ESF-II / 5 WS2014 5. Dst 12.11.2014

Anwendungen des SCNT beim Menschen und Gefahren

Georg Weitzer

Spekulationen was man mit SCNT beim Menschen alles machen können wird.

- Austausch von Mitochondrien mit defekten Genom. (Mts haben 37 Gene und über 250 Mutationen sind bereits bekannt.)
  - Durch Spindeltransfer
  - Durch Pronukleustransfer („Drei-Patienten-IVF“)
  - Durch Polarkörperchentransfer
  - Durch Keimbläschenltransfer*
- Zeugung von Mädchen durch zwei Frauen
- Zeugung von Kindern durch zwei Männer

* „Kinder für alte (<45a) Frauen aus jungen Oozyten“

Spindeltransfer

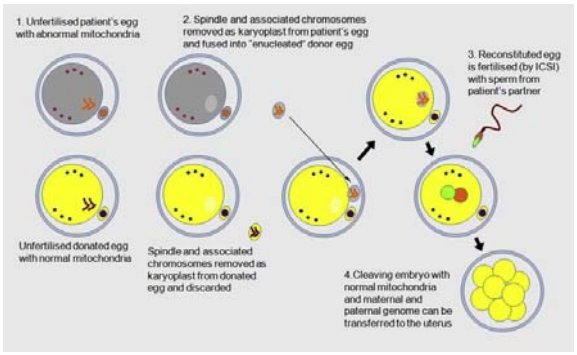

Figure 2 Spindle transfer technique. <ce:source> Image reproduced from http://www.hfea.gov.uk/6372.html

Paula Amato , Masahito Tachibana , Michelle Sparman , Shoukhrat Mitalipov

Three-parent in vitro fertilization: gene replacement for the prevention of inherited mitochondrial diseases

Fertility and Sterility, Volume 101, Issue 1, 2014, 31 - 35

http://dx.doi.org/10.1016/j.fertnstert.2013.11.030

Pronukleustransfer

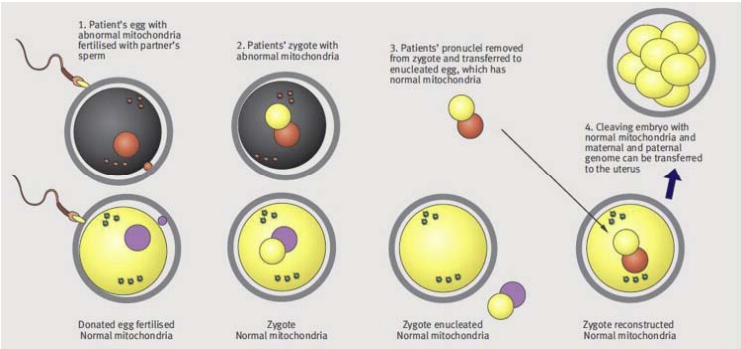

Figure 1 Pronuclear transfer technique.
<ce:source> Reproduced from Bredenoord and Braude <ce:cross-ref refid="bib18" id="crosref0150"> (18)</ce:cross-ref> , with permission from BMJ Publishing Group Ltd.</ce:source>

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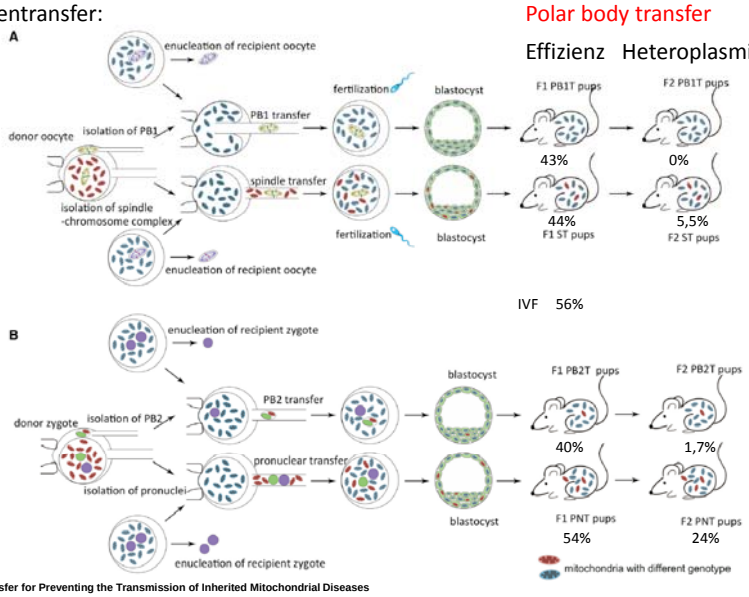
Polarkörperchentransfer:

Polar body transfer

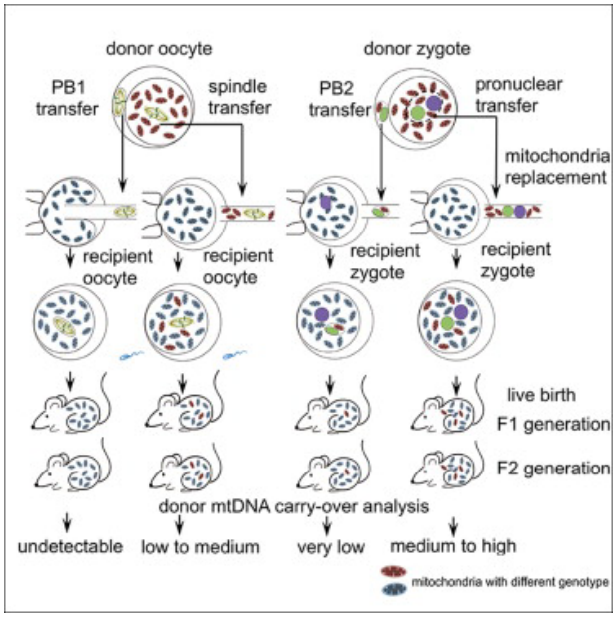
Effizienz Heteroplasmie



Polar Body Genome Transfer for Preventing the Transmission of Inherited Mitochondrial Diseases
Tian Wang , Hongying Sha , Dongmei Ji , Helen L. Zhang , Dawei Chen , Yunxia Cao , Jianhong Zhu
Cell, Volume 157, Issue 7, 2014, 1591 - 1604 http://dx.doi.org/10.1016/j.cell.2014.04.042

Polarkörperchentransfer:

Heteroplasmie



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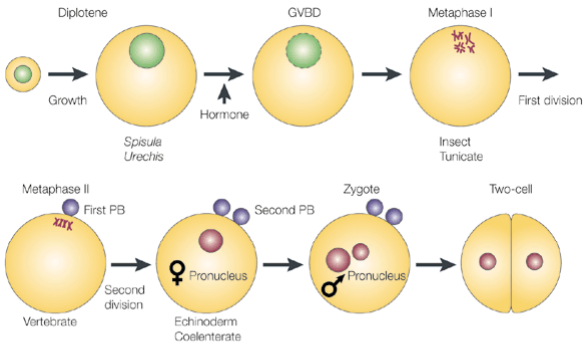
Keimbläschentransfer

Keimbläschen in embryonalen oder unreifen Oozyten



Nature Reviews | Molecular Cell Biology

Keimbläschentransfer

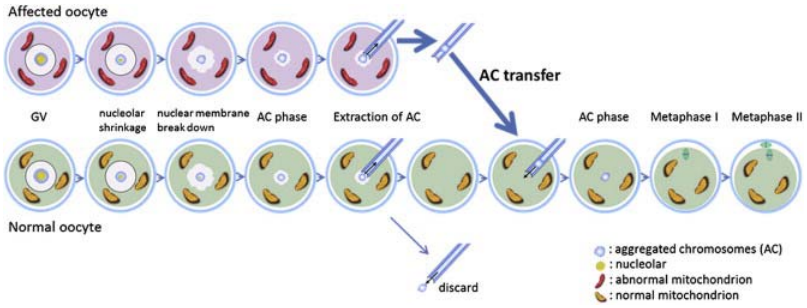


Figure 2 Schematic diagram of aggregated chromosomes transfer, depicting a proposed new chromosome replacement procedure, from oocytes that contain abnormal mitochondria into oocytes that contain normal mitochondria.

Junko Otsuki , Yasushi Nagai , Tadashi Sankai

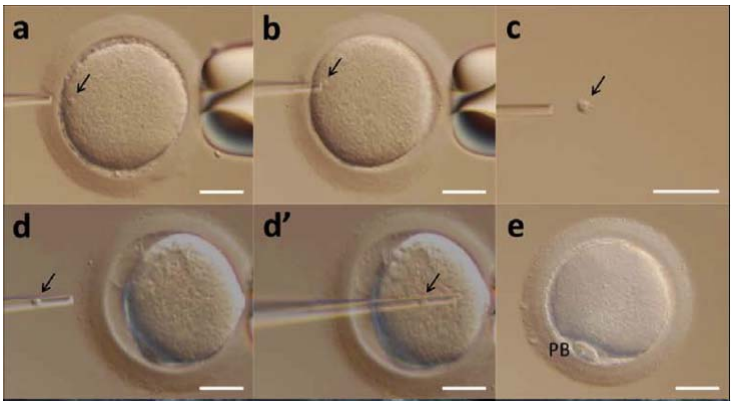
Aggregated chromosomes transfer in human oocytes

Reproductive BioMedicine Online, Volume 28, Issue 3, 2014, 401 - 404

http://dx.doi.org/10.1016/j.rbmo.2013.10.024

Keimbläschentransfer

Germinal vescile Transfer vor Meiose



Reproductive BioMedicine Online (2014) **28**, 401–404

Anwendungen von SCNT bei Tieren

Herstellung von humanen Proteinen in der Milk von Ziegen, Schafen und Kühen

Transgenic Res. 2014 Aug 20.
Production of transgenic dairy goat expressing human α-lactalbumin by somatic cell nuclear transfer.
Feng X[1], Cao S, Wang H, Meng C, Li J, Jiang J, Qian Y, Su L, He Q, Zhang Q.

Abstract
Production of human α-lactalbumin (hα-LA) transgenic cloned dairy goats has great potential in **improving the nutritional value** and perhaps increasing the yield of dairy goat milk. Here, a mammary-specific expression vector 5A, harboring goat β-lactoglobulin (βLG) promoter, the hα-LA gene, neo^r and EGFP dual markers, was constructed. Then, it was effectively transfected into goat mammary epithelial cells (GMECs) and the expression of hα-LA was investigated. Both the hα-LA transcript and protein were detected in the transfected GMECs after the induction of hormonal signals. In addition, the 5A vector was introduced into dairy goat fetal fibroblasts (transfection efficiency ≈60-70 %) to prepare competent transgenic donor cells. A total of 121 transgenic fibroblast clones were isolated by 96-well cell culture plates and screened with nested-PCR amplification and EGFP fluorescence. After being frozen for 8 months, the transgenic cells still showed high viabilities, verifying their ability as donor cells. Dairy goat cloned embryos were produced from these hα-LA transgenic donor cells by somatic cell nuclear transfer (SCNT), and the rates of fusion, cleavage, and the development to blastocyst stages were 81.8, 84.4, and 20.0 %, respectively. A total of **726 reconstructed embryos** derived from the transgenic cells were transferred to 74 recipients and pregnancy was confirmed at 90 days in 12 goats. Of six female kids born, two carried hα-LA and the hα-LA protein was detected in their milk. This study provides an effective system to prepare SCNT donor cells and transgenic animals for human recombinant proteins.

Nebenwirkungen der SCNT Technologie

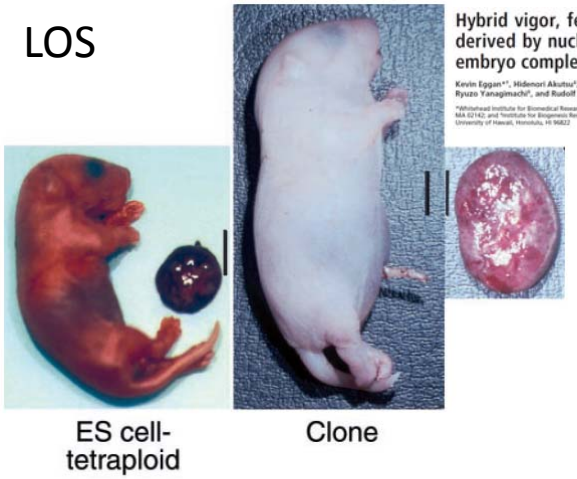
Pathologie der NT-Tiere

- Stark Kern-Spender Zelltyp abhängig.

- Sertoli Zellen → verfrühter Tod durch Leberversagen
- Cumulus Zellen → Fettleibigkeit
- Bei allen Zelltypen → Riesenwuchs (large offspring syndrom) durch fehlerhaftes gene silencing

- Phenotypen nicht auf die F1 Generation übertragbar → Pathologie ist epigenetisch bedingt.

LOS



Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation

Kevin Eggan*†, Hidenori Akutsu*, Janet Loring*, Laurie Jackson-Grusby*, Martina Klemm*, William M. Rideout 3rd*, Ryuzo Yanagimachi‡, and Rudolf Jaenisch*†§

*Whitehead Institute for Biomedical Research and ‡Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142; and †Institute for Biogenesis Research and Department of Anatomy and Reproductive Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96822

Fig. 4. ES cell clones display increased neonatal birth and placental weight. These two animals were derived from the same ES cell line, $F_{1.2-3}$, one cloned by nuclear transfer, the other derived by tetraploid embryo complementation. Note the dramatic increase in both neonatal and placental size in the cloned pup. (Bars = 1 cm.)

Toxizität von fremden Mitochondrien

Anim Biotechnol. 2014 Apr 3;25(2):139-49.

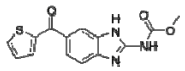
Incompatibility of nucleus and mitochondria causes xenomitochondrial cybrid unviable across human, mouse, and pig cells.

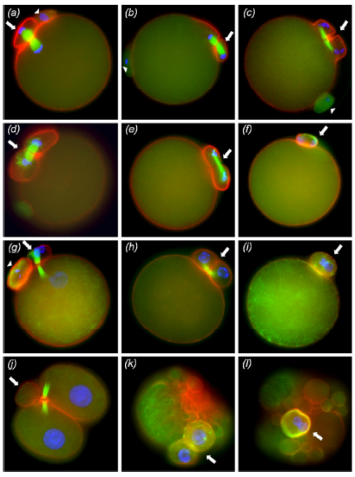
Yu G[1], Tian J, Yin J, Li Q, Zhao X.

Abstract

The nucleus and mitochondria are on correlative dependence; they interact in the process of protein transportation and energy metabolism. The compatibility of nucleus and mitochondria is essential for interspecies somatic cell nuclear transfer (iSCNT) and xenomitochondrial cybrid. In order to test the compatibility of nucleus and mitochondria among human, mouse, and pig cells, we compared the performances of cybrids that fused inter- and intra-species. The ρ0 cells from human and pig cell lines were created as nucleus donors which were transfected with GFP-neo for cell selective system in advance, and mitochondria donor cells were labeled by Mitochondria-RFP. Human and mouse platelets were also used as a mitochondrial donor. Results indicated that all interspecies cybrids declined to die in 2-4 d after the cell fusion in the selection medium, while intraspecies cybrid cells survived and formed stable clones. As a conclusion, the incompatibility between nucleus and mitochondria is the critical factor for the formation of interspecies cybrids.

Nocodazole bewirkt den Ausstoß von beiden Chromatiden in Oozyten.





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Theriogenology
www.theriojournal.com

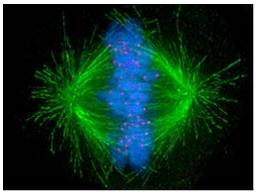
Demecolcine- and nocodazole-induced enucleation in mouse and goat oocytes for the preparation of recipient cytoplasts in somatic cell nuclear transfer procedures

Nuno Costa-Borges[a], Maria Teresa Paramio[b], Josep Santaló[a], Elena Ibáñez[a,*]
[a] Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biociències, Universitat Autònoma de Barcelona
[b] Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona
Received 10 August 2010; received in revised form 15 September 2010; accepted 20 September 2010

Nocodazole inhibiert Mikrotubulibildung
→ Missbildungen bei Tierföten

Fig. 1. Activated mouse oocytes that were exposed to DEM or NOC and then cultured in antimitotic-free medium up to 2, 6 or 20 h p.a. before being fixed and processed for the immunofluorescence detection of microtubules (green), microfilaments (red), and chromatin (blue). Arrowheads and arrows indicate the presence of the first and the second polar body (PB2), respectively. By 2 h p.a., non-enucleated oocytes presented only one set of chromosomes within the PB2 and the meiotic spindle was oriented perpendicularly *(a)* or in parallel *(b)* in relation to the oocyte cytoplasm. Activated oocytes showing all the chromatin inside one or sometimes two PB2s, were classified as partially enucleated *(c–d)* or completely enucleated *(e–f)*, depending on whether PB2s were partially or completely extruded, respectively. By 6 h p.a., non-enucleated oocytes presented one pronucleus in the cytoplasm and another one inside the PB2 *(g)*, similar to control oocytes non-exposed to the antimitotics, whereas in completely enucleated oocytes the two pronuclei were localized within a double *(h)* or single *(i)* completely extruded PB2. By 20 h p.a. non-enucleated oocytes, similar to control oocytes, had cleaved to the 2-cell stage *(j)*, whereas oocytes that remained irreversibly enucleated showed a vacuolized or fragmented cytoplasm due to the absence of the chromatin, which was localized within a double *(k)* or single *(l)* completely extruded PB2. Original magnification × 500.

Spindel Apparat in der Metaphase
der Meiose II ist möglicherweise nicht identisch mit „anderen“ Spindelapparaten.



…study revealed four proteins as being deficient in spindles of SCNT embryos in addition to those previously identified; these were clathrin heavy chain (CLTC), aurora B kinase, dynactin 4, and casein kinase 1 alpha.
J Proteome Res. 2010 Nov 5;9(11):6025-32

→ Was ist beim SCNT nicht perfekt?

Gefahrenquellen bei der Zeugung von Kindern mit Hilfe von SCNT

- Ist das Genom der Oozyte identisch mit dem in den 1. und 2. Polkörperchen ?
- Welche vielleicht einzigartigen Faktoren im Zytoplasma der gespendeten Oozyte beeinflussen die Embryogenese des Kindes und somit dessen Individualität?
- Fehlen dem Keimbläschen vielleicht essentielle Faktoren?
- Ist die Reprogrammierung des Genoms wirklich vollständig?
- Bewirkt SCNT das gehäufte Auftreten von Mutationen?