10

Expression of Insulin-like Growth Factor-I in Skeletal Muscle of Transgenic Swine

V.G. Pursel[1], R.J. Wall[1], A.D. Mitchell[1], T.H. Elsasser[1], M.B. Solomon[1], M.E. Coleman[2], F. DeMayo[3] and R.J. Schwartz[3]

[1]*USDA-ARS, Beltsville, Maryland, USA;* [2]*GeneMedicine, Inc., The Woodlands, Texas, USA;* [3]*Baylor College of Medicine, Houston, Texas, USA*

Although growth hormone is considered the primary growth-promoting hormone in mammals, many of its effects are thought to be mediated by insulin-like growth factor-I (IGF-I), which is a potent mitogen that stimulates cell proliferation and synthesis of DNA and protein. The aim of this research was to determine whether directing expression of IGF-I specifically to striated muscle would enhance lean muscle growth in swine. Transgenic pigs were produced by microinjection of zygotes with a fusion gene consisting of the regulatory sequences of an avian skeletal α-actin gene and a cDNA encoding human IGF-I. All but one of 13 transgenic pigs expressed the IGF-I transgene. Muscle IGF-I concentrations varied from 20 to 1702 ng g^{-1} muscle in transgenic pigs compared with less than 10 ng g^{-1} muscle in control pigs. Muscle IGF-I concentrations were in general agreement with abundance of IGF-I mRNA on Northern blots. Serum IGF-I concentrations in transgenic pigs (160 ± 6.8 ng ml^{-1}) did not differ from that of littermate control pigs (143 ± 6.5 ng ml^{-1}). Daily weight gain from 20 to 60 kg body weight was similar for transgenic and littermate control pigs (865 ± 29.6 g vs. 876 ± 18.9 g day^{-1}). Body composition of eight transgenic and eight control pigs was estimated by X-ray absorptiometry (DXA) scanning at 60 kg body weight. The DXA results indicated transgenic female pigs had significantly less fat and more lean tissue than female littermate controls (P <0.05 for each). However, body composition of transgenic and control boars did not differ. Subsequently nine of ten founders transmitted their transgene to G1 progeny, which will be used for evaluation of growth rate, feed efficiency and carcass composition. Transgenic and control pigs did not differ in general appearance, and no gross abnormalities, pathologies or health-related problems were encountered. Based on these results we conclude that enhancing IGF-I specifically in skeletal muscle may have a positive effect on carcass composition of swine.

 Transgenic Animals in Agriculture
(eds J.D. Murray, G.B. Anderson, A.M. Oberbauer and M.M. McGloughlin)

Introduction

Endocrine control of normal tissue development and growth in mammals is complex. A group of peptide hormones, i.e. growth hormone-releasing factor (GRF), somatostatin, growth hormone (GH), insulin, insulin-like growth factor-I (IGF-I), insulin-like growth factor-II (IGF-II), thyrotrophic hormone and gonadotrophic hormones work in concert to regulate and coordinate the metabolic pathways responsible for tissue formation and development. Even though correlations between growth and circulating levels of some of these peptide hormones have often produced conflicting results, the preponderance of data indicates that genetic capacity for growth is related to increased circulating levels of GH and IGF-I (Sejrson, 1986). Furthermore, injection of pigs, sheep and cattle with exogenous GH and stimulation of GH secretion in lambs by immunizing them against somatostatin have generally produced enhanced feed efficiency, increased growth rate, and reduced subcutaneous fat. These findings all suggest the possible usefulness of manipulating genes for the peptide hormones to modify the growth characteristics and carcass composition of farm animals.

Transfer of GH fusion genes has received the bulk effort in growth-related transgenic research with farm animals (see review by Pursel and Rexroad, 1993), however, IGF-I mediates many of the effects of GH, so enhanced expression of IGF-I may alter growth characteristics without such a dramatic affect on the systemic physiology of the animal. IGF-I is a mitogenic peptide that plays an important role in differentiation and postnatal development (Zapf *et al*., 1984). IGF-I has the potential of acting as an endocrine agent when its synthesis and secretion by the liver is stimulated by GH. In addition, elevated GH stimulates IGF-I synthesis in numerous tissues throughout the body where it may act locally as a paracrine and/or autocrine agent. The relative importance of these two modes of action in regulation of growth is unknown.

Mathews *et al*. (1988) produced a single line of human (h) IGF-I transgenic mice in which a mouse metallothionein (mMT) promoter was used to increase serum IGF-I by 50–60% above that of littermate control mice. This IGF-I expression resulted in a 1.3-fold increase in body weight in comparison with littermate control mice without an increase in skeletal growth. The elevated plasma IGF-I was effective in feeding back on the hypothalamus to inhibit GH synthesis and secretion, thus depressing serum GH to non-detectable levels, and may have confounded effects of IGF-I on muscle mass. Subsequently, the same mMT–hIGF-I construct was transferred into four pigs, only one of which expressed the transgene. Unfortunately, this pig died at an early age before growth performance could be evaluated (Pursel *et al*., 1989). Reiss *et al*. (1996) transferred hIGF-I under control of a rat α-myosin heavy chain promoter into mice to direct IGF-I expression

specifically to the heart. This strategy elevated plasma IGF-I by 84%, and increased body weight and heart weight compared with littermate control mice by about 15% and 50%, respectively.

Coleman *et al*. (1995) constructed an α-skeletal actin–hIGF-I transgene to direct IGF-I expression specifically in skeletal muscle of transgenic mice. The rationale behind this approach was to direct sufficient expression of IGF-I in skeletal muscle to act as a paracrine mitogenic agent without altering plasma IGF-I concentration sufficiently to have a systemic endocrine effect and depress growth hormone secretion and release.

A line of IGF-I transgenic mice was evaluated that had a 47-fold higher concentration of IGF-I per gramme of muscle than that of control mice. Over expression of IGF-I elicited hypertrophy of all classes of myofibres and a shift in myofibre type toward more oxidative fibre types. Neither IGF-I concentrations in the serum nor body weight were significantly increased in transgenic mice compared with sibling control mice.

This α-skeletal actin–hIGF-I transgene has now been transferred into swine to investigate its potential for improving productivity. This communication describes the production and characterization of the founder IGF-I transgenic pigs and the successful transmission of the fusion gene to G1 progeny.

Material and Methods

Animals

Seventy-six sexually mature DK-43 hybrid gilts (Dekalb Swine Breeders, Inc., Dekalb, Illinois) that had previously displayed oestrus two or more times were used for the experiment. Gilts were penned and fed outdoors in groups of ten, and checked for oestrus once daily with a mature boar before assignment to experimental use. The gilts were confined to a gestation stall for individual feeding of altrenogest (Regu-Mate®, Hoechst Roussel Pharmaceuticals Inc., Somerville, New Jersey). Experimental protocols used in this research were approved by the Beltsville Area Institutional Animal Care and Use Committee.

Synchronization of oestrus and ovulation

Oestral cycles of all ovum donors and recipients were regulated by feeding 0.17 mg altrenogest per kg body weight daily for 5 or 6 days starting on day 11–15 of the oestral cycle (onset of oestrus = day 0).

Donors received 1000, 1200 or 1500 IU eCG (Diosynth Inc., Chicago, Illinois) by subcutaneous injection at 29 h and 750 IU hCG by intramuscular (i.m.) injection at 111 h after the last feeding of altrenogest. Donor gilts were

bred by natural service or artificially inseminated twice between 7 and 33 h after the hCG injection. Twenty-eight donor gilts (designated D-R) were also used as recipients of microinjected zygotes that had previously been recovered from other donors. Micromanipulated ova were transferred into these donors immediately after their ova had been flushed from the oviducts.

Control recipient gilts (C-R) were injected with 750 IU hCG at 125 h after a last feeding of altrenogest (C-R gilts were last fed altrenogest 8 h before and were injected with hCG 6 h after donor gilts).

Ovum recovery, microinjection and transfer

Ova were recovered from donors between 50 and 55 h after the hCG injection. Anaesthesia was induced by administering the following per 100 kg body weight: 400 mg Ketamine HCl (Ketaset®, Aveco Co., Fort Dodge, Iowa); 200 mg xylazine (Rompun®, Haver Lockhart, Bayvet Division, Miles Laboratories, Shawnee, Kansas); 100 mg Telozol® (50 mg tiletamine HCl and 50 mg zolazepam HCl, Aveco Co., Fort Dodge, Iowa); 4 mg butorphanol tartrate (Torbugesic®, Fort Dodge Laboratories, Fort Dodge, Iowa); and 6 mg atropine sulphate (Butler Company, Columbus, Ohio). One-fifth of the anaesthetic dose was administered i.m. and the remaining dose was administered intravenously (i.v.) after 10 min. Oviducts were exteriorized by midventral laparotomy, and each oviduct was retrograde flushed with 20 ml of Beltsville embryo culture medium (without 5% CO_2), which was the only medium used for ovum culture from recovery to transfer (Pursel and Wall, 1996).

Ova were centrifuged at 15,000 $\times$ *g* for 10 min to permit visualization of the pronuclei (Wall *et al.*, 1985). One pronucleus of each zygote was microinjected with several hundred copies of the transgene. Within 2 h after microinjection, 12–35 injected ova were transferred into one or both oviduct of either D-R gilts or C-R gilts. Ova were recovered from four to six donors each day. Ova from early recoveries were transferred into subsequent donors (D-R), and ova from later recoveries were transferred into C-R gilts.

Gene construct

The fusion gene used for microinjection (Fig. 10.1) was composed of the avian skeletal α-actin (α-SkA) promoter −424 to +1, the natural capsite and the 5′-UTR, exon 1, first intron and portions of exon 2 up to the initiation ATG joined to human IGF-I class 1a cDNA and the avian skeletal α-actin 3′ UTR and contiguous 1.5 kb of non-coding sequences (Coleman *et al.*, 1995).

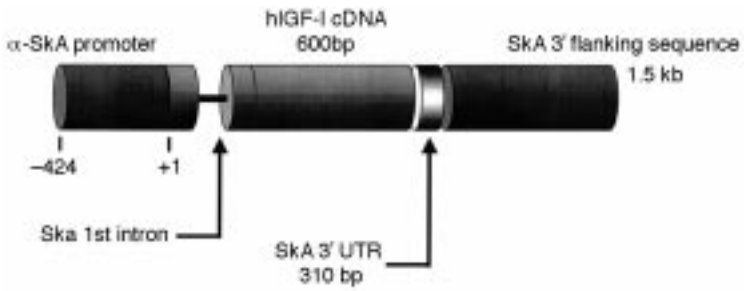

Fig. 10.1. Schematic diagram of the skeletal α-actin–IGF-I fusion gene. The construct was composed of the avian skeletal α-actin promoter −424 to +1, the natural capsite (+1), the 5′-UTR (exon 1, 60 bp), first intron (line, 123 bp), portions of exon 2 up to the initiation ATG (15 bp), human IGF-I cDNA (504 bp), skeletal α-actin 3′-UTR (310 bp) and contiguous 1.5 kb non-coding flanking sequences.

Farrowing

Recipients were brought into the farrowing barn on day 108 of pregnancy. One day after birth, the tip of each pig's tail was removed for DNA analysis by PCR. Pigs that were found to be positive for the transgene by PCR were subsequently confirmed by Southern blot hybridization analysis.

Animals and diets

At weaning (day 28) each transgenic was paired with a non-transgenic littermate of the same sex and housed in a nursery with free-choice feed and water. At about 20 kg body weight pigs were moved from the nursery and housed with two to four pigs per pen. Body weights were recorded at weekly intervals. The pigs were given *ad libitum* access to a pelleted corn–soybean meal diet containing 3.5 Mcal of digestible energy, 18% crude protein and 1.2% lysine until they reached 60 kg body weight, at which time they were fed at about 85% of *ad libitum*.

Carcass composition

At approximately 60 kg body weight, the mass of carcass fat, bone and lean of eight transgenic and eight littermate control pigs was estimated by dual-energy X-ray absorptiometry (DXA) analysis (Mitchell *et al*., 1996). Pigs were fasted overnight and then anaesthetized as indicated above to prevent movement during the scanning procedure. Total body DXA scans were

performed using a Lunar DPX-L instrument (Lunar, Madison, Wisconsin). The pig was positioned on its sternum with hindlegs extended caudally and forelegs held laterally away from the chest with foam rubber. The DXA fat measurements were adjusted to correct for previously reported inaccuracies using the formula % fat = 493 − [(348.8) (R_{st})], where R_{st} is the DXA soft tissue attenuation ratio. Bone content was calculated from DXA bone mineral (BMC) values (bone = BMC × 4.14) based on the 24.14% ash content of pork bones reported by Field *et al.* (1974). Lean content (including internal organs, connective tissue and gut fill) was calculated by subtracting fat and bone weights from total body weight.

Muscle and plasma IGF-I assay

A muscle biopsy was recovered from the longissimus muscle of each transgenic pig when they reached about 90 kg body weight using the biopsy device described by Schöberlein (1989). The tissue was frozen in liquid nitrogen immediately after biopsy and maintained at −70°C until protein and mRNA were extracted. Each muscle sample (100 mg wet weight) was lyophilized and powdered before homogenization in 1 ml 1 M acetic acid using a bead beater (Biospec Products Inc., Bartlesville, Oklahoma). The homogenized samples were held at −20°C for 1 h, centrifuged in a microfuge for 10 min, the supernatant transferred into a new tube and lyophilized to dryness. Each sample was solubilized in 0.1 × volume of 25 mM Tris-Cl (pH 8.0) and 1% (v/v) triton, and clarified by centrifugation before assay. The concentration of IGF-I was determined using 50 µl per assay by the non-competitive, two-site IRMA assay (kit DSL-5600, Diagnostic Systems Laboratories, Webster, Texas). Rat muscle spiked with rhIGF-I was used as a standard.

Total RNA was isolated from tissues by selective precipitation from phenol-extracted, guanidine thiocyanate homogenates. Northern blots were prepared as previously described by Coleman *et al.* (1995). Total RNA (10 µg) isolated from longissimus muscle biopsies was fractionated on agarose–formaldehyde gels and transferred to Gene Screen nylon membranes (Dupont NEN). Blots were hybridized with a ^{32}P-labelled hIGF-I probe and exposed overnight without intensifying screens.

Blood was collected from the anterior vena cava from each transgenic and control pig using a syringe that contained ethylenediamine tetraacetic acid (EDTA) to prevent coagulation. Plasma was separated by centrifugation and stored at −70°C until assayed. Plasma IGF-I was measured by double antibody RIA using rabbit anti-human IGF-I (UBK-487, NIDDK, Bethesda, Maryland) as the primary antiserum and recombinant human IGF-I (for standard and iodination tracer). Samples were prepared using the acid glycylglycine acidification technique (Elsasser *et al.*, 1988). The samples were all evaluated in a single assay. The intra-assay coefficient of variation was 6.7%.

Mating for G1 progeny

When transgenic gilts reached approximately 180 days of age they were housed in outside pens and oestrus was checked once daily with a mature boar. At the second or a subsequent oestrus transgenic gilts were mated to a non-transgenic boar (Orange Line hybrid, Dekalb Swine Breeders, Inc., Dekalb, Illinois).

When transgenic boars reached 8 months of age each boar was mated to two or three non-transgenic DK-43 hybrid gilts. When possible, each boar was mated twice per oestrus to each gilt.

Statistical analysis

Data were analysed using GLM procedures (SAS, 1988). The model used for analysis of birth weight, weaning weight, daily gain, body fat and plasma IGF-I concentrations consisted of the main effects of genotype, sex and resulting interactions with the pig considered to be the experimental unit. Means were compared by least significant differences. Frequency of transgene transmission to progeny was analysed using the chi-squared test.

Results and Discussion

Transgene integration

A total of 1207 zygotes were microinjected and transferred into 51 recipient gilts, 23 and 28 of which were C-R and D-R, respectively. Twenty-seven recipients became pregnant (11 C-R and 16 D-R) and produced 167 piglets (Table 10.1). The transgene integrated in 17 piglets, as determined by PCR and Southern blot hybridization, of which 14 and 13 were alive at birth and weaning, respectively. The 1.4% integration efficiency is well within the range of efficiencies reported by others (Ebert and Schindler, 1993).

Expression of IGF-I transgene

Expression of the transgene hIGF-I mRNA in longissimus muscle was detected in 9/12 transgenic pigs (Fig. 10.2). A subsequent Northern assay indicates that pig no. 50403, which was not included (Fig. 10.2), was expressing IGF-I at a level similar to pig no. 52006. In contrast, IGF-I concentrations in longissimus muscle were elevated more than twofold above control muscle in 11/12 transgenic pigs when expression was evaluated by the IRMA assay procedure after tissue extraction (Fig. 10.3). For the nine pigs in which IGF-I mRNA was detected, the quantity of hIGF-I

Table 10.1. Production of αACT-IGF-I transgenic founder pigs.

Item	Totals
No. of zygotes injected and transferred	1207
No. of recipients	51
No. of recipients farrowing	27
% of recipients farrowing	52.9
No. of pigs born (live)	167 (145)
No. of pigs per litter	6.2
% of injected zygotes developing (preg. gilts)	14 (26)
No. of transgenic pigs born (live)	17 (14)
% of pigs transgenic	10.2
% of injected zygotes	1.4

mRNA observed in the Northern blot was in general agreement with IGF-I concentrations found by the IRMA assay.

According to the assay results shown in Fig. 10.3, the range in levels of transgene expression among founders varied from about twofold to more than 170-fold above the IGF-I concentration detected in littermate control pigs (<10 ng IGF-I g^{-1} longissimus muscle). The wide range in expression

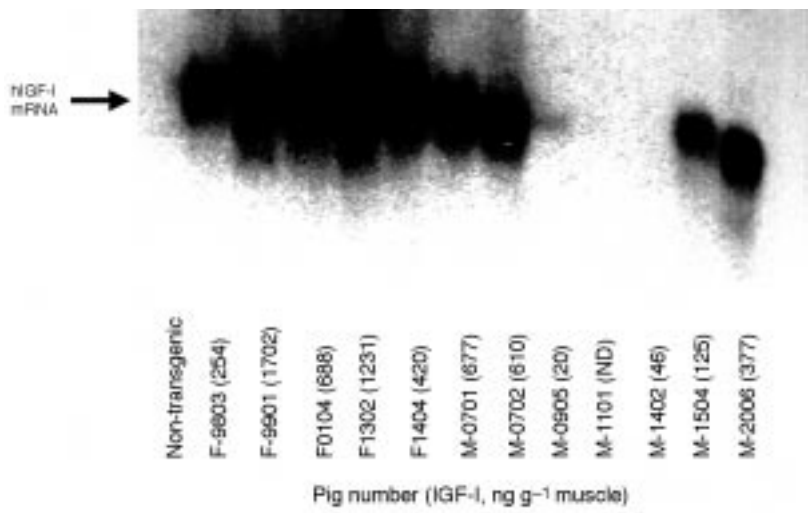

Fig. 10.2. Northern blot of total RNA (10 mg) isolated from longissimus muscle biopsies of five transgenic females (F), seven transgenic males (M), and a control pig (wild-type). The blot was hybridized with a ^{32}P-labelled hIGF-I probe that revealed a major transcript of approximately 1.0 kb IGF-I. For comparison with mRNA, IGF-I concentrations shown in Fig. 10.3 are indicated in parentheses for each animal.

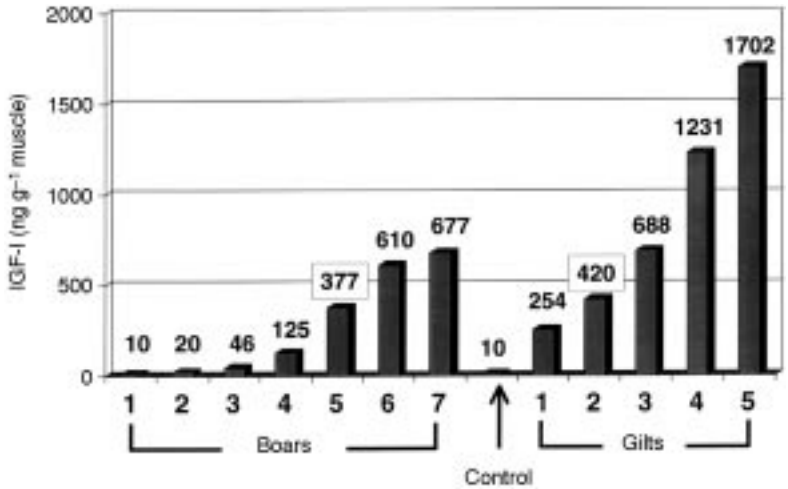

Fig. 10.3. IGF-I concentrations in longissimus muscle (ng IGF-I g^{-1} wet muscle) were determined by non-competitive, two-site IRMA assay.

among a group of transgenic founders is consistent with numerous other transgenes, and is thought to be the result of integration occurring in a wide variety of chromosomal locations, as has been shown by *in situ* hybridization analysis (Shamay *et al*., 1991; Kuipers *et al*., 1997), of mosaicism in some founders, and varying numbers of gene copies integrating (Palmiter and Brinster, 1986).

Plasma IGF-I concentrations of the transgenic founders (160 ± 6.8 ng ml^{-1}) did not differ from their littermates (143 ± 6.5 ng ml^{-1}; $P = 0.09$), but the trend for transgenics to be higher than controls was consistent with previous observations in the IGF-I transgenic mice (Coleman *et al*., 1995).

Growth performance and body composition

The birth and weaning weights of the transgenic pigs did not differ from those of their littermates ($P > 0.10$ for each). The average daily gain from 20 kg to 60 kg body weight for the six transgenic boars and six transgenic gilts that harboured the IGF-I transgene was similar to that of littermate controls (Table 10.2; 865 ± 29.6 vs. 876 ± 18.9 g day^{-1}, $P > 0.10$).

Body composition of transgenic and control female pigs differed significantly at 60 kg body weight according to estimations based on DXA analyses (Table 10.2). Transgenic gilts contained less fat (15.5 vs. 22.2, $P = 0.015$) and more lean tissue (74.5 vs. 68.1, $P = 0.034$) than control gilts. In contrast, body composition of transgenic and control boars did not differ. Estimated bone content was similar for transgenic and control pigs for both

Table 10.2. Least squares mean plasma IGF-I concentrations, daily weight gain and estimated fat content of IGF-I transgenic founder and control pigs.

		Plasma IGF-I		Weight gain day^{-1}[a]		Fat[b]		Lean[b]	Bone[b]
Gene	Sex	*n*	ng ml^{-1} ± SE	*n*	grammes ± SE	*n*	% ± SE	% ± SE	% ± SE
Control	F	6	140 ± 8.7	6	890 ± 36.0	2	22.2 ± 1.78[c]	68.1 ± 1.8[c]	10.3 ± 0.49
Transgenic	F	5	142 ± 9.6	6	886 ± 36.0	2	15.5 ± 1.98[d]	74.5 ± 2.0[d]	10.8 ± 0.55
Control	M	5	147 ± 9.6	6	863 ± 36.0	6	19.7 ± 1.03[d]	69.9 ± 1.0[c,d]	11.1 ± 0.29
Transgenic	M	6	178 ± 9.6	6	843 ± 36.0	6	19.5 ± 1.05[d]	70.1 ± 1.0[c,d]	10.8 ± 0.29

[a] Daily gain from 20 to 60 kg body weight during free-choice feeding.
[b] Estimated by dual energy X-ray absorptiometry at 60 kg body weight.
[c,d] Means in column with differing superscript differ significantly $P < 0.05$; gene × sex interaction for fat was significant at $P = 0.03$.

sexes. While these differences in body composition are based on relatively few animals, preliminary results with progeny produced by these transgenic founders confirm these results (A.D. Mitchell and V.G. Pursel, unpublished observations).

General health

The IGF-I transgenic and control pigs did not differ in physical appearance, general behaviour, ability to tolerate summertime heat stress or reproductive capacity. These observations differ from those reported previously for transgenic pigs expressing bovine growth hormone, who were lethargic, did not tolerate elevated ambient temperatures, and had reproductive problems (Pursel *et al*., 1989).

The general health of the transgenic pigs did not differ from their littermate controls. Of the 14 living transgenic pigs that were born, only one died before weaning; autopsy did not reveal the cause of death. Subsequently, one gilt (no. 1404) died suddenly at 6 months of age with no apparent cause, and another gilt (no. 9803) died just before the expected parturition. At necropsy the latter gilt was diagnosed with endocarditis of heart valves and cardiac haemorrhage. Whether these deaths were associated with expression of the IGF-I transgene in cardiac muscle is unknown. Based on results with this same fusion gene in mice we would expect IGF-I expression in cardiac muscle, but at a lower level than in skeletal muscle (Coleman *et al*., 1995).

Reproduction and gene transmission

Transgenic gilts exhibited their first oestrus at the same age as the littermate controls (213.8 days and 214 days, respectively) and conceived on the first or second breeding. The farrowing results for three transgenic gilts and 17 non-transgenic gilts, which were mated by seven transgenic boars, are presented in Fig. 10.4. In addition, gilt no. 9903 was carrying 11 fully formed pigs at the time of death just before parturition, and gilt no. 1405 became ill after entering the farrowing barn and farrowed two dead pigs after a protracted parturition. The 13 pigs produced by these two gilts were not assayed for presence of the IGF-I transgene.

Nine of ten transgenic founders transmitted the IGF-I transgene to one or more progeny (Fig. 10.4). The rate of transmission by seven of the transgenic pigs did not differ significantly from the 50% expected on the basis of Mendelian inheritance. One transgenic boar (no. 1504) failed to transmit the transgene to his 25 progeny, and two boars (nos 0905 and 0701) transmitted the transgene to significantly less than half of their progeny ($P < 0.01$ and $P < 0.02$, respectively). The failure to transmit and a

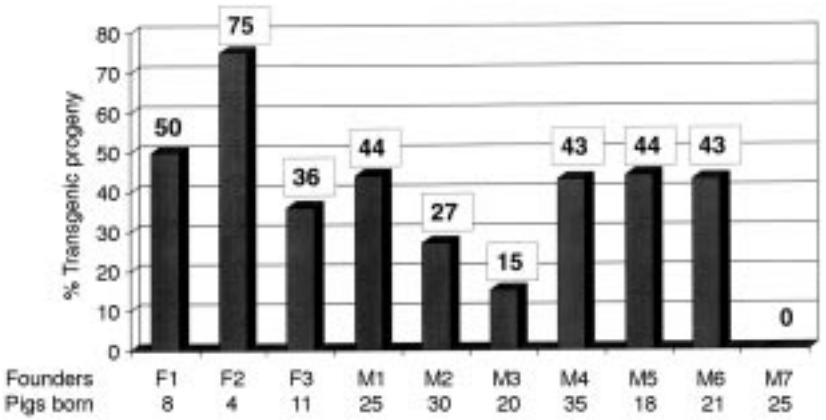

Fig. 10.4. The percentage of progeny that inherited the IGF-I transgene from the founder transgenic parent. The total number of progeny produced by the founder is indicated.

low rate of transmission probably results from transgene mosaicism in germ cells, as reported originally in mice (Palmiter *et al*., 1984) and more recently in pigs (Pursel *et al*., 1990).

Conclusions

The regulatory elements from avian skeletal α-actin gene and 5′ flanking sequences can provide a high level of IGF-I expression in skeletal muscle of transgenic pigs. Elevated IGF-I expression in skeletal muscle did not significantly increase serum IGF-I concentrations, alter the growth rate or adversely impact the general health of transgenic pigs in comparison with their littermate controls. Transgenic founder males and females appeared to have normal reproductive capacity, and 9/10 transgenic founders transmitted their transgene to progeny. These findings agree closely with those obtained when the same fusion gene was transferred into mice (Coleman et al., 1995). Further research is required to determine whether expression of this IGF-I transgene stimulates myofibre hypertrophy as it did in mice.

We believe the present findings, when combined with and compared with other IGF-I transgenic results, provide a strong indication as to whether circulating IGF-I (classical endocrine function) or locally produced IGF-I (paracrine function) is more important in regulation of animal growth. If one contrasts the results of Mathews *et al.* (1988) and Reiss *et al.* (1996), where transgene expression elevated circulating IGF-I (endocrine effect), with that of present results in pigs and in mice (Coleman *et al*., 1995), where IGF-I

expression is directed to skeletal muscle (paracrine effect), it appears that the endocrine effect has considerably more overall impact on growth and development.

References

Coleman, M.E., DeMayo, F., Yin, K.C., Lee, H.M., Geske, R., Montgomery, C. and Schwartz, R.J. (1995) Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofibre hypertrophy in transgenic mice. *Journal of Biological Chemistry* 270, 12109–12116.

Ebert, K.M. and Schindler, J.E.S. (1993) Transgenic farm animals: progress report. *Theriogenology* 39, 121–136.

Elsasser, T.H., Rumsey, T.S., Hammond, A.C. and Fayer, R. (1988) Influence of parasitism in plasma concentrations of growth hormone, somatomedin-C and somatomedin-binding proteins in calves. *Journal of Endocrinology* 116, 191–200.

Field, R.A., Riley, M.L., Mello, F.C., Corbridge, M.H. and Kotula, A.W. (1974) Bone composition in cattle, pigs, sheep and poultry. *Journal of Animal Science* 39, 493–499.

Kuipers, H.W., Langford, G.A. and White, J.G. (1997) Analysis of transgene integration sites in transgenic pigs by fluorescence *in situ* hybridization. *Transgenic Research* 6, 253–259.

Mathews, L.S., Hammer, R.E., Behringer, R.R., D'Ercole, A.J., Bell, G.I., Brinster, R.L. and Palmiter, R.D. (1988) Growth enhancement of transgenic mice expressing human insulin-like growth factor-I. *Endocrinology* 123, 2827–2833.

Mitchell, A.D., Conway, J.M. and Scholz, A.M. (1996) Incremental changes in total and regional body composition of growing pigs measured by dual-energy X-ray absorptiometry. *Growth, Development and Aging* 60, 113–123.

Palmiter, R.D. and Brinster, R.L. (1986) Germline transformation of mice. *Annual Review of Genetics* 20, 465–499.

Palmiter, R.D., Wilkie, T.M., Chen, H.Y. and Brinster R.L. (1984) Transmission distortion and mosaicism in an unusual transgenic mouse pedigree. *Cell* 36, 869–877.

Pursel, V.G. and Rexroad, C.E. Jr (1993) Status of research with transgenic farm animals. *Journal of Animal Science* 71 (Suppl. 3), 10–19.

Pursel, V.G. and Wall, R.J. (1996) Effects of transferred ova per recipient and dual use of donors as recipients on production of transgenic swine. *Theriogenology* 46, 201–209.

Pursel, V.G., Pinkert, C.A., Miller, K.F., Bolt, D.J., Campbell, R.G., Palmiter, R.D. and Brinster, R.L. (1989) Genetic engineering of livestock. *Science* 244, 1281–1288.

Pursel, V.G., Hammer, R.E., Bolt, D.J., Palmiter, R.D. and Brinster R.L. (1990) Genetic engineering of swine: integration, expression and germline transmission of growth-related genes. *Journal of Reproduction and Fertility* 41 (Suppl.), 77–87.

Reiss, K., Cheng, W., Ferber, A., Kajstura, J., Li, P., Li, B.S., Olivetti, G., Homcy, C.J., Baserga, R. and Anversa, P. (1996) Overexpression of insulin-like growth factor-1 in the heart is coupled with myocyte proliferation in transgenic mice. *Proceedings of the National Academy of Sciences USA* 93, 8630–8635.

SAS Institute (1988) *SAS/STAT User's Guide*, Release 6.03. SAS Institute, Cary, North Carolina.

Schöberlein, L. (1989) Die schußbiopsi-eine möglichkeit zur emittlung von qualitätsparametern und muskelstrukturmerkmalen am lebender tier. *Archiv fur Tierzucht, Berlin* 32, 235–244.

Sejrsen, K. (1986) Endocrine mechanisms underlying genetic variation in growth in ruminants. *Proceedings of the Third World Congress on Genetics and Applied Livestock Production, Lincoln*, Vol. XI, pp. 261–267. Lincoln, Nebraska.

Shamay, A., Solinas, S., Pursel, V.G., McKnight, R.A., Alexander, L., Beattie, C., Hennighausen, L. and Wall, R.J. (1991) Production of the mouse whey acidic protein in transgenic pigs during lactation. *Journal of Animal Science* 69, 4552–4562.

Wall, R.J., Pursel, V.G., Hammer, R.E. and Brinster R.L. (1985) Development of porcine ova that were centrifuged to permit visualization of pronuclei and nuclei. *Biology of Reproduction* 32, 645–651.

Zapf, J., Schmid, C. and Froesch, E.R. (1984) Biological and immunological properties of insulin-like growth factors (IGF) I and II. *Clinical Endocrinology and Metabolism* 13, 3–12.