RESEARCH PROJECT by HANEN KLITI

Notice of public competition for Stefania Spanò Career Springboard Scholarship.

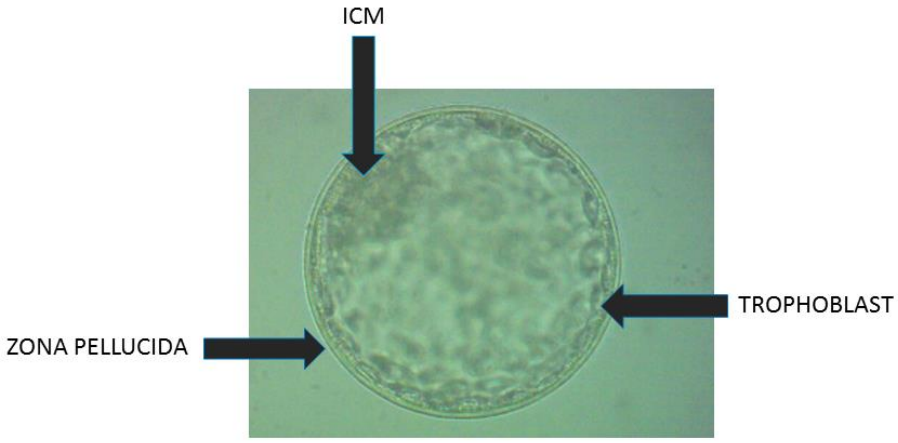
IN-VITRO DERIVATION OF CELL LINES FROM EMBRYOSBIPARENTALS, PARTENOGENOTES AND ANDROGENOTES, WITH DIFFERENTCHROMOSOMAL COMPOSITION (HAPLOIDS, DIPLOIDS ANDTETRAPLOIDS)

Introduction:

In-vitro blastocysts derivation is a process validated in a large number of species (Mondal et al.,2019). The experimental model that I studied during my internship for my experimental thesis is the Sheep (*Ovis Aries).* In this case we start from the oocytes recovery directly from the ovaries. Then we proceed with *in-vitro* maturation and *in-vitro* fertilization of the oocytes; after that the fertilized oocytes are *in-vitro* cultured until the blastocyst state. At the blastocyst state normally there is the implantation of that in the womb; at this stage we can distinguish two different kind of cell-lines:

1. Trophoblast's cells, that will form placenta and embryonal annexes;
2. Inner cell mass (ICM), that will form the embryo;

In-vitro embryo production is used both from applied research (for example income species) and from basic research. In particular I would like to concentrate my research project on the basic research.



Professor Loi's Laboratories (the which ones I attended) work from a long time on experimental embriology. One of the researching line is the production and characterization (both cellular and molecular) monoparental embryos (Zacchini et al.,Loi et al.). Monoparental embryos are constituted by the chromosomal kit only from the mother (*parthenogenetic)* or only from the father (*androgenetic).* Some species in nature are able to reproduce throught the parthenogenetic pathway, while the androgenetic embryos are produced exclusively in laboratory.

Monoparental embryos allowed to **discover the Genome Imprinting**; so thanks to the Imprinting in some genes only one allele in expressed: the maternal one or the paternal one (Barton et al). This means

that the maternal and paternal genes are complementary both in the embryonal development and in post-natal one.

In a recent work by Loi's laboratories shown that the parental genes have an important role also in some cellular fuctions regulation, in particular in autophagy's regulation (Ptak et al).

Objectives:

The objective is the embryonal cell-lines derivation from normal IVF embryos, and from androgenetic and parthenogenetic embryos in which cromosomal composition is modified, both haploid and tetraploid. In particular cultural condition will be standardize in order to obtain monoparental stable cellular line. Cellular lines from IVF embryos, both haploid and tetraploid, will be used as a control groups. Cellular lines will be characterized both genetically (kariotype) and functionally (autofagy, mitochondrial activity and proteomic).

Materials and Methods:

1. Biparental diploid embryos: for having biparental diploid embryos we have to *in-vitro* fertilize in-vitro maturated oocytes; then we have to colture fertilized oocytes untill the blastocyst stage following the standard protocol.
2. Biparental tetraploid embryos: also in this case we ave to fertilize in-vitro mature oocytes and at the 2-cells stage we have to operate an electro-mediated fusion of the 2 blastomers obteining a single tetraploid cell. In this way when the cellular division re-starts every new cell will be tetraploid.
3. Partenogenetic haploid embryos*: in-vitro* maturated oocytes are activated without the second polar globule (PG) extrusion block. In this way with the PG extrusion and without fertilization we obtain an haploid oocytes that will start segmentation reaching the blastocyst stage in which every cell is haploid.
4. Partenogenetic diploid embryos: in this case when we activate the oocyte we have to block the PG extrusion using the 6-DMAP. In this way the oocyte mantein its diploid chromosomal composition and will start segmentation reaching the blstocyst stage in which every cell is diploid.
5. Partenogenetic tetraploid embryos: as I previously described for the biparental tetraploid embryos, also in this case we have to proceed with the electro-fusion of the blastomers at the 2-cells stage. So we have to activate the mature oocyte, we have to block the PG extrusion and after 24 hours from that we have to fuse the 2 diploid blastomers.
6. Androgenetic haploid embryos: we have to enucleate the in-vitro matured oocytes (matured oocytes are at the MII stage) and then we have to fertilize them throught the intaciplasmatic sperm injection (ICSI). In this case we inject only one sperm into the enucleated oocyte.
7. Androgenetic diploid embryos: the mechanism is the same that I described in the point 6 but in this case for having the diploid chromosomal composition we have to inject 2 sperms inside the enucleated oocyte.
8. Androgenetic tetraploid embryos: as for all the tetraploid embryos described also in this case we have to fuse the diploid blastomeres at the 2-cells stage.

All the embryos obteined will be put in colture using the coltural conditions that allow to obtein the outgrowth. The outgrowth is the first stage necessary for having cell-lines. The cell-lines obteined will be analyzed for the kariotype evaluation, mitochondrial activity evaluation, autophagy and all the cellular activities that will be evident in all the different cellular types.

Results:

During my internship I already started to put in colture monoparental embryos with different chromosomal composition, so there are preliminary datas. Having these kind of cellular line could be a new model for studying cellular functions under one of the parents controll. The different chromosomal composition could generate different cellular phenotypes that indicate the parental effect on a specific function. We already know that cellular-lines from androgenetic or partenogenetic have a totally different behaviour, in particular for senescence, proliferation and strenght of the cell-cycle (Hernandez et al.,2003). Thanks to the improvement of the Epigenetic studies we clearly know that the maternal and paternal contribution are not equivalent. Improving or reducing the number of chromosomes should show better the maternal or paternal different contribution also in cellular function still unknown.

References:

1. Hernandez L, Kozlov S, Piras G, Stewart CL. Paternal and maternal genomes confer opposite effects on proliferation, cell-cycle length, senescence, and tumor formation. Proc Natl Acad Sci U S A. 2003 Nov 11;100(23):13344-9. Epub 2003 Oct 27.
2. Ptak GE, Toschi P, Fidanza A, Czernik M, Zacchini F, Modlinski JA, Loi P. Autophagy and apoptosis: parent-of-origin genome-dependent mechanisms of cellular self-destruction. Open Biol. 2014 Jun;4(6):140027
3. He W, Chen J, Gao S. Mammalian haploid stem cells: establishment, engineering and applications. Cell Mol Life Sci. 2019 Jun;76(12):2349-2367. Epub 2019 Mar 19
4. Elling U, Woods M, Forment JV, Fu B, Yang F, Ng BL, Vicente JR, Adams DJ, Doe B, Jackson SP, Penninger JM, Balmus G. Derivation and maintenance of mouse haploid embryonic stem cells. Nat Protoc. 2019 Jul;14(7):1991-2014. Epub 2019 Jun 3.
5. Cui T, Li Z1, Zhou Q, Li W4. Current advances in haploid stem cells. Protein Cell. 2019 Apr 19. [Epub ahead of print]
6. Mondal S, Mor A, Reddy IJ, Nandi S, Gupta PSP, Mishra A. In Vitro Embryo Production in Sheep. Methods Mol Biol. 2019;2006:131-140.
7. Zacchini F, Czernik M, Iuso D, Toschi P, di Egidio F, Scapolo PA, Loi P, Ptak G. . Efficient production and cellular characterization of sheep androgenetic embryos. Cell Reprogram. 2011 Dec;13(6):495-502
8. Loi P, Ledda S, Fulka J Jr, Cappai P, Moor RM. Development of parthenogenetic and cloned ovine embryos: effect of activation protocols. Biol Reprod. 1998 May;58(5):1177-87 9. Barton SC, Surani MA, Norris ML. Role of paternal and maternal genomes in mouse development. Nature. 1984 Sep 27-Oct 3;311(5984):374-6