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ESTABLISHMENT OF PREGNANCIES IN LARGE WHITE SOW RECIPIENTS AFTER TRANSFER OF CLONED EMBRYOS OF DIFFERENT PIG BREEDS

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Introduction

Production of cloned piglets using normal or transgenic donor cells has been of limited efficiency. One reason could be breed differences between recipient and embryos. The aim of this study was to compare pregnancies after transfer to Large White (LW) sow recipients of cloned LW embryos or cloned, transgenic minipig embryos.

Materials and methods

Large White donor cells were from LYxD, and minipig cells were transgenic with 1 of 5 genes related to different human diseases and from either Göttingen or Yucatan. The cells were used on Day 0 for handmade cloning and were cultured *in vitro* until transfer on Day 5 to 6. As recipients, 70 LW sows were weaned and anaesthetized 4 days after natural heat. Through an abdominal incision the embryos were slowly introduced into the uterus via a catheter inserted 5 to 6 cm into the tip of the horn. To each of 35 recipients, 40 to 100 LW embryos were transferred, and 37 recipients each received 50 to 100 transgenic minipig embryos. Pregnancies were examined by ultrasound scanning every second week. Abortions were defined as absence of earlier confirmed scanning or delivery of aborted fetuses. Caesarean sections were performed on Day 114 (minipig) or Day 116 (LW) 24 hr after injection of a prostaglandin analogue. At delivery, placental gross morphology was recorded with samples taken for later histology. The piglets were fed every 3 hr with colostrum for the first 12-24 h and then by the recipient LW sow (Figure 1). Data was analyzed by Fisher's Exact test with a significance level of $P < 0.05$.

Figure 1. Mini piglets at their LW recipient sow

Table 1. Pregnancies and deliveries

Type of Embryos	No of transfers to LW sows	Pregnancies	Abortions	Deliveries/transfer
LW	35	16 46%	2	14 40%
Mini	37	20 54%	8	12 32%
Total	72	36 50%	10	26 36%

Figure 2. Oedematous placenta

Results

The overall pregnancy rate (see Table 1) had an abortion rate of 28% (8 aborted + 2 resorbed/34) from Day 30 to 45, giving 36% deliveries of the transfers. There was no difference between minipig and LW pregnancy results, although there tended to be more abortions with minipig pregnancies ($P = 0.133$) resulting in 12 minipig and 14 LW litters. Mean no. of piglets/no. transferred embryos was $7.1 \pm 0.6\%$. The total litter sizes ranged from 1 to 12 piglets and the minipig litter size tended to be the highest ($p=0.089$) (Table 2). The perinatal mortality of all piglets was 45/124 (36%) and thus higher than that on the farm (15-20%). In almost every recipient the placenta showed abnormal thick and oedematous morphology (Figure 2). The piglets appeared normal with respect to birth weight and weight gain, but in 7 of the litters some of the piglets showed malformations such as syndactyly, chryptorchidism, diaphragmatic hernia or joint contractures (Figure 3).

Discussion and conclusion

These results demonstrate that, in spite of a rather high abortion rate and perinatal mortality, an acceptable number of viable piglets can be achieved after transfer to LW recipients of cloned LW embryos as well as cloned, transgenic minipig embryos. Therefore a breed difference between the embryos and their recipient sow seems not to influence the pregnancy results.

Table 2. Piglet mortality and bodyweight

Type of embryos	No of litters	Litters with 1-6 dead piglets	Born alive	Born dead	Dead < 10 days	All born	mean bodyweight kg	Full term piglets/litter
LW	14	4	45	12 +3 mummies	7	57 +3 mummies	1.65 ± 0.07	4.1 ± 0.6 (1-12)
Mini	12	6	56	11 +9 mummies	15	67 +9 mummies	0.78 ± 0.03	5.6 ± 0.9 (1-12)
Total	26	10	101	23 +12 mummies	22	124 +12 mummies		4.8 ± 0.6 (1-12)

Figure 3. Joint contracture of forelegs

ESTABLISHMENT OF PREGNANCIES IN LARGE WHITE SOW RECIPIENTS AFTER TRANSFER OF CLONED EMBRYOS OF DIFFERENT PIG BREEDS

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Production of cloned piglets, using normal or transgenic donor cells, has been of limited efficiency. One reason could be breed differences between recipient and embryos. The aim of this study was to compare pregnancies after transfer to Large White (LW) sow recipients of cloned LW embryos or cloned, transgenic minipig embryos. Large White donor cells were from LYxD, and minipig cells were transgenic with 1 of 5 genes related to different human diseases and from either Göttingen or Yucatan. The cells were used on Day 0 for handmade cloning (Du *et al.* 2005 Cloning Stem Cells **7**, 199–205). The reconstructed embryos were cultured *in vitro* until transfer on Day 5 to 6. As recipients, 70 LW sows were weaned and anesthetized 4 days after natural heat. Through an abdominal incision the ovaries were controlled (CL formation, absence of cysts) and the embryos slowly introduced into the uterus via a catheter inserted 5 to 6 cm into the tip of the upper horn. To each of 33 recipients, 40 to 60 LW embryos were transferred, and 37 recipients each received 50 to 100 transgenic minipig embryos. Pregnancies were examined by ultrasound scanning every second week. Abortions were defined as absence of earlier confirmed scanning or delivery of aborted fetuses. Caesarean sections were performed on Day 114 (minipig) or Day 116 (LW) 24 h after injection of a prostaglandin analogue. At delivery, placental gross morphology was recorded with samples taken for later histology. The piglets were fed every 3 h with colostrum for the first 24 h and then by the recipient LW sow. Data were analyzed by Fisher's Exact test with a significance level of $P < 0.05$. The overall pregnancy rate was 49% (34/70) with an abortion rate of 29% (8 aborted + 2 resorbed/34) from Day 30 to 45 giving 24/70 deliveries (34% of the transfers). There was no significant difference between minipig (54%, 20/37) and LW pregnancy results (42%, 14/33), although there tended to be more abortions with minipig pregnancies (8/20 v. 2/14; $P = 0.14$) resulting in 12 minipig and 12 LW litters of which 4 and 9, respectively, have grown up to adulthood. In almost every recipient the placenta and fetal membranes showed abnormal thick and edematous morphology. The total litter sizes ranged from 1 to 10 piglets (mean 4.4 ± 0.6), and in 13 of 24 litters there were 1 to 5 stillborn piglets. Except for one litter of 9 transgenic Yucatan piglets that all died within their first 2 weeks, the postnatal mortality of both LW- and mini-piglets seemed similar to that of farm piglets of the same age, and the piglets appeared normal with respect to weight gain, gross morphology, and behavior. These results demonstrate that, in spite of a rather high abortion rate and some fetal mortality, an acceptable birth rate can be achieved after transfer to LW recipients of cloned LW embryos (36%) as well as cloned, transgenic minipig embryos (32%). Therefore, a breed difference between the embryos and their recipient seems not to influence the pregnancy results.

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