid zygotes in vitro, in which I described endoreduplication in the maternal pronucleus as a possible cause of digynic triploidy (Rosenbusch, 2008). From our cytogenetic analyses of abnormally fertilized oocytes, there was only evidence for a maternal contribution but I concluded that, in accordance with assumptions of Petignat et al. (2003), endoreduplication in the paternal pronucleus is likewise conceivable. Independent of whether the maternal or paternal genome will be affected, the resulting pronuclear stage would be characterized by only two pronuclei (one maternal and one paternal, respectively) and then develop into a triploid 1-cell zygote.

On closer examination, the two models are not at all consistent concerning the predicted number of pronuclei. To explain the discrepancy in detail, it is pertinent to recapitulate some of the early steps of fertilization. Briefly, the spermatozoon carries the paternal genome in the form of a tightly packed chromatin what has been achieved with the aid of histone replacement by protamines. In the ooplasm, the sperm chromatin is decondensed, i.e. the protamines have to be replaced again by histones. The decondensing sperm DNA is enclosed within the membrane of the male pronucleus. Here, DNA replication will occur. To the best of my knowledge, this is the normal process and therefore I wonder whether Niemann et al. (2008) have found evidence in the literature for sperm chromatin decondensation followed by an immediate DNA replication without requiring a pronuclear membrane. In addition, the time frame for such a phenomenon appears rather narrow because, for instance, time-lapse video cinematography revealed that 5 h after ICSI, 51% of the normally fertilized oocytes had already formed two pronuclei. Thirteen hours after ICSI, formation of two adjacent pronuclei was complete in all oocytes (Payne et al., 1997).

One may now rightly ask for an alternative explanation of the above-mentioned findings if we follow the postulate that one sperm should yield one paternal pronucleus. Let us further assume that replication and endoreduplication of the sperm DNA occur within the male pronucleus whereas the