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Correspondence and requests for materials should be addressed to Y.S. (e-mail: sakaki@ims.u-tokyo.ac.jp) or H.I. (e-mail: iskw@biol.s.u-tokyo.ac.jp). The complete sequence and the annotated data are available on our website (http://buchnera.gsc.riken.go.jp/). The sequence has been deposited with DDBJ under accession number AP000398, AP001070 and AP001071 for chromosome, the pTrp plasmid and the pLeu plasmid, respectively.

Cloned pigs produced by nuclear transfer from adult somatic cells

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Since the first report of live mammals produced by nuclear transfer from a cultured differentiated cell population in 1995 (ref. 1), successful development has been obtained in sheep[2,3], cattle[4], mice[5] and goats[6] using a variety of somatic cell types as nuclear donors. The methodology used for embryo reconstruction in each of these species is essentially similar: diploid donor nuclei have been transplanted into enucleated MII oocytes that are activated on, or after transfer. In sheep[2] and goat[6] preactivated oocytes have also proved successful as cytoplast recipients. The reconstructed embryos are then cultured and selected embryos transferred to surrogate recipients for development to term. In pigs, nuclear transfer has been significantly less successful; a single piglet was reported after transfer of a blastomere nucleus from a four-cell embryo to an enucleated oocyte[7]; however, no live offspring were obtained in studies using somatic cells such as diploid or mitotic fetal fibroblasts as nuclear donors[8,9]. The development of embryos reconstructed by nuclear transfer is dependent upon a range of factors. Here we investigate some of these factors and report the successful production of cloned piglets from a cultured adult somatic cell population using a new nuclear transfer procedure.

To date, the efficiency of somatic cell nuclear transfer, when measured as development to term as a proportion of oocytes used, has been very low (1–2%)[10]. A variety of factors probably contribute to this inefficiency. These include laboratory to laboratory variation, oocyte source and quality, methods of embryo culture (which are more advanced in some species (such as cows) than others (such as pigs)), donor cell type, possible loss of somatic imprinting in the nuclei of the reconstructed embryo, failure to reprogram the transplanted nucleus adequately, and finally, the failure of artificial methods of activation to emulate reproducibly those crucial membrane-mediated events that accompany fertilization.

In the pig, there is the additional difficulty that several (>4) good quality embryos are required to induce and maintain a pregnancy[11]. As fully developmentally competent embryos are rare in nuclear transfer procedures, there is every chance of squandering those good embryos unless very large numbers of reconstructed embryos are transferred back into recipients. Even if it were possible in the pig to select good quality blastocysts for transfer (after, for example, the use of a temporary recipient), most blastocysts formed from reconstructed embryos in other species are not competent to proceed to term[10]. The co-transfer of reconstructed embryos with 'helper', unmanipulated embryos, parthenotes or tetraploid embryos has been suggested as an aid to inducing and maintaining pregnancy. However, studies in mice after zygote pronuclear injection have suggested that the manipulated embryos are 'compromised' and selected against[12]. An alternative to the use of 'helper' embryos is the hormonal treatment of recipient sows to maintain pregnancy with low embryo numbers[13].

We cannot currently address all of the methodological problems, and, to improve our chances of success in pig nuclear

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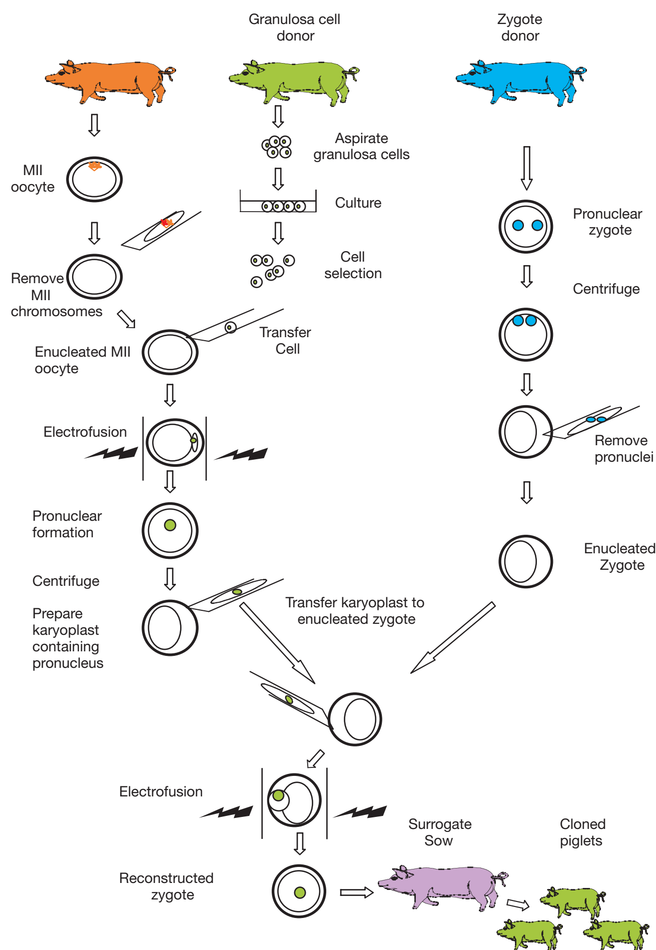

Figure 1 Representation of the double nuclear transfer procedure for the production of viable piglets using cultured adult somatic granulosa cells as nuclear donors. The outer circle in all the oocytes and embryos denotes the zona pellucida; the inner circle denotes the cell membrane.

Figure 2 A litter of five live piglets derived by nuclear transfer using cultured adult granulosa cells as nuclear donors. A total of 72 reconstructed embryos were transferred to the surrogate sow.

transfer, we chose to focus on four areas: activation, choice of donor cell, embryo culture, and induction and maintenance of pregnancy.

In all species, when using MII oocytes as recipients, the method of activation is crucial for subsequent development. In the pig, although current activation protocols stimulate pronuclear formation, cleavage, and development to the blastocyst stage, both the frequency of development and the quality of the embryos produced are low[14]. A system that involves the use of fertilized zygotes as cytoplast recipients would bypass the inefficiencies of artificial activation procedures and might promote more successful development. The technique of pronuclear exchange between zygotes showed that the manipulations involved were compatible with development[15]; however, when donor nuclei from later developmental stages were transferred there was restricted development[16]. One explanation is that factors required for development, which are absent in the donor nuclei, are removed with the pronuclei. But if a pronucleus-like structure could be produced from the donor nucleus, this might prove a suitable nuclear donor for transfer. Such a system was described in mice by Kwon and Kono[17], who first fused mitotically arrested blastomere nuclei with enucleated MII oocytes. The reconstructed oocytes were subsequently activated in the presence of cytochalasin B, preventing polar body extrusion and resulting in the formation of two diploid pseudo-pronuclei. Each pseudo-pronucleus was then transferred into an enucleated, *in vivo* produced zygote, which was transferred into a surrogate recipient for development to term. Effectively, this latter procedure mimics pronuclear exchange and allows the

Table 1 Development of porcine embryos

	Double NT							Single NT		
Cell isolate	Pool1	GR5	GR8	GR12	GR21Z	GR18	GR18	GR1	GR8	GR18
Cell treatment	CI	CI	CI	CI	CI	SS (74)	SS (51)	CI	CI	SS (51)
No. of oocytes	245	344	269	291	311	193	216	123	109	N/A
No. of attempted reconstructions day 1 (%)	183 (75)	217 (63)	207 (77)	226 (78)	221 (71)	122 (63)	192 (89)	94 (76)	83 (76)	N/A
No. of fused embryos day 1 (%)	124 (68)	153 (70)	186 (90)	97 (43)	163 (74)	90 (74)	162 (84)	87 (93)	61 (73)	N/A
No. of day-1 embryos with single pronucleus (%)	87 (70)	88 (57)	120 (64)	62 (64)	69 (42)	23 (26)	102 (63)	–	–	–
No. of reconstructed embryos day 2	74	57	105	61	55	22	45*	–	–	–
No. of fused embryos day 2 (%)	72 (97)	56 (98)	100 (95)	53 (87)	54 (98)	22 (100)	44 (98)	–	–	–
No. of embryos transferred to recipient	72	56	100	53	54	22	44	85	61	39†
Pregnancy (no. of fetuses observed)	+ve (3)	–ve	–ve	–ve	–ve	+ve (6)	–ve	–ve	–ve	–ve
No. of live births (%)	5 (7)	0	0	0	0	0	0	0	0	0

Development of porcine embryos reconstructed using a single or double nuclear transfer protocol with adult granulosa cells cultured to confluence (CI), or serum starved (SS) as nuclear donors.
* Insuffici9ent zygotes to reconstruct day 1 embryos.
† Due to insufficient zygote numbers 39 day-1 recosntructed embryos were transferred to a single recipient.

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formation of a final reconstructed one-cell embryo whose membrane has been activated during fertilization.

The use of cultured cell populations for the production of animals by nuclear transfer is now well documented in a number of species. We have considerable experience in the production of sheep and cattle from primary cell populations and genetically modified primary cell populations. Analysis of these studies has shown considerable variation in development between individual cell populations and at present has provided no definitive method for the identification of cell populations that are suitable for nuclear transfer. Factors that are thought to influence the suitability of a particular cell population include the effects of oxidative damage associated with cellular metabolism, genome instabilities and chromosomal pathologies. All of these factors may be influenced by the method of isolation and culture, and the number of population doublings in culture. On consideration of these factors and our previous observations, we chose to use granulosa cells as nuclear donors. Granulosa cells are suitable nuclear donors in cattle[18], and require the minimum of manipulations to establish in culture. Because of differences between cell populations, we initially decided to use a pool of cells isolated from a group of four donors. In later experiments, cell populations from individual animals were also examined. To minimize the culture period, early passage, never-frozen cells were used.

For embryo reconstruction, we attempted to minimize the potential inefficiencies at each step of the nuclear transfer procedure and adopted an approach that (1) uses *in vivo* derived material, (2) seeks to avoid artificial activation, and (3) minimizes the period of *in vitro* culture of manipulated embryos. To do this we used a two-stage nuclear transfer procedure modified from Kwon and Kono[17] (Fig. 1). In the first stage, donor cells were fused to *in vivo* derived, enucleated MII oocytes obtained from superovulated crossbred gilts. The pseudo-pronucleus formed in the first nuclear transfer embryo was then subsequently transplanted into an *in vivo* produced, enucleated zygote (second nuclear transfer embryo). The second nuclear transfer reconstructed embryo was transferred to the oviduct of a synchronized sow within 2 h of fusion. Because of the expected low developmental rate, we transferred up to 100 reconstructed embryos to a single recipient. Each recipient was treated with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) to maintain pregnancy[13] in the event that fewer than four reconstructed embryos were viable at implantation.

Coordination of the cell-cycle stages of the recipient cytoplasm and the donor nucleus are essential for maintaining correct ploidy and preventing DNA damage in nuclear transfer reconstructed embryos[19]. Various combinations of donor and recipient cell-cycle stages can prevent DNA damage and uncoordinated DNA replication, and result in formation of a pseudo-pronucleus. It has been suggested that the use of MII oocytes may improve 're-programming' of the donor genetic material owing to the occurrence of nuclear envelope breakdown and premature chromosome condensation, thus exposing the donor chromatin to maternally derived oocyte factors involved in early development. To take advantage of this here, we used MII oocytes as cytoplast recipients for the first nuclear transfer embryo reconstruction. To maintain ploidy in this situation, we chose diploid donor nuclei as nuclear donors.

Previous studies have suggested that diploid cells arrested in the G0 phase of the cell cycle may be beneficial[2]. Using flow cytometry, we examined the cell-cycle distribution of porcine granulosa cells under three different culture conditions: sub-confluent actively growing, 100% confluent, and cells starved of serum for 48 hours (see Figure in Supplementary Information). After serum starvation, the population contained a large proportion (7.2%) of cells with a DNA content lower than that consistent with a diploid cell (termed sub-G1). In contrast, in the population synchronized by contact inhibition, 90.3% of the cells had a diploid DNA content (G1/G0) and there were fewer sub-G1 cells (1.6%). We analysed DNA synthesis in serum-starved and contact-inhibited cell populations by 5-bromo-2′-deoxyuridine (BrdU) incorporation. These experiments revealed that 45% of the contact inhibited cell population compared with 0% of the serum-starved population incorporated BrdU. An analysis of BrdU incorporation after an additional 24 h of contact inhibition revealed that the fraction of BrdU-positive cells was reduced to 5%. These observations suggest that the diploid cells in the contact-inhibited granulosa cell population used as nuclear donors for embryo reconstruction contained a mixture of cell-cycle-arrested diploid cells (G1/G0) and unarrested diploid cells (G1), which were able to undergo a further round of DNA synthesis. In

Table 2 Microsatellite analysis of pigs and cell donors

Loci	S0059	S0070	S0122	S0226	SW24	SW72	SW840	SW936	TNFB
Samples									
PGR1	152	275	178	178	103	102	129	95	161
	152	295	182	198	111	110	129	97	164
PGR2	148	275	180	178	103	110	129	97	158
	154	275	182	198	111	112	129	111	185
PGR3	146	275	180	178	103	110	125	97	N/A
	152	275	182	198	109	112	125	111	
PGR4	152	275	178	198	103	102	129	97	158
	152	295	182	198	111	110	129	109	161
NTP1	152	275	178	178	103	102	129	95	161
	152	295	182	198	111	110	129	97	164
NTP2	152	275	178	178	103	102	129	95	161
	152	295	182	198	111	110	129	97	164
NTP3	152	275	178	178	103	102	129	95	161
	152	295	182	198	111	110	129	97	164
NTP4	152	275	178	198	103	102	129	97	158
	152	295	182	198	111	110	129	109	161
NTP5	152	275	178	198	103	102	129	97	158
	152	295	182	198	111	110	129	109	161
Recipient (54B)	152	275	178	192	111	102	N/A	103	158
	152	295	182	198	95	102		109	161
Boar	134	265	178	180	115	102	129	97	164
	156	273	182	180	115	112	129	109	185

Microsatellite analysis was performed on genomic DNA from the four individual populations of granulosa cells (PGR1, PGR2, PGR3, PGR4), the piglets (NTP1–5), the surrogate sow (54B) and the boar responsible for inseminating the zygote donors. Primers corresponding to nine polymorphic loci were used. Two numbers are shown for each sample at each locus, which represent the PCR product size for each of the two alleles at that particular locus.

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contrast, when the serum-starved populations were used as nuclear donors most the diploid cells were cell-cycle arrested (G1/G0).

Production of the first nuclear transfer embryos requires activation of the *in vivo* derived oocytes. Activation experiments carried out in control oocytes showed that electrical stimulation applied between 51.5 and 60 h after hCG administration, promoted similar cleavage and development to blastocyst (see Table in Supplementary Information). For embryo reconstruction, MII oocytes were collected 46–54 h after hCG and the first nuclear tranfer embryo reconstruction was carried out between 50 and 58 h after hCG. Reconstructed embryos were cultured overnight in NCSU-23 medium[22]; we then checked them for the presence of a pronucleus and used them for the second nuclear transfer embryo reconstruction. The development of single nuclear transfer and double nuclear transfer embryos reconstructed from contact-inhibited and serum-starved granulosa populations were compared (see Table 1). In total, 185 single nuclear transfer embryos were transferred to 3 recipient sows and 401 double nuclear transfer embryos to 7 recipients. Two recipients of the double nuclear transfer embryos became pregnant as determined by ultrasound visualization of fetuses at day 35 of gestation. One of these maintained the pregnancy to term, and five piglets (Fig. 2) were delivered by Caesarean section on day 116 of gestation. The average birth weight of the piglets was 2.72 lb (range 2.28–3.08 lb); this is about 25% lower than that observed in the same population of pigs under natural mating conditions (average litter size average 10.9, average birth weight 3.6 lb, range 3.3–3.9 lb).

The live piglets were produced from a pooled population of cells derived from four animals. We carried out microsatellite analysis of genomic DNA from the various samples (Table 2). The comparison of the pattern of alleles in the piglets with that of the granulosa cell populations indicated that three of the nuclear transfer piglets (NTP1, NTP2 and NTP3) were derived from the porcine granulosa (PGR)1/cell line, as there was 100% identity at all nine microsatellite markers. The other 2 nuclear transfer piglets (NTP4 and NTP5) showed perfect identity with the genotype of the PGR4 cell population. All five of the nuclear transfer piglets were significantly different from the surrogate mother (54B). Some of the loci (S0059, S0070, S0122 and TNFB) were not highly polymorphic indicating a degree of homogeneity or inbreeding within the population of pigs used in these studies (all of which come from the same commercial supplier).

We think that the principal reasons for the success of this modified nuclear transfer procedure in pigs is its lack of reliance on current artificial activation protocols and *in vitro* culture techniques. Although elaborate, the double nuclear transfer does not add another major inefficiency (the second step fusion is very efficient). Direct transfer of a somatic nucleus to an enucleated zygote will not work because (in addition to reprogramming difficulties) of the loss of important factors sequestered within the removed pronuclei. The cell population used successfully as nuclear donors in these experiments were not quiesced by serum starvation. Cell-cycle analysis showed that most cells in control cultures had a diploid DNA content, and a high percentage were able to undergo a further round of DNA synthesis suggesting that most cells in the population were in the G1 phase of the cell cycle and not arrested in G0. All five of the pigs, now three months old, are extremely healthy, in contrast to the (usual) 50% postnatal loss of nuclear transfer animals[10]. It is tempting then to speculate that this modified method may have general utility in other species, even those where single nuclear transfer has been shown to work.

The successful development of nuclear transfer in pigs opens the door for the application of gene-targeting technology, thus allowing for very precise genetic modifications, including gene knockouts. We have recently reported gene targeting in cultured ovine somatic cells and the successful development to term of offspring produced by nuclear transfer using these cells[20]. In pigs, a gene of great interest for the application of knockout technology is that for α-1,3-galactosyl transferase (α-1,3-GT)—the enzyme responsible for adding the xenogeneic sugar, galactose α-1,3-galactose, to the surface of porcine cells. This gene is inactive in certain monkeys and humans, and their blood contains anti-gal antibodies, which trigger (in monkeys) early rejection of transplanted organs[21]. We have achieved targeted disruption of the α-1,3-GT gene in primary porcine cells (unpublished data) and this will allow the production of α-1,3-GT-deficient pigs, whose organs should show improved resistance to rejection. Overcoming antibody-mediated rejection is the first critical step in improving xenograft survival, towards the ultimate goal of providing an unlimited supply of compatible pig organs for human transplantation. □

Methods

Modified NCSU-23 medium

The published NCSU-23 medium[22] was modified for use as a phosphate-buffered benchtop medium without $NaHCO_3$. Physiological pH phosphate buffer is made using a 3:1 molar ratio of dibasic to monobasic phosphate anions. These changes induced alterations in the Na and K concentrations, which were corrected by adjusting the NaCl and KCl concentrations to maintain osmolarity and Na/K ratio (all chemicals purchased from Sigma unless otherwise noted).

Superovulation of donor gilts for collection of oocytes and zygotes

Crossbred gilts (280–320 lbs) were synchronized by oral administration of 18–20 mg Regu-Mate (Altrenogest, Hoechst) mixed into the feed. Regu-Mate was fed for 5–14 d using a scheme dependent on the stage of the oestrous cycle. Estrumate (250 μg, Bayer) was administered intramuscularly (i.m.) on the last day of the Regu-Mate treatment. Superovulation was induced with a single i.m. injection of 1,500 IU of PMSG (Diosynth) 15–17 h after the last Regu-Mate feeding. One thousand units of hCG (Intervet America) were administered i.m. 82 h after the PMSG injection.

We collected oocytes 46–54 h after the hCG injection by reverse flush of the oviducts using pre-warmed Dulbecco's phosphate buffered saline (PBS) containing bovine serum albumin (BSA; 4 g l^{-1}). For the collection of zygotes, 24–36 h after the hCG injection the gilts were either artificially inseminated or bred naturally. We flushed zygotes from the oviduct 52–54 h after the hCG injection using PBS containing BSA (4 g l^{-1}).

Isolation and culture of porcine granulosa cells

Follicular fluid was aspirated from 2–8-mm diameter follicles of superovulated crossbred gilts (Large White (1/2), Landrace (1/4), White Duroc (1/4)), 7–8 months old, 280–320 lb, 28–51 h post hCG injection. Granulosa cells were collected by centrifugation at 1,040 *g* for 10 min, re-suspended in DMEM (Gibco), containing 10% fetal calf serum (FCS; Summit Biotech), 0.1 mM non-essential amino acids (NEAA Gibco), 2 ng ml^{-1} basic fibroblast growth factor (bFGF) (Beckton Dickinson) and 6 μl ml^{-1} Gentamycin (Sigma). Cells were expanded for several days and then cryo-preserved.

For nuclear transfer, we plated the granulosa cells at $1–5 \times 10^4$ cells per 35 mm dish in DMEM medium supplemented with NEAA (0.1 mM), bFGF (2 ng ml^{-1}) and 10% FCS, and cultured them to 100% confluency at 37 °C. For experiments where serum starvation was evaluated, cells were starved of serum for 48–72 h in DMEM containing 0.5% FCS. We collected cells by trypsinization and stored them in suspension in modified NCSU-23 phosphate medium at 38.5 °C for 20–120 min, before use as nuclear donors.

Activation of oocytes

Activation of control oocytes was achieved by application of two 1.0 kV cm^{-1} DC electric pulses for 60 μs each at an interval of 5 s in activation medium (0.3 M D-sorbitol supplemented with 0.1 mM Mg SO_4 and 0.05 mM $CaCl_2$ in H_20).

Reconstruction of first nuclear transfer embryo

Recovered oocytes were washed in PBS containing 4 g l^{-1} BSA at 38 °C, and transferred to calcium-free phosphate-buffered NCSU-23 medium at 38 °C for transport to the laboratory. For enucleation, we incubated the oocytes in calcium-free phosphate-buffered NCSU-23 medium containing 5 μg ml^{-1} cytochalasin B (Sigma) and 7.5 μg ml^{-1} Hoechst 33342 (Sigma) at 38 °C for 20 min. A small amount of cytoplasm from directly beneath the first polar body was then aspirated using an 18-μM glass pipette (Humagen, Charlottesville,Virginia). We exposed the aspirated karyoplast to ultraviolet light to confirm the presence of a metaphase plate. A single granulosa cell was placed below the zona pellucida in contact with each enucleated oocyte. The couplet was transferred to a fusion chamber (model no. BT-453, BTX Inc., San Diego) containing 700 μl of 0.3 M mannitol, 0.1 mM $MgS0_4$ and 0.1 mM $CaC1_2$ in deionized water. Fusion and activation were induced by application of an AC pulse of 5 V for 5 s followed by two DC pulses of 1.5 kV Cm^{-1} for 60 μs using an ECM2001 Electrocell Manipulator (BTX Inc., San Diego). Couplets were then washed in bicarbonate buffered NCSU-23 medium, and incubated in this medium for 0.5–1 h at 38.6 °C in a humidified atmosphere consisting of 5% CO_2 in air. We checked couplets for fusion at ×300 magnification using an inverted microscope. Fused embryos were given a second activation stimulus of two successive DC pulses of 1.2 kV cm^{-1} for 60 μs each, and cultured overnight in NCSU medium[20].

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Reconstruction of second nuclear transfer embryo

Zygotes were centrifuged at 14,900 *g* for 15 min in a Biofuge 13 centrifuge and then incubated in phosphate buffered NCSU-23 medium containing 5.0 μg ml^{-1} cytochalasin B (Sigma) at 38 °C for 20 min. Zygotes containing two pronuclei were enucleated using a 25–35-μm glass pipette by aspirating a membrane bound karyoplast containing both pronuclei (and the second polar body if present). Karyoplasts containing the pseudo-pronucleus were prepared from the day 1 nuclear transfer embryos as described for zygote enucleation with the modification that a 30–45-μm enucleation pipette was used for manipulation. A single karyoplast was placed into the perivitelline space of each enucleated zygote. Fusion was induced by application of an AC pulse of 5 V for 5 s followed by two DC pulses of 1.2 kV cm^{-1} for 60 μs. Couplets were then washed and cultured in NCSU-23 medium for 0.5–1 h at 38.6 °C in a humidified atmosphere of 5% CO_2. We transferred fused couplets as soon as possible to the oviduct of an oestrus-synchronized recipient gilt.

Treatment of recipient sows

Pregnancy was maintained by using a combination of PMSG and hCG. PMSG (1,000 IU) was injected i.m. on day 10 of the oestrous cycle (day 1 being the day of oestrus), hCG was injected i.m. 3–3.5 d later (day 13 of the cycle)[15].

Microsatellite analysis

DNA (25 ng μl^{-1}) from each of the five piglets (24-h tail samples), the four granulosa cell lines mixed to make pool 1 (PGR1, PGR2, PGR3 and PGR4), the recipient sow (54B) and a boar, 'Ranger', that was used for artificial insemination purposes, were sent in individually coded vials to Celera-AgGEN (Davis, California), a company that specializes in parentage verification for swine. The microsatellite analysis consists of a multiplexed set of nine polymorphic porcine loci, each of which consists of different multimers of short tandem repeats (dinucleotides). The polymorphic loci are designated: S0059, S0070, S0122, S0226, SW24, SW72, SW840, SW936 and TNFB (PCR primer sequence information is proprietary to Celera-AgGEN). This multiplex set contains nine different PCR primer pairs, amplified in two PCR reactions, a five-plex and a four-plex. Ten nanograms of template DNA was amplified in each multiplex PCR. The forward primers were labelled on the 5′ end with a fluorescent dye (either FAM, JOE or TAMRA). The entire battery for each DNA sample was loaded in a single lane and co-electrophoresed with the internal size standard GeneScan 350 Rox. All multiplexing and loci evaluations were performed on an ABI PRISM 377 DNA Sequencer and analysed with Genotyper 2.0 software.

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Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice

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Animal donors such as pigs could provide an alternative source of organs for transplantation. However, the promise of xenotransplantation is offset by the possible public health risk of a cross-species infection[1,2]. All pigs contain several copies of porcine endogenous retroviruses (PERV)[3,4], and at least three variants of PERV can infect human cell lines *in vitro* in co-culture, infectivity and pseudotyping experiments[3,5–7]. Thus, if xenotransplantation of pig tissues results in PERV viral replication, there is a risk of spreading and adaptation of this retrovirus to the human host. C-type retroviruses related to PERV are associated with malignancies of haematopoietic lineage cells in their natural hosts[8]. Here we show that pig pancreatic islets produce PERV and can infect human cells in culture. After transplantation into NOD/SCID (non-obese diabetic, severe combined immunodeficiency) mice, we detect ongoing viral expression and several tissue compartments become infected. This is the first evidence that PERV is transcriptionally active and infectious cross-species *in vivo* after transplantation of pig tissues. These results show that a concern for PERV infection risk associated with pig islet xenotransplantation in immunosuppressed human patients may be justified.

Juvenile-onset diabetes mellitus is a major health problem and exogenous insulin therapy is only partially successful in preventing its many complications. Although islet transplantation holds great promise for a cure, the number of potential human pancreas donors are extremely unlikely to provide enough islet tissue to treat the millions of patients worldwide. The xenotransplantation of pig

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