

Short Communication

Two cases of Reciprocal Chromosomal Translocation (4; 7)(p+; q-) (2; 8)(q-; q+) in Piglets Produced by ICSI

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Contents

In this study, the karyotypes of 14 piglets from four different litters produced by intracytoplasmic sperm injection (ICSI) and embryo transfer were analysed. The chromosome analysis was based on a classical cytogenetic examination following the standard protocols of lymphocyte cultures. Two cases of reciprocal translocation [(4; 7)(p+; q-) and (2; 8)(q-; q+)] were detected in two female transgenic piglets. These animals showed neither anatomical nor physiological alterations and had normal growth. To our knowledge, this is the first karyotype study of piglets produced by ICSI.

Introduction

Intracytoplasmic sperm injection involves the fertilization of oocytes in metaphase II by injecting a single sperm into the cytoplasm. This technique was first described in hamster, and since then, ICSI procedure has been used for *in vitro* production (IVP) in several livestock species with a variable efficiency (reviewed by García-Roselló et al. 2009). It has formed a great contribution to reproduction of mammalian species for scientific study and commercial benefit, increasing the number of offspring from selected high-quality animals and allowing production of cloned and transgenic animals (reviewed by Galli and Lazzari 2008; Gadea and García-Vázquez 2010). ICSI efficiency in pig is still very low, and there have been only a few litters with a small number of piglets born using the ICSI technique (Galli and Lazzari 2008; García-Roselló et al. 2009).

Previous studies have analysed the incidence of chromosomal abnormalities in pigs under commercial conditions (Ducos et al. 2008), in porcine embryos produced *in vivo* (van der Hoeven et al. 1985; Zudova et al. 2003) and *in vitro* by IVF (Ulloa Ulloa et al. 2008; Hornak et al. 2009) and ICSI (Park et al. 2005). However, to our knowledge, karyotype studies of the animals generated using ICSI have not been conducted. This study reports the cytogenetic analysis of 14 piglets born by ICSI and sperm-mediated gene transfer (ICSI-SMGT) method.

Material and methods

The animals used in this study came from embryos obtained through the ICSI-SMGT, which were transferred immediately to four recipient gilts. In brief, the *in vitro* matured porcine oocytes were injected with a spermatozoon previously incubated with linealized EGFP (Enhanced Green Fluorescent Protein) transgene

(García-Vázquez et al. 2009; García-Vázquez et al. 2010). Approximately 100 injected oocytes were surgically transferred to the oviducts of recipient pre-puberal females previously synchronized with hormonal treatment (García-Vázquez et al. 2010).

Peripheral blood samples of piglets were obtained from 2 months of age for the morphological analysis of chromosomes. The chromosome analysis was based on a classical cytogenetic examination following the standard protocols of lymphocyte cultures, Leishman staining and Giemsa-Trypsin-Leishman (GTL) banding techniques (Rodríguez et al. 2010). Metaphase was examined under an Olympus microscope and analyses made using CytoVision Genus[®] (Applied Imaging, Sunderland, UK) and Ikaros (MetaSystems, Altlußheim, Germany) software. To make the pairing, guidelines established by the Committee for the standardization of karyotype of domestic swine (*Sus Scrofa* SSC) were followed, examining at least 10 metaphasic plates per animal.

Results

A total of 14 piglets (nine females and five males) were analysed. Metaphase analysis of cultured peripheral blood lymphocytes allowed the identification of two female transgenic piglets from different litters carrying a reciprocal chromosome translocation (14.28% prevalence). In case 1, the q-arm of one of the number 7 chromosomes appeared shortened, with the absent distal fragment found at the tip of the p-arm of one of the number 4 chromosomes (Fig. 1a; Fig. S1). In case 2, the q-arm of one of the number 2 chromosomes appeared shortened, with the absent distal fragment found at the top of the q-arm of one of the number 8 chromosomes (Fig. 1b; Fig. S1). The chromosomal rearrangement was described according to standard nomenclature as 38, XX, rcp (4; 7)(p+; q-) and 38 XX, rcp (2; 8)(q-; q+). These animals showed neither anatomical nor physiological alterations and had normal growth. The remaining 12 piglets evaluated were cytogenetically normal (38, XX; 38 XY).

Discussion

The prevalence of balanced structural constitutional chromosomal rearrangements in commercial pigs is 0.47% (Ducos et al. 2007). However, IVP of embryo techniques is associated with a higher incidence of

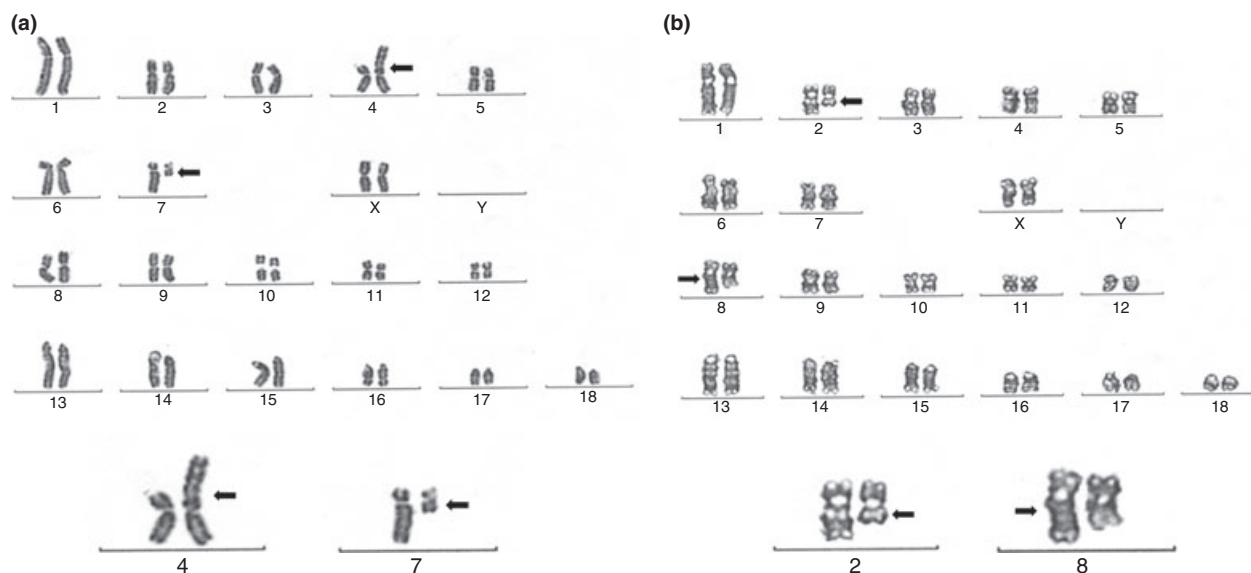


Fig. 1. GTL banding karyotype showing the reciprocal translocations: (a) rcp (4; 7)(p+; q-), (b) rcp (2; 8)(q-; q+) and a detail of the latter. Arrows indicate the chromosomal breakpoints

embryonic and foetal loss (Hyttel et al. 2000), and some authors suggest the *in vitro* conditions of culture could increase the prevalence of cytogenetic abnormalities. In this sense, cytogenetic analysis of porcine blastocysts produced *in vitro* by IVF has revealed that 39.1–45.9% of the blastocysts are chromosomally abnormal embryos (Ulloa Ulloa et al. 2008; Hornak et al. 2009) showing that these embryos can develop up to the blastocyst stage despite having these abnormalities. This percentage is higher than the 7.3–11.4% reported for *in vivo*-derived embryos (van der Hoeven et al. 1985; Zudova et al. 2003). It had been reported in several species a higher incidence of embryos with structural chromosome aberrations using ICSI in comparison with IVF method (Tateno and Kamiguchi 2007).

In pigs, the most frequently reported chromosomal rearrangements are reciprocal translocations, as a characteristic feature of this species (Ducos et al. 2007). However, to our knowledge, this is the first time that the reciprocal translocation detected in our study [(2q-; 8q+) and (7q-; 4p+)] have been reported in pigs. In general terms, reciprocal translocations will produce a high proportion of genetically unbalanced gametes leading to an early mortality of the embryos produced (Villagómez and Pinton 2008).

There are several possible explanations for this observation. In ICSI procedures, the acrosome is usually introduced into the oocyte when incapacitated spermatozoa are injected. It has been shown that the acrosome can alter sperm chromatin remodelling in the ooplasm following ICSI in mouse, pig and rhesus monkey (Tateno 2008). In addition, ICSI using membrane-damaged spermatozoa could cause structural chromosome aberrations in resultant embryos (Tateno et al. 2000). The occurrence of structural chromosome aberrations in mouse ICSI embryos was also dependent on the kind of medium and sperm incubation time (Tateno and Kamiguchi 2007). In this ICSI-SMGT study, the

sperm were incubated with exogenous DNA; it has been suggested that treatment of spermatozoa with exogenous DNA leads to paternal chromosome damage in the zygote (Szczygiel et al. 2003).

The incidence of chromosomal abnormalities obtained in this study using IVP of embryos by ICSI (14.28%) was higher than reported for human where approximately 5% of children born by ICSI carry a risk of chromosomal abnormalities (Bonduelle et al. 1999). This result may support previous studies in mouse (Tateno 2008) and humans (Bonduelle et al. 1999), but the present data seem to be too small to conclude a high prevalence of chromosome anomaly in swine ICSI. Further studies must be carried out to confirm these preliminary observations.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

FA García-Vázquez and J Gadea had contributions to research design, piglets production, drafted the manuscript. I Hernández-Caravaca assisted in piglets production, document revision and responsible for the animal material. M Martín, E Gómez, A Rodríguez and R Sánchez-Sánchez assisted in cytogenetic analysis, interpretation of data and document revision.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. GTL banding karyotype showing the reciprocal translocations and ideograms of rearranged chromosomes: a) $\text{rcp}(4;7)(\text{p}+; \text{q}-)$, b) $\text{rcp}(2;8)(\text{q}-; \text{q}+)$.

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References

- Bonduelle M, Camus M, De Vos A, Staessen C, Tournaye H, Van Assche E, Verheyen G, Devroey P, Liebaers I, Van Steirteghem A, 1999: Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. *Hum Reprod* **14**(Suppl. 1), 243–264.
- Ducos A, Berland H, Bonnet N, Calgareo A, Billoux S, Mary N, Garnier-Bonnet A, Darré R, Pinton A, 2007: Chromosomal control of pig populations in France: 2002–2006 survey. *Genet Sel Evol* **39**, 583–597.
- Ducos A, Revay T, Kovacs A, Hidas A, Pinton A, Bonnet-Garnier A, Molteni L, Slota E, Switonski M, Arruga M, van Haeringen W, Nicolae I, Chaves R, Guedes-Pinto H, Andersson M, Iannuzzi L, 2008: Cytogenetic screening of livestock populations in Europe: an overview. *Cytogenet Genome Res* **120**, 26–41.
- Gadea J, García-Vázquez FA, 2010: Methodologies for generating transgenic pigs: Revista de la asociación interprofesional para el desarrollo agrario-ITEA. **106**, 31–45.
- Galli C, Lazzari G, 2008: The manipulation of gametes and embryos in farm animals. *Reprod Domest Anim* **43**(Suppl. 2), 1–7.
- García-Roselló E, García-Mengual E, Coy P, Alfonso J, Silvestre M, 2009: Intracytoplasmic sperm injection in livestock species: an update. *Reprod Domest Anim* **44**, 143–151.
- García-Vázquez FA, Ruiz S, Matas C, Izquierdo-Rico M, Grullón L, De Ondiz A, Vieira L, Aviles-Lopez K, Gutierrez-Adan A, Gadea J, 2010: Production of transgenic piglets using ICSI-SMGT in combination with recombinase RecA. *Reproduction* **140**, 259–272.
- García-Vázquez FA, García-Roselló E, Gutiérrez-Adán A, Gadea J, 2009: Effect of sperm treatment on efficiency of EGFP-expressing porcine embryos produced by ICSI-SMGT. *Theriogenology* **72**, 506–518.
- van der Hoeven F, Cuijpers M, de Boer P, 1985: Karyotypes of 3- or 4-day-old pig embryos after short in-vitro culture. *J Reprod Fertil* **75**, 593–597.
- Hornak M, Hulinska P, Musilova P, Kubickova S, Rubes J, 2009: Investigation of chromosome aneuploidies in early porcine embryos using comparative genomic hybridization. *Cytogenet Genome Res* **126**, 210–216.
- Hyttel P, Viuff D, Laurincik J, Schmidt M, Thomsen P, Avery B, Callesen H, Rath D, Niemann H, Rosenkranz C, Schellander K, Ochs R, Greve T, 2000: Risks of in-vitro production of cattle and swine embryos: aberrations in chromosome numbers, ribosomal RNA gene activation and perinatal physiology. *Hum Reprod* **15**(Suppl. 5), 87–97.
- Park C, Uhm S, Song S, Kim K, Hong S, Chung K, Park C, Lee H, 2005: Increase of ICSI efficiency with hyaluronic acid binding sperm for low aneuploidy frequency in pig. *Theriogenology* **64**, 1158–1169.
- Rodríguez A, Sanz E, De Mercado E, Gómez E, Martín M, Carrascosa C, Gómez-Fidalgo E, Villagómez D, Sánchez-Sánchez R, 2010: Reproductive consequences of a reciprocal chromosomal translocation in two Duroc boars used to provide semen for artificial insemination. *Theriogenology* **74**, 67–74.
- Szczygiel M, Moisyadi S, Ward W, 2003: Expression of foreign DNA is associated with paternal chromosome degradation in intracytoplasmic sperm injection-mediated transgenesis in the mouse. *Biol Reprod* **68**, 1903–1910.
- Tateno H, 2008: Chromosome aberrations in mouse embryos and fetuses produced by assisted reproductive technology. *Mutat Res* **657**, 26–31.
- Tateno H, Kamiguchi Y, 2007: Evaluation of chromosomal risk following intracytoplasmic sperm injection in the mouse. *Biol Reprod* **77**, 336–342.
- Tateno H, Kimura Y, Yanagimachi R, 2000: Sonication Per Se Is Not as Deleterious to Sperm Chromosomes as Previously Inferred. *Biol Reprod* **63**, 341–346.
- Ulloa Ulloa C, Yoshizawa M, Komoriya E, Mitsui A, Nagai T, Kikuchi K, 2008: The blastocyst production rate and incidence of chromosomal abnormalities by developmental stage in *in vitro* produced porcine embryos. *J Reprod Dev* **54**, 22–29.
- Villagómez D, Pinton A, 2008: Chromosomal abnormalities, meiotic behavior and fertility in domestic animals. *Cytogenet Genome Res* **120**, 69–80.
- Zudova D, Rezacova O, Kubickova S, Rubes J, 2003: Aneuploidy detection in porcine embryos using fluorescence *in situ* hybridization. *Cytogenet Genome Res* **102**, 179–183.

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