reagent and protector in the process of tissue cloning, human differentiated cells may grow as totipotent cells with human gene and cell characteristics. The successful possibility of tissue cloning may increase with the experiments and the modified techniques, and may decrease with the differentiated cells from elder people.

Supported by: None

P-1013

HUMAN PARTHENOGENETIC BLASTOCYSTS DERIVED FROM NON FERTILIZED CRYOPRESERVED HUMAN OOCYTES. E. S. Polak de Fried, P. Ross, G. Zang, F. B. Denaday, G. Arenas, J. B. Cibelli. CER Medical Institute, Buenos Aires, Argentina; Michigan State Univ., East Lansing, MI.

OBJECTIVE: Parthenogenesis is the process through which an oocyte can develop into an embryo in the absence of sperm. The objective of this presentation is to report for the first time the production of human parthenogenetic blastocysts and in vitro attachment, generated from chemical activation of non inseminated cryopreserved human oocytes.

DESIGN: Prospective study.

MATERIALS AND METHODS: Thirty eight non inseminated fresh human metaphase II (MII) oocytes were cryopreserved using the 1,2-propanediol slow freeze - rapid thaw method with 0.3M sucrose and stored in liquid nitrogen at -196°C. The oocytes were provided from five healthy donors (mean age ± SD was 32.2 ± 3.4) who underwent controlled ovarian stimulation and guided ultrasound oocyte recovery. All donors participating in the present study signed an informed consent form previously approved by the Institutional Review Board on Human Subjects Research and Ethics Committee which had previously accepted the research. After thawing, non inseminated oocytes were activated with 10uM of Ionomicyn and 2mM of 6-Dimethylamino Purine to induce pronucleus (PN) formation. Activated oocytes (parthenos) were cultured at 37°C, 6% CO2 in air to achieve blastocyst formation. Parthenos blastocysts were plated on top of mitotically inactive human umbilical cord fibroblasts. All parthenos were checked every day to follow their growth and attachment ,and to test the medium renewal as well as having a photographic register.

RESULTS: Thirty six out of 38 cryopreserved non inseminated oocytes survived after thawing (survival rate: 94.7%). Thirty one out of 36 oocytes showed only one PN (activation rate: 86.1%). Thirty out of 31 cleaved (cleavage rate: 96.8%). Three parthenos on day 6 and 2 parthenos on day 7 after activation, showed cavitation. Human partheno blastocyst were plated. After plating one partheno blastocyst exhibited incipient attachment and the other one showed complete attachment. No further development was observed. Fifteen non evolutive cleaved parthenos were plated on day 9 after activation. Six out of 15 showed attachment. Two days after, three parthenos continue attached until the day of the present communication (56 days).

CONCLUSION: The use of parthenogenetic human embryos for therapeutically indications (p. e. source of stem cells) could give the countries with ban restrictions towards the use of human gametes and human embryos the possibility to work in this field. On the other hand, cryopreserved non inseminated human oocytes could be a permanent and safe source for parthenos production avoiding, in this way, the long process of donor recruitment, selection and treatment, as well as the possibility to use the oocytes after rechecking the patient of any viral infectious disease. It also offers the possibility to store oocytes highly immunological compatible for own future uses. According to the present study, the high survival rate of non inseminated cryopreserved oocytes allows a high rate of parthenogenetic formation, and also blastocyst development and attachment. This approach provides a new challenge to obtain non adult human stem cells without generating controversial issues.

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P-1014

OBTENTION OF THE FIRST LATIN AMERICAN HUMAN EMBRYONIC STEM CELL LINE. C. Lucena, K. Andersson, C. Esteban, J. Hyllner, E. Lucena. Cecolfes, Bogota, Colombia; Cellartis, Goteborg, Sweden.

OBJECTIVE: To demonstrate that stem cell research is possible in Latin America. Here we present Cecol-14 , the first latin american human embryonic stem cell line.

DESIGN: The Colombian IVF center, Cecolfes has together with Cellartis AB established the first human embryonic stem (hES) cell line in Latin America. This unique possibility of establishing hES cell lines at Cecolfes IVF center will increase the accessibility to genetically diverse hES cell lines, a necessary objective in order to develop hES cell based therapies and research tools for a wider market. Research conducted on hES lines is also an excellent tool for the IVF technology development.

MATERIALS AND METHODS: The hES cell line has been derived from IVF surplus blastocysts with informed donor consent. Donated embryos were cultured to the age of 5-7 days at the Cecolfes laboratory. Immunosurgery was performed as described in the mouse and humans. Blastocysts that had not hatched and with intact zona pellucidae were treated in Pronase (Sigma-Aldrich:1mg/ml). All treated blastocysts were placed on a layer of mytomicin inactivated mouse embryos fibroblasts (MEFs) with hESC medium for six days. The expanded colonies were mechanically dispersed into small clumps, and cultured on fresh MEF layers. hESCs colonies were passaged by mechanical dissociation and removed to fresh feeder layers.

RESULTS: A total of 43 embryos were cultured. Of these, 30 reached the blastocyst stage at 5-6 days (69.7%). Most of them were graded 3AA, according to Gardner′s classification. One cell line was obtained, cecol-14. Immunocharacterization was performed on undifferentiated colonies with commonly used markers indicative of pluripotent hESC, Oct-4, SSEA1, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81. A normal chromosomal set up for chromosomes X, Y, 13,18, and 21 was shown with FISH analysed with Vysis multivysion. Pluripotency tests in vitro and in vivo are in progress. Results on embryoid bodies will be shown.

CONCLUSION: Taken together the results indicate that Cecol-14 is a normal genetically cell line. A bank of Latin American cell lines is in project at Cecolfes. This will allow basic research on a genetically different cell line of those already existent.

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P-1015

OOCYTE DONORS' PERSPECTIVES REGARDING EMBRYO DISPOSITION OPTIONS: GRANDFATHERING EMBRYOS FOR STEM CELL RESEARCH? J. E. Zweifel, M. Christianson, A. S. Jaeger, N. Fost, D. Olive, S. R. Lindheim. Univ. of Wisconsin-Madison, Madison, WI; Bio Law Group, Santa Fe, NM.

OBJECTIVE: For those who have completed IVF treatment, disposition of excess cryopreserved embryos can create a decision-making dilemma whether to donate to other couples, discard, or consent to stem cell research. Since 2003, cryopreserved embryos donated for stem-cell research originated from donor ooctyes and donor sperm in 17% and 26%, respectively. Well-meaning professionals clamor for consent from the third party gamete donors, however, given the average length of time of embryo cryopreservation (4.8 year, range 1-13) follow-up contact may be unrealistic, potentially rendering embryos created with third-party gametes unusable. Unfortunately, clinics have not been sufficiently attentive to details in donor agreements to ensure the relinquishment of donor rights including consent to donate to research. We report our program's assessment of oocyte donor's perspectives regarding embryo(s) disposition options and how it may apply to issues of consent regarding stem cell research.

DESIGN: Questionnaire based evaluation in university based practice.

MATERIALS AND METHODS: During the Program's initial screening process (n=112) and exit interview (n=15), oocyte donors were queried regarding disposition options of their donated ooctyes and for excess cryopreserved embryos following completion of the recipient's treatment including: donation to other couples (Embryo Donation); discarding embryos; donating to general research; and donating to stem-cell research. Results were tabulated as "yes" or "no". Donors were further asked if they had any reservations regarding participation; had reservations but would participate; or would not participate in the program knowing the options available to recipients. Comparison between groups and initial screening and exit interview was performed using chi-square for categorical comparisons.