fertilisation procedure which was IVF. Exclusion criteria were : endometriosis, polycystic ovary syndrome (PCOS), hydrosalpinx, myoma intramural or submucosal, thrombophylia, severe masculin factor necesitating ICSI. The study was approved by our Institutional Review Board. All hysteroscopies were performed in the follicular phase preceding second IVF cycle with a flexible 4 mm hysteroscope (Karl Storz, Germany) and saline was used for distention. Patients underwent stimulation using gonadotropin releasing hormone (GnRH)- long agonist protocol from the mid luteal phase with decapeptyl 0,1mg s.c. daily and started on day 2 of stimulation cycle using 150 UI urinary gonadotropins. This dose was fixed for the first five days of treatment, then it was adequately change. Urinary hCG (10 000 UI) was administered for final oocyte maturation as soon as at least three follicles of 17 mm were observed by ultrasound scan (USS). Luteal phase support with progesterone 600 mg/day vaginally was started on the day of oocytes pick-up and continued for 12 weeks or up to a negative pregnancy test. Clinical pregnancy rate as assessed by USS was the primary end-point. Other parameters evaluated included dose of FSH, duration of stimulation, number and size of follicles, the incidence of miscarriage. Data were analysed by statistical package for social science SPSS 16 using the student's t-test and the chi-square test as appropriate. Results are expressed as mean ± SD, $P < 0.05$ was considered significant.

Results: At hysteroscopy, the subtle lesions in both groups were: 41.6% hypervascularisation and strawberry pattern, 23.3% elevation, 15% polyposis, 13.3% endometrial defects, 6.8% necrotic tissue. There were no differences ($P > 0.05$) between groups for mean age, body mass index, years of infertility, total days or doses of gonadotropins, number and size of mature follicles, or sperm parameters. Mean age (SD) of the patients was 32.5 (2.2) years and BMI was 24.2 (3.1) kg/m^2. The average duration of infertility was 3.5 (2.3) years. On day 2 of normal cycle, no differences were observed between the groups in baseline serum hormone levels. The mean number of oocytes (10.2 ± 3 in group A versus 10.1 ± 1 in group B) did not differ significantly between the two groups ($p > 0.05$). Also the quality of the embryos 45% in group A vs 42% in group B and the number of embryo transfer was comparable for both groups ($p > 0.05$). Clinical pregnancy rate was higher in group A vs. group B (36.6% vs 13.3%, $p < 0.05$). Biochemical pregnancy was 46.6% vs 23.3% ($p < 0.05$). The incidence of miscarriage was similar 10% in both groups.

Conclusions: In patients with IVF failure and subtle lesions found at hysteroscopy the treatment with antibiotics and sequential hormone therapy, improve outcome of IVF at second attemp.

POSTERS:

STEM CELLS

P-570 Spermatogonial stem cell line from a patient with Klinefelter's Syndrome

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Introduction: Spermatogonial stem cells (SSCs) are the unique population of cells that transmit genetic information to the next generation in the men. Recent studies have suggested that SSCs can be isolated from human testis, where germ line cells can be reprogrammed and turned to pluripotent cells capable of giving rise to many different cells types. The objective of this work is to derive a spermatogonial stem cell (SSC) line from an adult human testicular biopsy at the time of infertility diagnosis in a patient with Klinefelter's syndrome.

Materials and Methods: Testicular biopsy was obtained from an azoospermic patient, as a routine diagnosis method. After manipulation and microscopic analysis to obtain sperm for ICSI, the sample was mechanically dissected in PBS with 1% Pen/Strep and enzymatically dissected with 0.5g/ml Collagenase type 1A for 20min and TripLE Select for 15 min at 37ºC on a shaker, and then filtered with a 30um filter and later centrifugation at 1.000 rpm for 5 min at 4ºC. The pellet was then resuspended in ESC (embryonic stem cell) medium with 4ng/ml GDNF (glial cell line-derived neurotrophic factor) for 5 days on 37ºC. After purification by FACS, CD49f + cells was cultured in 0.1% gelatine with LIF.

Results: Histology diagnosis revealed a Sertoly Only syndrome. Hormonal levels were FSH 30,8; LH 7,17 and Testosterone 5,7. Characterization of the Klinefelter SSC cell line revealed that cells were positive for the immunostaining with CD49f, CD90, SSEA-4, TRA 1-81, TRA 1-60, and negative for CD15, CD34, CD133, CD44, CD117. Molecular characterization revealed the presence of OCT4, NANOG and SOX2 at the mRNA level. Quantitative telomerase activity expression analysis was comparable to that shown in hESC. Pluripotency was also assessed by embryoid bodies formation and differentiation to the different three germ layers demonstrated by smooth muscle actin, α-fetoprotein, and β-tubulin III immunostaining. Karyotyping showed a tetraploid pattern, as confirmed by Fish analysis which demonstrated the presence of 1818XY in 60.9% of the cells and 1818XXY in 32.4%. The cell cycle studies demonstrate that 95% of cells were in the G1 phase. After injection of 1.10^5 cells in passage 3 and 7, teratomas were not observed in SCID mice.

Conclusion: We have derived the first Klinenfelter SSC line from a testicular biopsy from an azoospermic patient with non mosaic Klinenfelter syndrome in peripheral blood, but with a mosaicism in the testicular tissue. Immunophenotyping and molecular analysis, as well as in vitro pluripotency assays, show embryonic stem cells -like pattern, only lacking in vivo pluripotency properties, probably due to the low number of cells injected.

P-571 Spermatogonial stem cell lines from human testicular biopsies

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Introduction: In mammalian testes, spermatogonial stem cells (SSCs) consists of a small number of single cells located on the basal compartment of the seminiferous tubules surrounded by Sertoli cells. SSCs have been isolated successfully from neonatal and adult mice as well as from human testis but in this case the complete gonad has been dissected.

The objective of this work is derive human SSCs from the testicular biopsies routinely use in the infertility clinic as a therapeutic/diagnostic tool in azoospermic patients for sperm isolation and cryopreservation to be used for ICSI.

Materials and Methods: Testicular biopsies (n = 30) were obtained from azoospermic patients as part of their diagnosis and work-up after obtaining a signed informed consent. A portion of the samples were mechanically dissected in PBS with 1% Pen/Strep. For enzymatic digestion samples were incubated with 0.5g/ml Collagenase type 1A for 20min and TripLE Select for 15 min at 37ºC on a shaker and then filtered with 30um and centrifuged at 1.000 rpm. The pellets were seeded in embryonic stem cell media (ESC) with glial cell line-derived neurotrophic factor (GDNF 4ng/mL) for 5-7 days on 37ºC. To identify and isolate SSCs they were incubated with CD49f antibody and FACS sorted. Positive cells were cultured in gelatine plates containing ESC media with leukaemia inhibitory factor (LIF1000U/mL). During passages, SSC lines were characterized by PCR, RT-PCR, Immunohistochemistry, Karyotype and telomerase activity. In vitro differentiation was performed using embryoid bodies (EB) formation and in vivo differentiation by injecting cells into testis of SCID mouse.

Results: We have generated 8 adult human SSC lines. They were positive for undifferentiation markers OCT4, NANOG, SOX2 and negative for germ markers VASA and DAZL. The lines expressed specific SSC markers such as STRA-8 and PLZF. Immunohistochemistry also demonstrated that they were positive for SSEA-4, SSEA-1, TRA 1-81 and TRA 1-60 and have middle-high telomerase activity. Karyotype analysis was normal (46,XY). In vitro differentiation of these cells shows the spontaneous formation of EB´s that differentiated into cells from all three layers (ectoderm,endoderm,mesoderm). Teratoma formation is underway.

Conclusions: We have successfully derived 8 SSC cells lines from a fraction of adult human testicular biopsies. That resolves the problems and opens new possibilities in the future clinical therapies with germ cell differentiated for infertility treatment.

MR was founded by Santiago Grisolía Program from the Valencian Government, Spain

P-572 Effective cryopreservation of human embryonic stem cell-derived cardiac precursors using neurotrophin 4

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Introduction: As human embryonic stem cells (hESCs) have been regarded as a attractive material for cell therapy based on their capacity to differentiate into cardiomyocytes (CMs). Despite many studies reported differentiation into CMs from hESCs, protocols for the long-term storage of hESC-derived cardiac precursors (CPs) are still unknown. In this study, we tried to develop a protocol for cryopreservation of hESC-derived CPs.
Materials and Methods: We cryopreserved and thawed hESC-derived CPs at two different stages, before (day 12) and after (day 16) the appearance of contraction, using trophic factor, neurotrophin (NT) 4. Briefly, cells were pre-incubated for 1hr with NT4 and dissociated into small mass. Dissociated cells were plunged into LN2 until thawing. Thawed CPs were incubated with NT4 for 2 days and cultured for 5 more days. Their post-thaw viability was measured using BrdU assay.
Results: Post-thaw CPs at both stages recovered their morphology and were successfully cultured. The survival was higher if CPs were cryopreserved prior to the beginning of contraction. Thawed CPs expressed cardiac-specific transcription factor and proteins such as Nkx2.5, α-actinin and myosin heavy chain (MHC).
Conclusions: We demonstrated a possibility that cryopreservation of hESC-derived CPs using NT4 can be applied for long-term storage, if frozen prior to initiation of contraction (SC1150 and No. 2009-0071924).

P-573 Acquisition of a trisomy 20 in the human embryonic stem cell line HD90

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Introduction: The chromosomal stability of human embryonic stem cells (hESCs) is an essential prerequisite for both therapeutic and research applications. The occurrence of chromosomal aberrations in dividing cells is not an unusual phenomenon, and several studies of hESCs have shown karyotypic aberrations over extended culture. Here, we report the cytogenetic analysis of the hESC line HD90 carrying a germline Von Hippel Lindau (VHL) mutation, and the early acquisition of a trisomy 20 in HD90 culture.
Materials and Methods: The HD90 hESC line was derived from the inner cell mass of a 5 days-old-preimplantation-blastocyst stage embryo harbouring a germline VHL mutation. Colonies were continuously cultured on a feeder layer of human foreskin fibroblasts. Manual dissection rather than an enzymatic technique was used to passage the line. The cytogenetic analysis were performed using standard R-banding technique and FISH techniques combining locus probes and painting probes, specific for chromosome 20.
Results: The R-band karyotyping of HD90 cells was carried at passage 26. Fifty metaphase spreads were analysed and all indicated the presence of a trisomy 20. The defect was confirmed by dual colour FISH in 200 interphasic cells from passage 23. No trisomic cells were observed at the earlier passage 6, whereas the trisomy 20 was detected in 19% of the cells at passage 12. This indicates that this chromosomal abnormality was progressively acquired during long-term culture.
Conclusions: Several chromosomal abnormalities have already been reported in hESC lines, especially trisomy 12 and trisomy 17, which appear to give a proliferative growth advantage in hESC cultures. The occurrence of numerical chromosomal abnormalities constitutes a major problem in cultures of hESCs, since extra or missing chromosomes can compromise cell viability or lead to adaptative advantage in cultures. Cells with trisomy 20 could have a similar proliferative advantage since they increase at the expense of the normal cells until they dominate the HD90 cell line. In addition, the presence of the VHL mutation could promote chromosomal instability and aneuploidy, by impairing spindle checkpoint function and proper spindle orientation. Consequently, the analysis of this hESC line could be a valuable model to study the process of progressive culture adaptation of self-renewing cells, in combination with the occurrence of aneuploidy. These data also indicate that the monitoring of hESC lines by cytogenetics is an essential part of cell lines quality control. Cells with chromosomal imbalances in hESC lines are usually undistinguishable from normal cells by appearance, and also display typical markers of pluripotency. The early detection of these cells is then essential, since trisomy cells can replace the normal cell population in a few passages. Although molecular methods such as micro-array and single nucleotide polymorphism (SNP) analysis, offer high resolution in terms of detecting genetic changes, karyotyping and interphase FISH constitute more sensitive method for identifying small cell populations with clonal chromosomal abnormalities.

Supported by the "Fondation Pour La Recherche Médicale", the "Association Française contre les Myopathies", and the "Agence de la Biomédecine"

P-574 Human amniotic fluid derived mesenchymal stem cells are resistant to a panel of DNA damage agents

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Introduction: Mesenchymal stem cells(MSCs)are important cell population that are thought to have multiple functions in cell transplantation and gene therapy. Traditionally MSC can be obtained from various human tissues such as bone marrow, adipose tissue, muscle, brain and dermis of mammalian. They are able to self-renew *in vitro* and differentiate into osteocytes, chondrocytes, adipocytes, myocytes and even neural cells under appropriate conditions. However, these cells are often present in low numbers and can be difficult to maintain in culture, and they do not display the pluripotency of embryonic stem cells. Recently human amniotic fluid(AF) and placenta tissues have attracted increasing attention as a possible reserve for MSC that may be easily isolated and cultured *in vitro* and several reports have demonstrated that amniotic fluid derived MSC(AF-MSC) display similar phenotype and differentiation potential to those from other places and this may suggest that amniotic fluid and placenta may be another sources for MSC in terms of clinical application in regenerative medicine. As many studies have demonstrated that human bone marrow derived MSCs are resistant to apoptosis induction by DNA damage agents such as chemotherapeutic substances used in bone marrow transplantation, the response of MSC from amniotic fluid or placenta tissues to high dose DNA damage agents is largely unknown. Here we investigated the character of MSC from human trimester amniotic fluid and placenta tissues and *in vitro* response of them to chemotherapeutic substances.
Materials and Methods: MSC were isolated from human trimester amniotic fluid and placenta during diagnostic procedures(amniocentesis). We analyzed their morphology/phenotypes and differentiation potential using methods of flow cytometry and RT-PCR before and after DNA damage agents treatment; We assayed the acute direct chemosensitivity and recovery potential of AF-MSC to individual DNA-damage agents using XTT in comparison with a sensitive human tumor cell line. Also the DNA damage-induced apoptotic cell death were examined in the AF-MSCs by method of TUNEL. Finally we analyzed the expression of p53 before and after the treatment of DNA-damage agents by western blot.
Results: Our results showed that AF-MSC were resistant to DNA-damage agents cisplatin/vincristine and camptothecin compared to sensitive tumor cell line K562 especially at apoptosis induced dosage. The phenotype and osteogenic or adipogenic differentiation potential were not altered by DNA-damage agents treatment *in vitro.* There was a significant recovery in AF-MSCs after treatment of cisplatin/vincristine and camptothecin respectively. Finally we demonstrated that *in vitro* exposure of AF-MSCs to increasing dose of cisplatin/vincristine and camptothecin could cause an increased p53 expression but there is no apoptosis-related cell death simultaneously.
Conclusion: Our findings suggested that amniotic fluid derived MSCs are resistant to several DNA damage induced apoptosis response. The overexpression of apoptosis induced protein p53 in AF-MSC by DNA damage agents might not be the reason for their chemoresistance and thus the underlying mechanism on how AF-MSCs response to DNA damage agents needs further investigation.

P-575 BMP-4 induced differentiation of spermatogonial stem cells reveals changes in adhesion properties

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Introduction: Spermatogonial stem cells (SSCs) are at the basis of the spermatogenic process and allow the continuous production of spermatozoa throughout life. The mechanisms governing SSC self-renewal and differentiation are largely unknown. Previous studies have focused on the role of different growth factor in regulating the balance between self renewal and differentiation. However, all these studies have used mixed culture systems due to lack of markers to identify SSCs and therefore the lack of effective methods to obtain and culture a pure population of SSCs. As a consequence, it is currently unknown whether the growth factors tested act directly on SSCs or whether the observed effects are mediated through other cell types. One of the growth factors that plays a role in SSCs fate decision is bone morphogenetic protein 4 (BMP-4). In mixed testicular cell cultures, BMP-4 is able to induce c-KIT expression and reduce the number of colonies after transplantation to the testis of recipient mice. In this study, we aimed at investigating whether BMP-4 can directly induce SSC differentiation and if so, which pathways are involved.
Matherial & Methods: We used the rat SSC line GC-6spg which was previously established in our laboratory. GC-6spg cells were cultured in the presence or absence of 50ng/ml of BMP-4 for 2-4-8-12-24-48-168h. Western Blot and qPCR analysis were used to determine the expression of c-kit, which is an early marker of differentiating spermatogonia. A time point micro-array experiment was then performed, and data were analysed using Pathway-express software to identify possible pathways involved in SSCs differentiation.
Results: Rat SSCs cultured in the presence of BMP-4 for 2, 4, 8, 12, 24, 48 and 168h showed a time-dependent increase in c-Kit expression, on both mRNA and protein level Pathway analysis of the micro array data revealed that pathways involved in adhesion properties of the SSCs were highly affected. Interestingly, one of the genes affected was *cdh1*, which was upregulated more than 2,5 fold at mRNA level, also the relative protein content increased of about 50 to 60%.
Discussion: Overall, our results show that BMP-4 induces early differentiation of SCCs in a direct manner and suggest a role for CDH1 and adhesion pathways during early SSC differentiation.

P-576 Long-term cultivation of human embryonic stem cells (hESCs) - signs of an adaptation to in vitro culture conditions at 3 cell lines

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Introduction: Human embryonic stem cells have been derived from human blastocysts, they can proliferate indefinitely in culture when kept in an undifferentiated state. On the other hand, they can differentiate into most cell types of human body under certain conditions. Some recent studies indicate, that long-term cultivation of hESCs may potentially affect their phenotype which is considered to be a manifestation of an adaptation to culture conditions (at preserved genomic stability). In present study, early and late passages of 3 hESCs lines were investigated to compare their morphology at submicroscopic level and to assess possible alterations in biological properties of the cell culture after long-term cultivation.
Materials and Methods: The hESCs (lines CCTL10, CCTL12 and CCTL14) were cultured according to the standard protocol, mouse embryonic fibroblasts as feeder cells were used. Colonies of hESCs of early (30) and late (234 and 354) passages were processed by standard techniques for both transmission and scanning electron microscopy on day 3 and day 5 after replantation into culture. In addition, 5 in vitro developed human blastocysts were used to compare the ultrastructure of inner cell mass (ICM) with hESCs. The capacity of hESCs to differentiate into three germ layers was assessed by embryoid bodies formation. Genetic stability and markers of undifferentiated state (Oct4 and SSEA4) were tested continuously.
Results: Generally, submicroscopic characteristics of 3 hESCs lines were similar but not identical, especially cells of CCTL12 line tended to single cilia formation and more extensive cell-to-cell communication via gap junctions - contrary to other lines.

In all 3 lines, the growth of colonies was accelerated in late passages (hESCs of late passages reached on day 3 the same size of colonies as cells of early passage reached on day 5). High proportion of cells in colonies expressed undifferentiated state markers, however electron microscopy reveals relatively high rate of partially differentiated cells (on day 5 in early passages and on day 3 in late passages). Cells in central part of colonies exhibited ultrastructure typical for undiffentiated cells strongly resembling ICM of blastocysts (high nucleus/cytoplasm ratio, numerous polyribosomes, thin mitochondria, small Golgi apparatus and almost no granular endoplasmic reticulum). Partially differentiated cells of epitheloid type were found mostly at the periphery of colonies with well developed junctional complexes and tonofilaments. Long microvilli covered the surface of some cells.

The prominent feature of hESCs of all lines and passages was abundant glycogen accumulation with heavy deposits in cytosol or in membrane-limited vesicles. There is a significant difference in comparison with ICM cells, glycogen is accumulated in blastocysts only by trophectoderm cells.

Scanning electron microscope revealed numerous microvilli extending from surface of some cells, their lenght and number increased with time from replantation and with passage number. Rate of microvilli formation also differed between cell lines (low in CCTL10, highest in CCTL14). Other types of surface specializations (blebs, filopodia, shedding vesicles) were observed.

Differentiation potential of hESCs was not reduced up to 234 passage, but it slightly decreased at 354 passage as revealed investigation of embryoid bodies.
Conclusions: Acceleration of cell cycle in late passages of all 3 lines, early morphologic marks of spontaneous differentiation as well as extensive microvilli formation may be considered to be signs of an adaptation to culture conditions. The differentiation capability of hESCs was not reduced up to 234 passage. Analysis of submicroscopic structure of hESCs colonies showed certain differences in cell phenotype between individual lines. Glycogen accumulation represents the conspicuous ultrastructural difference between undifferentiated hESCs and ICM cells of blastocyst.

Supported by Research Project MSM0021622430 of the Ministry of Education, Youth and Sports of the Czech Republic.

P-577 Human and mouse embryonic stem cells suppress T cell responses via Arginase I - dependent mechanism - implications for implantation and transplantion

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Introduction: Embryonic stem cells (ESCs) can proliferate extensively in culture and give rise to progeny of the three germ layers. Several past reports had demonstrated that mouse and human ESCs attenuate immune responses *in vitro*. However, *in vivo* studies showed variable results for xenogeneic and allogeneic transplantations. In this study we show that human and mouse ESCs inhibit T cell responses both *in vivo* and *in vitro*, via Arginase I-dependent mechanism and the implications of this inhibition for implantation and transplantation.
Materials and Methods: *In vitro* studies – human ESCs (hESCs) were co-cultured for 72 hours with peripheral blood mononuclear cells, which were obtained from healthy donors, in the presence of T cell activators: SEB super-antigen or the T cell activating antibody OKT-3. T cell activation was then monitored by quantification of IFN-γ secretion using ELISA and by the study of T cell proliferation. T cell receptor-associated CD3-ζ was analyzed using flow cytometry. *In vivo* transplantation studies – teratomas were obtained by sub-cutaneous injection of allogeneic C57BL/6 mouse ESCs into BALB/c mice. Teratoma-infiltrating T cells were isolated and studied for T cell receptor ζ-chain using flow cytometry. *Ex vitro* studies – mouse blastocysts were immobilized in fibrin-gel, and stained with an anti-Arginase I antibody.
Results: Using co-culture experiments, we demonstrated that hESCs inhibit IFN-γ secretion and T cell proliferation in response to potent T cell activators. Furthermore, we showed that hESCs down-modulate the T cell receptor-associated CD3-ζ chain. These effects are maintained when hESCs are replaced

by their conditioned media, and can be restored by the addition of L-Arginine to hESCs-conditioned media or by treatment of hESCs with a specific Arginase inhibitor. Moreover, we showed Arginase-I expression and activity in hESCs of three independent lines. We further demonstrated that mouse ESC similarly inhibit T cell activation via Arginase I, and that this enzyme is expressed by the inner cell mass and the trophectoderm of mouse blastocysts and by hESCs-derived trophectodermal cells. Finally, *in vivo* allogeneic transplantation studies of mouse ES cells showed that T cells which infiltrate teratoma tumors have significantly lower levels of CD3-ζ chain then splenic T cells, supporting the expression of Arginase I in these teratomas.

Conclusions: ESCs suppress T cell responses, via Arginase I activity and local L-Arginine depletion, resulting in TCR ζ-chain down-modulation. This mechanism, that is utilized by tumors to evade local T cell responses, may potentially also serve ESC to inhibit T cell activity in the co-culture system, and in teratoma-infiltrating T cells. Arginase I-dependent T cell suppression may serve as a universal mechanism to augment the survival of the pre-implantation embryo by attenuating maternal T cell activity in the embryo's microenvironment.

P-578 Factor analysis:a good practical way for selection of appropriate housekeeping gene in rat mesenchymal stem cell and its differentiated lineages

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Introduction: One way for detecting the rate of mesenchymal stem cell differentiation into its derived lineages is the assay of mRNA level for some specific genes. Although DNA content is the same in all of the cells in an organism, mRNA content of cells is different upon the cell type. Now, RT-PCR is very efficient and useful technique to measure the gene expression, but factors like amount of starting material, enzymatic efficiencies, and differences between tissues or cells can affect the expression bringing up the need for precise normalization to a reference gene called housekeeping gene for correcting results of different amounts of mRNA. Along with PCR technique, we need a qualified and useful statistical analysis instrument to find the best housekeeping gene. Mean of candidate genes and geometric mean of the genes have been used for normalizing but, these methods need at least two genes to obtain the mean to be used as housekeeping control. Principal component analysis and Factor Analysis has been implicated to detect a single gene as housekeeper. Both methods can be viewed as attempts to approximation the covariance matrix structure. We chose factor analysis as elaborated method.

Material and Methods: Bone marrow was collected from the femurs of 10 Wistar rats with 8 to 10-weeks-old followed by culture of mesenchymal stem cells for each rat. Differentiation into osteocytes, cartilage cell and adypocytes was directed afterward by well known standard protocols. Quantitative RT-PCR was used to measure the variability in mRNA levels of 6 housekeeping genes in 4 different cells type of rat both differentiated and undifferentiated. We chose 6 different housekeeping genes that were previously suggested by other researches for evaluation. Factor analysis and other statistical procedures was used to find the best housekeeping gene among the following six evaluated genes Beta Tubulin(β-tub), Beta-2-microglobulin precursor(b2m), cyclophilin(PPIA), Glyseraldehyde 3 phosohate dehidrogense(GAPDH), Beta-glucoronidase and transferin receptor protein. Housekeeping genes were used as the variables and the C_t values of each cell sample as the observations. Results were analyzed in two dimensions.

Results: For this assay, we should consider housekeeping genes which has lower C_t (higher expression) and lower standard deviation(SD) means higher stability. The lower C_t mean was related to GAPDH and β-tub (18.84, 19.49) respectively and the lowest C_t standard deviation was related to β-tub (0.44). In the two-dimensional analysis, the first and second principal factors after rotation explained 46% and 37% of the variability respectively (83% in total). Greatest load in the first factor was related to β-tub (0.94). Also, loadingplot for the first two factors showed a pattern of β-tub, b2m, PPIA. Using determination coefficient and scatter plot matrix, β-tub showed the highest value of determination coefficient for all cell samples together ($r^2 \geq 0.96^2$). In addition, correlation was uniformly high in the mesenchymal stem cells and its lineages. The mean difference between C_t of β-tub and the mean C_t of other two genes (-6.9 ± 0.9) showed β-tub had higher expression than the mean expression of the other two genes (about 7 lower PCR cycles were needed to reach fluorescence threshold). Additionally, this mean difference C_t (-6.9) had the highest accuracy ($2 \times SD = 1.8$).

Conclusions: This study showed β-tub as an appropriate housekeeping gene among six candidate genes in mesenchymal stem cell and its differentiated lineages. Factor analysis and the other complimentary statistical procedures seem the powerful tools to detect a housekeeping gene among the others.

P-579 Neonatal-derived Sertoli cells support more expansion of mouse spermatogonial stem cells

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Introduction: Spermatogonial stem cells (SSCs) are unique population of adult stem cells in mammalian testes which continuously provide gametes and transfer genetic material to the next generation. Various feeder layers have been tested to support the in vitro culture of SSCs, however age effect of feeder cells has been remained a controversial issue. This study was initiated to compare Sertoli cells derived from neonatal and adult mice to examine age effect of Sertoli cells in the maintenance of mouse SSCs in vitro.

Materials and Methods: SSCs were isolated from testes of 6 day-old mice and cultured in vitro at the presence of glial-derived neurotrophic factor (GDNF) for 10 days. Then, the 10-day isolated SSCs transferred onto Setoli cells isolated by DSA lectin from neonatal (6 day-old) and adult (6-8 week-old) mice and STO (as common feeder layer) and cultured for further 10 days.

Results: Immunostaining analysis of 10-day isolated SSCs showed that our culture system contained SSC colonies as they were positive for α6-Integrin? ß1-Integrin and negative for C-kit. In addition, these stem cells were able to migrate to seminiferous basal membrane after transplantation to testes of busulfan-induced infertile adult mice. After 10 days co-culturing of SSCs, the area of colonies and the number of colonies and the number of cells pre each colony were significantly higher on neonatal-Sertoli cells. Flowcytometry analysis revealed that there was significant enhancement in the number of α6-Integrin and ß1-Integrin positive cells -positive cells in the culture with neonatal-Sertoli cells in comparison with other groups. There was no significant difference in the number of C-kit–positive cells between groups. Colony efficiency results showed that the number of colonies derived per seeded single SSCs was significantly higher on neonatal-Sertoli cells. Moreover, the co-cultured SSCs on neonatal-Sertoli cells were engraftmented in seminiferous basal membrane after transplantation to testes of busulfan-induced infertile adult mice.

Conclusions: These results showed that neonatal-Sertoli cells might be support more stemness and ex vivo expansion of mouse SSCs in comparison adult-Sertoli cells.

P-580 Has the introduction of legislation prescribing ethical consent adversely affected approval rates for these procedures?

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Introduction: New Zealand is unique in the Western World in the way that it requires national ethical approval for surrogacy, embryo donation and some inter-family gamete donations.

In 2004 New Zealand passed into law the Human Assisted Reproductive Technology Act 2004. This Act mandated for and regulated ethical approval of certain applications for assisted reproductive techniques including surrogacy and some inter-family gamete donations. It established the Ethics Committee Assisted Reproductive Technology (ECART) and Advisory Committee on Assisted Reproductive Technology (ACART). These committees work together. ACART sets policy and ethical guidelines for ECART and ECART receives applications from clinics. All couples who wish to participate in surrogacy and/or inter-family gamete embryo donations are now required to comply with guidelines under the Act and submit an application to ECART for ethical approval before treatment. The study analyses the applications received by the predecessor to ECART, the National Ethics Committee on Assisted Human Reproduction ("NECAHR") which had no statutory ability to bind fertility clinics to its decisions and the applications received under the new legislation. The study examines the outcomes of those applications to see whether there has been any

real difference in the percentage of applications approved and any conditions imposed upon them between the regulated system and unregulated system. The paper examines whether the introduction of a structure to ethical application has really made any difference to the ethical outcome. The question it poses is whether ethical considerations are impacted by legislative change?

Material and Methods: This paper is a retrospective analysis of the anonymous applications for surrogacy and inter-family gamete donations received by the Ministry of Health from 1998 to 2008. It examines the nature of the applications and ethical outcome of the applications in the unregulated and regulated environment. These applications were received by the NECAHR (National Ethics Committee on Assisted Human Reproduction) and ECART (Ethics Committee Assisted Reproductive Technology) over a ten year period.

Results: The paper concludes that the introduction of the guidelines has not adversely impacted on the outcome of ethical applications but has lead to an increase in the number and complexity and cost of applications.

Conclusions: The study concludes that the impact of the change in legislation has not been reflected in any change in applications approved.

P-581 Rapid and efficient reprogramming of human amnion-derived cells into pluripotency by three factors OCT4 /SOX2/NANOG

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Introduction: Reprogramming human somatic cells to pluripotency represents a valuable resource for research aiming at the development of *in vitro* models for human diseases and regenerative medicines to produce patient-specific induced pluripotent stem (iPS) cells. Seeking appropriate cell resources for higher efficiency and reducing the risk of viral transgene activation, especially oncogene activation, are of significance for iPS cell research. In this study, we tested whether human amnion-derived cells (hADCs) could be rapidly and efficiently reprogrammed into iPS cells by the defined factors: *Oct4 /Sox2/Nanog*.

Material and Methods: hADCs from normal placenta were isolated and cultured. The 3rd passage cells were infected with the lentiviral vectors for the delivery of *Oct4, Sox2*, and *Nanog*. Afterwards, the generated iPSCs were identified by morphology, pluripotency markers, global gene expression profiles, and epigenetic status both *in vitro* and *in vivo*.

Results: Our data showed that we were able to reprogram hADCs by the defined factors (*Oct4/Sox2/Nanog*). The efficiency was significantly high (0.11%), and the typical colonies appeared on the 9th day after infection. They were similar to human embryonic stem (ES) cells in morphology, proliferation, surface markers, gene expression, and the epigenetic status of pluripotent cell-specific genes. Furthermore, these cells were able to differentiate into various cell types of all three germ layers both *in vitro* and *in vivo*.

Conclusions: These results demonstrate that hADCs were an ideal somatic cell resource for the rapid and efficient generation of iPS cells by *Oct4 /Sox2/ Nanog*.