

Short communication

**Distribution of polymorphic forms at the porcine GH locus
in a population of day-10 pig embryos**

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Abstract. The present study describes an analysis of genotype and allele distribution at the porcine GH locus among day-10 pig embryos. Embryos were collected post mortem from 6 crossbred (Danish Landrace × Yorkshire) sows inseminated with mixed Duroc semen and individually frozen for later analysis. After extraction, DNA was subjected to PCR amplification and restriction analysis with Msp I and Hae II enzymes. The genotype frequencies were: Msp I CD 0.17, DD 0.83; and Hae II AA 0.33, AB 0.58; and BB 0.09. The Msp I CC genotype was not found among analysed embryos. To our knowledge, this is the first report on the genotype and allele distribution at the GH locus among early pig embryos.

Key-words: day-10 embryo, GH gene, pig, polymorphism.

Point mutations have been reported in the porcine growth hormone gene sequence, and can be revealed by PCR-RFLP analysis (KIRKPATRICK 1992). The distribution of alleles and genotypes at the porcine GH locus varies greatly among adult pigs of different breeds (NIELSEN, LARSEN 1991, SCHELLANDER et al. 1994, MACKOWSKI 2001). Moreover, GH genotypes are associated with variations in production traits (KNORR et al. 1997).

The extent of embryonic death in the preimplantation period of gestation in the pig may vary from 25% to 40% of fertilised ova (LAMBERT et al. 1991). A number

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of factors have been suggested to contribute towards this loss (for review see WILMUT et al. 1986). One such factor is the asynchronous development of pig embryos from early cleavage, leading to a great diversity in embryo size in littermates by the day-10 stage of development (PERRY 1962, HUNTER 1972). This phenomenon is related to the duration of fertilisation process and accessory sperm count (SOEDE, KEMP 1993). It has been suggested that within a litter the more developed embryos start to produce oestrogen earlier, and thus advance the uterine secretions, to the detriment of their smaller, slower-growing littermates (POPE et al. 1990).

The aim of this study was to investigate genotype and allele frequencies at the porcine GH locus among day-10 pig embryos.

Day-10 embryos from six crossbred sows (Danish Landrace \times Yorkshire) inseminated with a commercial dose of mixed Duroc semen (2×10^9 sperm) were collected post mortem (NISSEN et al. 1997). The embryos were individually frozen for future DNA analysis. The DNA was recovered by proteinase K treatment followed by phenol/chloroform extraction and ethanol precipitation. A pair of primers corresponding to the pGH gene with the sequences published by KIRKPATRICK (1992) was used according to Kirkpatrick's PCR protocol. The two restriction enzymes used in the present work were Msp I and Hae II. The PCR was carried out in a final volume of 30 μ l consisting of a 4.0 μ l aliquot out of 300 μ l of embryonic DNA template, 0.2 μ M of each dNTP, 0.5 μ M of each primer, 1 U of Taq polymerase and 1 \times PCR buffer. Half of each PCR product was subjected to Msp I digestion (4U/15 μ l of the PCR product) and the other half to Hae II digestion (2.5U/15 μ l of the PCR product). The pGH genotypes were visualised on 2% agarose gel.

A total of 90 day-10 embryos were genotyped at the pGH locus. The PCR reaction yielded a 506bp product. Digestion with Msp I revealed the presence of alleles: C (284bp, 222bp) and D (222bp, 147bp, 137bp), whereas digestion with Hae II produced alleles: A (506bp only) and B (333bp, 173bp). The distribution of pGH genotypes within the litters is shown in Table 1. Since the restriction anal-

Table 1. The distribution of pGH genotypes and allele variants in the analysed day-10 pig embryos

Litter	Number of analysed embryos	Msp I genotypes			Hae II genotypes		
		CC	CD	DD	AA	AB	BB
I	24	–	3	21	12	10	2
II	27	–	–	27	13	14	–
III	15	–	8	7	–	15	–
IV	13	–	–	13	–	8	5
V	4	–	–	4	4	–	–
VI	7	–	7	–	–	7	–
Total	90	–	18	72	29	54	7

Table 2. Msp I and Hae II genotype and allele frequencies in purebred and crossbred pigs

Breed	Genotypes						Alleles				References
	Msp I			Hae II			Msp I		Hae II		
	CC	CD	DD	AA	AB	BB	C	D	A	B	
Purebred											
1	0.01	0.03	0.96	0.71	0.24	0.05	0.01	0.99	0.83	0.17	SCHELLANDER et al. 1999
2	0.07	0.46	0.47	0.25	0.44	0.31	0.3	0.7	0.47	0.53	SCHELLANDER et al. 1999
3	–	–	–	–	–	–	0.15	0.85	0.5	0.5	KIRKPATRICK 1992
4	0.02	0.18	0.8	0.51	0.4	0.09	0.11	0.89	0.71	0.29	MAĆKOWSKI et al. 2001
5	0.14	0.34	0.52	0.17	0.45	0.38	0.31	0.69	0.4	0.6	MAĆKOWSKI et al. 2001
6	0.19	0.44	0.37	0.13	0.5	0.37	0.41	0.59	0.37	0.63	MAĆKOWSKI et al. 2001
Crossbred											
7	0.11	0.44	0.45	0.05	0.35	0.6	0.33	0.67	0.22	0.78	PIERZCHAŁA et al. 1999
8	0.03	0.32	0.65	0.08	0.53	0.39	0.19	0.81	0.34	0.66	KRENKOVA et al. 1999
9	0.15	0.58	0.27	0.16	0.58	0.26	0.44	0.56	0.45	0.55	KUCIEL et al. 1998
10	–	–	–	–	–	–	0.26	0.74	0.18	0.82	KIRKPATRICK 1992
11	0.21	0.54	0.25	0.08	0.63	0.29	0.48	0.52	0.4	0.6	MAĆKOWSKI et al. 2001
12	0.1	0.38	0.52	0.19	0.76	0.05	0.29	0.71	0.57	0.43	MAĆKOWSKI et al. 2001
13	0.0	0.17	0.83	0.33	0.58	0.09	0.08	0.92	0.58	0.42	present study

Breeds: 1 = Austrian Landrace; 2 = Austrian Edelschwein; 3 = Yorkshire; 4 = Polish Landrace; 5 = Polish Large White; 6 = Pietrain; 7 = Polish Large White Zlotnicka Spotted; 8 = Large White Landrace Pietrain; 9 = Large White Landrace Pietrain Hampshire; 10 = 5 crossbreeds; 11 = Duroc Pietrain; 12 = Hampshire Pietrain; 13 = Landrace Yorkshire Duroc.

ysis was carried out a few years after embryo collection, it was not possible to genotype the parents of the litters. The majority of embryos were DD homozygotes (0.8) and AB heterozygotes (0.58). The CC genotype was not observed in the analysed population of embryos, whereas the frequency of the BB homozygotes was very low (0.09).

There is a great variation in pGH genotype and allele frequencies among purebred and crossbred pigs (Table 2). However, there is a limited space for making conclusions basing on the presented results due to the lack of parental genotypes. Therefore we do not know if the low frequency of the C allele and the absence of the CC genotype is caused by the small sample size or rather reflects the unknown genotypes of the parents. For example, parents with DD and CD genotypes would produce offspring with a predominance of the D allele and none of them would have the CC genotype. Interestingly, the frequency of this genotype appears to be low in adult pigs as well. It ranges between 0.01 in Austrian Landrace and 0.21 in Duroc × Pietrain crossbreed, whereas the average frequency rarely exceeds

0.15 (Table 2). Besides, there is no published data on the genotype and allele distribution in the Duroc breed and in Danish Landrace × Yorkshire crossbred pigs.

In conclusion, the distribution observed in Day-10 pig embryos falls into a wide range of frequencies previously reported for adult pigs. To our knowledge, this is the first report on the genotype and allele distribution at the GH locus among early pig embryos.

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