Physiologal aspects of two candidate genes for litter size in pigs

ESR and PRLR

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Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
prof. dr. ir. L. Speelman,
in het openbaar te verdedigen
op dinsdag 2 oktober 2001
des namiddags te half twee in de Aula

Physiological aspects of two major genes for litter size in pigs: *ESR and PRLR*

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ISBN: 90-5808-432-9

Printed by Van Gils, Wageningen, The Netherlands

Cover:	BTTM van Rens	
Pictures:	BTTM van Rens	p. I, 196, 198
	W Valen	p. 188, 194, 198, 214

Abstract. In the present thesis, physiological aspects of two candidate genes for litter size, estrogen receptor (ESR) and prolactin receptor (PRLR) gene were investigated in gilts at D 35 of pregnancy and at term. For both genes two alleles (A and B) were described. The results clearly indicate that the two polymorphisms affect different components of litter size that are expressed at different stages of pregnancy, i.e. ovulation rate (PRLR gene) and fetal survival (through an effect on placental size: ESR gene). This implies there are favorable and unfavorable PRLR/ESR genotype combinations for litter size. The favorable PRLR/ESR genotype combination is AA/BB for L93 Meishan Synthetic gilts, and AA/AB for Large White x Meishan F2 crossbred gilts. In contrast with the general opinion, there actually seem to be traits and relations between traits that are dependent on both maternal and fetal ESR genotype, which might lead to a difference in litter size. In ESR AA gilts, for example, the fraction of ESR AA fetuses that are growth retarded at D35 of pregnancy is larger than the fraction of their AB littermates. Therefore a distortion in genotype ratio of liveborn piglets was predicted. Combining the results with information from literature has resulted in the hypothesis that the ESR gene is a marker for litter size, while the possibility of PRLR gene being a major gene rather than a marker for a closely linked major gene for litter size can not be excluded. Since other genes (e.g. ESR gene) and also environmental factors might change the effect caused by the PRLR polymorphism within the 112 days to parturition, however, at present it is preferable to state that PRLR gene is a major gene for ovulation rate rather than for litter size. Like every marker assisted selection, selection on the favorable genotype combination will lead to an enlargement of litter size once-only. Furthermore, the present thesis shows examples of marker alleles having positive and negative effects at the same time, making it difficult to use the marker for selection. The favorable PRLR allele for litter size for example, appears to be the unfavorable allele for age at first estrus and litter average of teat number of the piglets, while the favorable ESR allele for litter size appears to be the unfavorable allele for growth until weaning. This problem seems to be a biological reality animal scientists will have to live with. It clearly demonstrates the importance of physiological research parallel to and coherent with the search for QTLs and markers for any trait.

aan mamma en pappa

NN08201, 3040

STELLINGEN

1. Het ESR gen is een merker voor foetale overleving bij gelten.
   Dit proefschrift.

2. Hoe breder het scala aan fysiologische functies van het product van een polymorf gen, hoe groter de kans op neveneffecten bij selectie op het voor het kenmerk van interesse gunstige allel.
   Dit proefschrift.

3. Het ei was er eerder dan de kip.

4. Gebruik van genetisch gemodificeerd leven in het veld is als de introductie van het konijn in Australië; de gevolgen zijn niet te overzien.
   N.a.v. NP Louwaars and M Minderhoud. When a law is not enough: biotechnology patents in practice. Biotechnology and Development Monitor No. 46, Juni 2001, p 16-19.

5. Statistiek is geen exacte wetenschap.

6. Het feit dat de ervaring van een zintuigelijke waarneming door de mens afhangt van zijn/haar gemoedstoestand op het moment van waarnemen, maakt onderzoek van de samenleving d.m.v. neuroesthetica[1] of m.b.v. de electronische neus[2] onmogelijk.
   [1] *S. Zeki. Artistic creativity and the brain. Science 293 (2001) 51-52.*
   [2] *S. Montag et al. "Electronic nose" detects major histocompatibility complex-dependent prerenal and postrenal odor components. PNAS 98 (2001) 9249-9254.*

7. Om zowel angst als euforie te temperen, zou men bij maatschappelijke discussies over klonen de realiseerbaarheid van de techniek voor eenieder meer voelbaar kunnen maken door het aantal eicellen nodig voor één gezonde, levendgeboren kloon te vermelden.

8. Wanneer een restauratieve dienst van een instantie huur gaat vragen voor het glas waaruit men haar consumpties nuttigt, is de euroautomaat aan de WC-deuren van die instantie niet ver meer verwijderd.

9. Een emailtje versturen lijkt op het laten van een windje: eenmaal losgelaten, kun je 't niet meer terugvangen.
   De vluchtigheid van een nieuwe vorm van communiceren.

B.T.T.M. van Rens. Physiological aspects of two candidate genes for litter size in pigs: *ESR and PRLR.*
Wageningen, 2 oktober 2001

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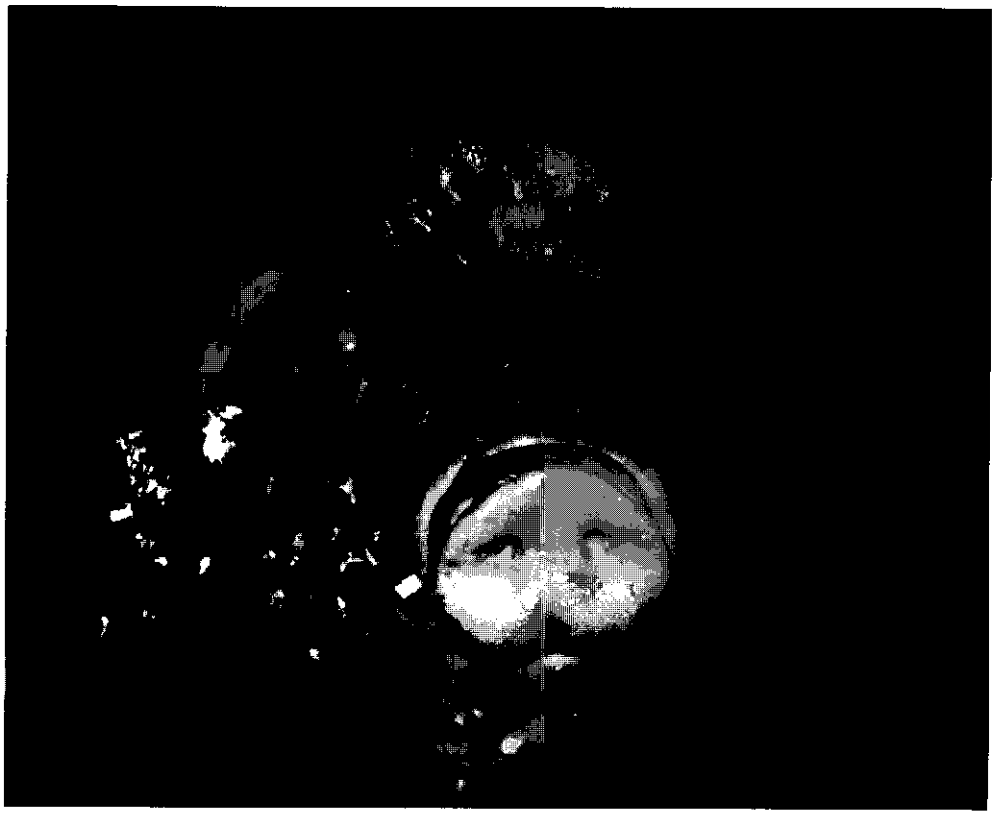

Chapter 1

General introduction

Litter size is an economically important trait in pig production, and thus much effort is made to improve this trait. The heritability for litter size, however, is very low, around 0.1 (Haley et al., 1988). Furthermore, the trait is only expressed in females and is not measurable until sexual maturity. Newly developed molecular technologies make it possible to select directly for genes that control litter size, provided that such genes or markers for such genes are known. One of the approaches to gather genomic information that might be used in genetic improvement of litter size, is the candidate gene approach (De Vries and Plastow, 1998). In this approach, a gene is suggested to be a potential candidate major gene for litter size because of the important physiological role its product plays in reproduction. The gene will be examined for the presence of a polymorphism, and if the result is positive, the polymorphism will be examined for association with litter size. If an association is found, the candidate gene is suggested to be a major gene (or a marker for a closely linked major gene) for litter size.

One of the most discussed candidate genes for litter size in pigs is the estrogen receptor (ESR) gene. For the porcine ESR gene, a polymorphism has been detected (Rothschild et al., 1991) and two alleles (A and B) were described. Several studies have been published in which this polymorphism has been associated with total number of piglets born and(or) number born alive in various genetic lines tested (Rothschild et al., 1994, 1995, 1996, Southwood et al., 1995, Short et al., 1997). Since all differences in litter size found in gilts were in favor of the B allele (for more details, see chapter 4), the gene is suggested to be a major gene for litter size, and selection for the favorable allele is recommended (Rothschild et al., 1996, Short et al., 1997). The mechanism through which this gene affects litter size, however, is not known. Furthermore, it is not known, whether this ESR polymorphism itself causes differences in litter size, or whether the polymorphism provides a marker for a closely linked major gene for litter size.

Another candidate gene for litter size of the pig, is the prolactin receptor (PRLR) gene. A polymorphism has been detected, and two alleles, (A and B) were described (Vincent et al., 1998). The polymorphism was associated with total number of piglets born and(or) number born alive in three commercial lines tested. The direction and magnitude of the genotype effects, however, varied between these lines, suggesting that PRLR gene is a marker, or that background genes play a role (Vincent et al., 1998). Nevertheless, PRLR gene has been described as having "the potential to be a powerful tool when used in conjunction with traditional selection methods for some lines" (Rothschild et al., 1998, Vincent et al., 1998).

Again, however, nothing is known about the physiological mechanism through which litter size is affected.

For a better understanding of the overall effects of using one or both polymorphisms for selection on litter size (including possible correlated responses for other economically important traits), knowledge about the physiological background of the effects of ESR and PRLR loci on reproduction is essential. To increase this knowledge, in the present study gilts with different ESR or PRLR genotypes are compared for several reproductive traits.

AIMS OF THE THESIS

The aims of the present thesis are:

1) Increase knowledge about the physiological background of the effect of ESR polymorphism on litter size in gilts
2) Study the interaction between ESR genotype of the mother and offspring for various reproductive traits
3) Get an impression about the usability of existing physiological data for a first screening of another candidate gene for litter size, in this case PRLR gene

OUTLINE OF THE THESIS

The chapters of this thesis are based on the results of two experiments, both designed to compare several physiological traits in gilts with different ESR genotypes. The first experiment was performed with L93 Meishan synthetic gilts. These gilts belonged to a genetic line in which the ESR B allele is favorable for litter size, resulting in a difference of 2.9 piglets between the two homozygous genotypes in gilts (Southwood et al., 1995). The main aim of this experiment was to identify which component of litter size (ovulation rate, implantation rate, embryonic survival, fetal survival, or a combination of these traits) was affected by ESR genotype on the one hand and to obtain as much information as possible about the underlying reproductive physiology on the other hand. Hereto periovulatory hormone profiles, components of litter size (ovulation rate, embryonic survival), and uterine, placental and embryonic development at Day 35 of pregnancy were compared for gilts with ESR genotype AA and BB (Chapter 2). Subsequently, the effect of fetal ESR genotype nested within maternal ESR genotype on fetal traits at

Day 35 of pregnancy was examined, in order to study the interaction between maternal and fetal ESR genotype (Chapter 3). Furthermore, the relations of fetal weight and fetal heart weight to different placental traits were evaluated relative to fetal and maternal ESR genotype (Chapter 3).

For the second experiment, Large White x Meishan F2 crossbred gilts were bred at our own experimental farm. Based on the previous results (Chapters 2 and 3), placental traits at term were the main subject of interest. Since the effect of ESR genotype on litter size of the F2 crossbreds was not known, however, litter sizes of the gilts had to be studied also (Chapter 4). For part of the gilts, placentae were labeled during farrowing in order to examine the effect of ESR genotype of the mother on average placental characteristics (weight, size, efficiency, number of areolae) of the liveborn piglets (Chapter 4). Subsequently, the effect of piglet ESR genotype nested within maternal ESR genotype on placental traits at term, piglet birthweight and growth until weaning was studied (Chapter 5). Furthermore, the relation of birthweight to various placental traits and the relation between placental traits were evaluated relative to their ESR genotype (Chapter 5).

For the third aim of this study, the L93 Meishan synthetic gilts of the first experiment, and part of the F2 crossbred gilts of the second experiment were genotyped for PRLR. For the L93 Meishan synthetic gilts, the effect of PRLR on components of litter size (ovulation rate, embryonic survival), uterine development and average placental and embryonic development at Day 35 of pregnancy were examined, without knowing the effect of PRLR on litter size of these gilts (Chapter 6). For the Large White x Meishan F2 crossbred gilts, the effect of PRLR on age at first estrus, teat number, litter size and on average piglet and placental characteristics was examined (Chapter 7).

The results of the two experiments have led to six chapters of this thesis (Chapters 2 - 7). The major conclusions of these chapters are combined and discussed in Chapter 8. Furthermore, the discussion on the questions why litter size differences are more pronounced in gilts than in sows with different ESR genotypes, whether it is wise to select for candidate genes like ESR and PRLR, and whether the two genes are major genes or markers, is opened (Chapter 8).

REFERENCES

De Vries AG, and Plastow GS (1998). Major genes for quality. The evidence and the opportunities. Pig progress 14:43-48.

Haley CS, Avalos E, and Smith C (1988). Selection for litter size in the pig. Animal Breeding Abstracts 56:317-332.

Rothschild MF, Jacobson C, Vaske DA, Tuggle CK, Short TH, Sasaki S, Eckardt GR and McLaren DG (1994). A major gene for litter size in pigs. Proc World Congr Genet Appl Livest Prod 21:225-228.

Rothschild M, Jacobson C, Vaske D, Tuggle C, Wang L, Short T, Eckardt G, Sasaki S, Vincent A, McLaren D, Southwood O, Van der Steen H, Mileham A, and Plastow G (1996). The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc Natl Acad Sci USA 93:201-205.

Rothschild MF, Larson RG, Jacobson C, Pearson P (1991). Pvu II polymorphisms at the porcine oestrogen receptor locus (ESR). Anim Genet 22:448.

Rothschild MF, Vaske DA, Tuggle CK, Messer LA, McLaren DG, Short TH, Eckhardt GR, Mileham AJ, and Plastow GS in collaboration with OI Southwood and HAM van der Steen (1995). Estrogen receptor locus is a major gene for litter size in the pig. 46th Ann Mtg Eur Assoc Anim Prod; 5pp.

Rothschild MF, Vincent AL, Tuggle CK, Evans G, Short TH, Southwood OI, Wales R, and Plastow GS (1998). A mutation in the prolactin receptor gene is associated with increased litter size in pigs. 26th International Conference on Animal Genetics, ISAG (International Society for Animal Genetics), August 9-14, 1998, Auckland, NZ;pp 105 (Abstr).

Short TH, Rothschild MF, Southwood OI, McLaren DG, De Vries A, Van der Steen H, Eckardt GR, Tuggle CK, Helm J, Vaske DA, Mileham AJ, and Plastow GS (1997). Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. J Anim Sci 75:3138-3142.

Southwood OI, Van der Steen HAM, Mileham AJ, Plastow GS, Cuthbert-Heavens D (1995). Evaluation of the oestrogen receptor (ESR) gene in Meishan synthetic pigs. 46th Ann Mtg Eur Assoc Anim Prod;53 (Abstr).

Vincent AL, Evans G, Short TH, Southwood OI, Plastow GS, Tuggle CK, and Rothschild MF (1998). The prolactin receptor gene is associated with increased litter size in pigs. 6th WCGALP 27:15-18.

Chapter 2

Periovulatory hormone profiles and components of litter size in gilts with different estrogen receptor (ESR) genotypes

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Published in : Theriogenology 53 (2000) 1375-1387

ABSTRACT

Estrus, endocrine changes during the periovulatory period, and components of litter size at Day 35/36 of pregnancy were studied in gilts with estrogen receptor genotype AA (AA gilts) or BB (BB gilts), in which the B allele is associated with a larger litter size. Neither estrus length nor estrous cycle length was affected by estrogen receptor genotype. No differences in periovulatory plasma LH, estrogen or progesterone profiles between the AA and BB gilts were detected. Furthermore, temporal aspects of these profiles were not different for both genotypes.

Although the B allele is associated with a larger litter size, no differences in number of corpora lutea or number and percentage of vital Day 35/36 embryos were found in this study. This indicates that the difference in litter size is not due to differences in oocyte maturation, fertilization, implantation or embryonic survival, but is likely caused by a difference in fetal survival. Thus, uterine capacity might be different for both genotypes. The available uterine space per embryo seems to be the same for both genotypes, as is endometrial folding of uterine surface area. However, a difference in placental size was found. Embryos of BB gilts had significantly longer placentae than embryos of AA gilts. These results suggest a higher chance for placental insufficiency in AA gilts, leading to the expected higher fetal mortality compared with the BB gilts. The difference in placental size might have been related to a difference in the timing of embryonic mortality.

Key words: pigs, estrogen receptor gene, ovulation rate, embryo survival, placental development

Acknowledgments

The authors thank PIC for providing the experimental animals; Piet de Groot and Henk Vos for their assistance in taking all the blood samples; Frits Rietveld, Corrie Oudenaarden, Elène Vos and Jo Erkens for technical advice.

INTRODUCTION

In pigs, a polymorphism at the estrogen receptor (ESR) locus related to variation in litter size has been detected (7). Two ESR alleles (A and B) were described, of which the B allele, discovered initially in Chinese pigs (9), is significantly associated with a higher litter size (7,8,11,13,14). The mechanism through which this gene affects litter size is not yet known. Furthermore, it is not known whether this ESR polymorphism itself causes differences in litter size or whether this polymorphism provides a marker for a closely linked major gene for litter size.

Because of the considerable economic value of litter size for pork producers, marker assisted selection by pig breeding companies for the favorable allele may be expected (8,11). For a better understanding of the overall effects of this selection, including possible correlated responses for other economically important traits, knowledge about the physiological background of the effect of ESR locus on reproduction is essential.

The objective of this study was to obtain more information about differences in reproductive physiology of gilts with different ESR genotypes. Hereto periovulatory hormone profiles; components of litter size (ovulation rate, embryonic survival); and uterine, placental and embryonic development in gilts with ESR genotype AA (AA gilts) and BB (BB gilts) were compared.

MATERIALS AND METHODS

Animals

Seventy-nine synthetic L93 gilts (31 AA and 48 BB) were provided in 4 batches at 7- to 8-wk intervals by Pig Improvement Company (50% Meishan/50% Landrace synthetic L93, PIC W Europe). Animals of each batch were treated similarly, except for additional canulation of animals in Batches 2 and 3.

At the day of arrival, the gilts (AA gilts 195±4 d old, weighing 106±4 kg and BB gilts 196±3 d old, weighing 106±3 kg) were housed in groups of 3. Each gilt was given 1.25 kg normal sow ration (12.5 MJ metabolizable energy/kg, crude protein 141 g/kg, ileal digestible lysine 5.8 g/kg) twice daily at 0830 and 1630 h. Water was available ad libitum.

Experimental Design

Starting at the day after arrival, the gilts were checked for estrus by the back pressure test in presence of a vasectomized adult boar twice daily (at 0800 and 1600 h). No information on estrus prior to arrival at the experimental farm was available. Animals that did not show estrus within the first 3 wk after arrival (5 gilts) were removed from the experiment. One day after the second estrus, the gilts were housed individually. The gilts from Batches 2 and 3 were then canulated, as described below.

At third estrus the gilts were artificially inseminated twice at an interval of 24 h with pooled semen of 3 Great Yorkshire sire line boars ($3x10^9$ sperm cells per dose). When the first signs of estrus were detected in the morning, first insemination took place on the same day (at 1530 h); when estrus was detected in the afternoon, first insemination took place on the next day (at 1530 h).

On Day 35 or 36 after the first insemination the animals that did not return to estrus were slaughtered to study reproductive parameters as described below. Reproductive tracts were collected immediately after slaughter and transported to the laboratory on ice. Transport lasted on average 20 min.

All gilts were weighed on the day of arrival at the experimental farm, the day of individual housing and the day before slaughter.

Canulation of the Jugular Vein

The gilts of Batches 2 and 3 were surgically fitted with a permanent jugular vein catheter between 4 and 11 d after they had shown their second estrus. Canulation was performed as described by Soede et al. (12). Blood samples were collected at 4-h intervals from Day 16 after second estrus until Day 2 after second insemination. Thereafter, blood samples were collected at 12-h intervals until Day 10 after second insemination. During the 4-h interval sampling period, the gilts were checked for estrus 3 instead of 2 times daily (at 0800, 1600 and 2400 h).

Blood Sampling

At each sampling, approximately 10 mL of blood was collected into ice-cooled polypropylene tubes containing 100 µL saline with 14.4% EDTA. After collection, blood samples were immediately placed on ice until centrifugation (30 min after collection) at 900 g for 10 min at 4 °C. Plasma was collected and stored at -20 °C until analysis for LH, estradiol and progesterone as described below. After every blood collection, the canula was filled with 10 mL of heparinized saline (33 IU/mL, Heparin Leo, Leopharmaceutical Products B.V., Weesp, The Netherlands). Prior to blood collection the heparinized saline together with the first milliliter of blood were discarded.

Hormone Assays

LH. Plasma concentrations of LH were measured by a double-antibody radio-immunoassay as described by Niswender et al. (6). Porcine LH (pLH, H028/H, iodinationgrade batch 006/2, UCB, Brussels, Belgium) was used as standard and as tracer after radioiodination (specific activity 54 µCi/µg). Rabbit anti-porcine LH (A528/R1H, batch 004/1, UCB, Brussels, Belgium) was used at a final dilution of 1:65000, which gave an initial binding of the labeled hormone of approximately 40%. As second antibody, Sac-cel (A-sac-1 anti rabbit, Lucron, Nijmegen, The Netherlands) was used at a dilution of 1:3. The sensitivity of the assay was 0.5 ng/mL at the 80% $B:B_0$ concentration. The minimal detectable dose at the 90% $B:B_0$ concentration was 0.13 ng/mL. The inter-assay coefficient of variation was 13.8% and the intra-assay coefficient was 10.6%. Triplicate standard curves were run in each assay and all serum samples were assayed in duplicate at 200 µL per assay tube.

Estrogen. Plasma concentration of estradiol-17β was measured by radioimmunoassay after extraction, using a modification of the method described by Helmond et al. (4). Duplicate 1-mL samples were extracted with dichloromethane after adding 1000 c.p.m. of [2,4,6,7-^{3}H] estradiol (TRK.322, Amersham International plc, Buckinghamshire, England) for estimating procedural losses. After mixing and centrifugation (10 min, 2000 g), the dichloromethane fraction was isolated and dried under a stream of nitrogen. It was then redissolved in 500 µL of PBS with 0.1% BSA. An aliquot of 150 µL was taken to determine the recovery of [^{3}H] estradiol. On average the recovery was 45%. The antibody used in

the radioimmunoassay was a rabbit antiserum against estradiol-17β (Human and Animal Physiology Group, WAU, Wageningen, The Netherlands). The main crossreacting steroids were estrone (4.39%) and estriol (0.14%). The antiserum was used at a final dilution of 1:125000, and 17β estra-1,3,5 (10) triene 3,17 diol (E1132, Sigma Chemical Co, Zwijndrecht, The Netherlands) was used as standard and [2,4,6,7-^{3}H] estradiol as tracer. The intra- and inter-assay coefficients of variation were 12.4 and 17.4%, respectively, and the detection limit was 1.25 pg/mL. The amount of estradiol was expressed in pg/mL after correction for procedural losses.

Progesterone. Plasma concentrations of progesterone were estimated by a direct solid-phase ^{125}I RIA method (Cóat-A-Count TKPG; Diagnostic Products Corporation, Los Angeles, CA, USA) in 100-µL plasma samples in duplicate. The main cross-reactivities were 2.4, 2.0, 1.7 and 1.3% for deoxycortisol, 20α-hydroxypregn-4-ene-3-one, deoxycorticosterone and 5β-pregnane-3,20-dione, respectively, and <1% for other steroids tested, according to the manufacturer. The sensitivity was 0.15 nmol/L, and the inter-assay coefficient of variation was 11% (n=20). A sample with a high value (20 ng/mL), added to plasma with a low progesterone level, showed a high degree of parallelity and an average precision of 4.4% (coefficient of variation). Over the whole standard curve different amounts of the high sample added to plasma with a low progesterone level showed an intra-assay coefficient of variation of 8% and an average recovery of 114.5%.

Collection of Data after Slaughter

Morphometry and weight analyses were performed according to Van der Lende et al. (15). Ovaries, oviducts and mesometrium were separated from the utero-cervical tract, and the length and weight of both uterine horns were measured separately. The position of embryos was determined by palpation and was expressed as the distance from the utero-cervical junction. Each uterine horn was opened longitudinally at the anti-mesometrial side. Embryos were isolated from extra-embryonic membranes, weighed and measured for crown-rump length. Embryos that were morphologically normal and not degenerating are referred to as "vital embryos" in this paper.

After removing the extra-embryonic membranes from the endometrium, their length was determined by measuring the length of the allanto-chorion (excluding the

necrotic tips) under minimal stretching. The extra-embryonic membranes were then weighed.

After removal of all embryos and extra-embryonic membranes, the uterine horns were weighed again. The length and width of implantation sites were measured as well as their distance from the utero-cervical junction. In addition, the length of both empty uterine horns was measured.

Immediately after removal from the reproductive tract, the ovaries were weighed. The ovaries were maintained on ice until the morphometry and weight analysis of the reproductive tract was complete (after approximately 1 h). The corpora lutea were then dissected from the ovaries to be counted and weighed individually.

Statistical Analysis

All data were analyzed with SAS (10). The procedure GLM was used for analyzing linear models.

Endocrine analyses. All results of calculations concerning endocrine changes were based on 25 gilts (9 AA and 16 BB gilts), unless mentioned differently. Endocrine profiles were standardized to the time of onset of the LH surge.

The frequent sampling period was used to establish the basal LH concentration per gilt, and the surge onset in each case was defined as a consistent increase above this mean basal value (i.e., consecutive hormone levels remained above the base line). The duration of the LH surge was defined as the interval over which the surge was sustained above the basal level. The area under the LH curve was defined as the area between average basal line and the LH curve.

The onset of the estradiol surge for some of the canulated animals had already started before the first blood sampling. Thus, for calculation of the basal estradiol level per gilt (9 AA and 15 BB gilts), only the samples after the estradiol surge (i.e., 100 h after beginning of LH surge) were used. The onset of the estradiol surge was defined as a consistent increase above the mean basal values. The duration of the estradiol surge (8 AA and 12 BB gilts) was defined as the interval over which the surge was sustained above the basal level. The area under the estradiol curve (8 AA and 12 BB gilts) was defined as the area between average basal line and the

estradiol curve. Because not all canulated animals showed a full estradiol peak during the blood sampling period, also ascending and descending slopes of estradiol peak (9 AA and 15 BB gilts) were calculated by means of linear regression.

For calculation of basal progesterone values, the samples within the interval of 48 h before to 48 h after onset of the LH surge were used. The onset of the post-ovulatory progesterone rise was defined as the first consistent increase above the basal level. For calculation of the ascending slope of the progesterone rise by means of linear regression, the data within the interval of the onset of the progesterone rise to 150 h after the onset of the LH surge were used.

The following endocrine characteristics were compared between genotypes by analysis of variance: basal LH, estradiol and progesterone values, duration of LH and estradiol surge and duration of the period with basal progesterone values, peak surge values (the highest concentration observed) for LH and estradiol, area under LH and estradiol curves, and slopes for ascending and descending parts of the estradiol curve and progesterone rise. Results are expressed as least squares means and standard errors of least squares means, estimated using the following model:

$$y_{ik}=m+genotype_i+batch_k+genotype*batch_{ik}+e_{ik}$$

in which 'batch' (k=1,2) is the batch in which the pig was obtained from PIC.
Estimates were based on the model without the interaction term when it was not significant.

Analyses of estrus and components of litter size. For calculations concerning estrus and components of litter size, data were available on 56 gilts (17 AA and 39 BB). Estrous cycle length was defined as the time interval between the first day of estrus and the first day of the subsequent estrus. The time of onset of estrus was defined as the first time the gilt showed estrus minus half the time interval from the previous estrus detection. The end of estrus was defined as the last time the gilt showed estrus plus half the time interval to the subsequent estrus detection. As far as parameters for conceptus development are concerned, all statistical analyses were performed on average values per gilt. These values represent only the vital embryos. Results are expressed as least squares means and standard errors of

least squares means, estimated after stepwise elimination of nonsignificant effects (except for 'genotype' and 'batch'), using the following model:

$$y_{ijkl}=m+genotype_i+day_j+batch_k+genotype*day_{ij}+genotype*batch_{ik}+day*batch_{jk}+e_{ijkl}$$

in which 'day' (j=1,2) is day after first insemination on which the pig was slaughtered and 'batch' (k=1,4) is the batch in which the pig was obtained from PIC. Including bodyweight at individual housing in the model as a covariable did not change the results.

RESULTS

Gilts

In total, 63 gilts were inseminated (19 AA and 44 BB) of which 56 gilts (17 AA and 39 BB) became pregnant (pregnancy rate 89.5 versus 88.6% for AA and BB gilts, respectively). Of the pregnant gilts, 25 (9 AA and 16 BB) had been canulated.

Age at insemination and bodyweight at relocation to individual cages (245±5 versus 248±3 d and 117.0±4.0 versus 118.7±2.8 kg for AA and BB gilts, respectively) and age and bodyweight at slaughter (280±5 versus 283±3 d and 153.0±4.3 versus 153.2±2.8 kg for AA and BB gilts, respectively) were not significantly different between genotypes (P>0.5).

Estrus

In total, 3 estruses were detected for each gilt. Genotype did not affect the length of estrus (P>0.3). The first estrus lasted on average 55±4 and 60±3 h, the second 62±3 and 64±2 h, and the third 54±3 and 55±2 h for AA and BB gilts, respectively. The canulated gilts had a significantly longer third estrus (i.e., the estrus after canulation; P=0.0001) than the noncanulated gilts (63±3 and 46±3 h, respectively). Estrous cycle length was not different (P>0.5) between genotypes (20.7±0.3 versus 20.6±0.2 d for AA and BB gilts, respectively).

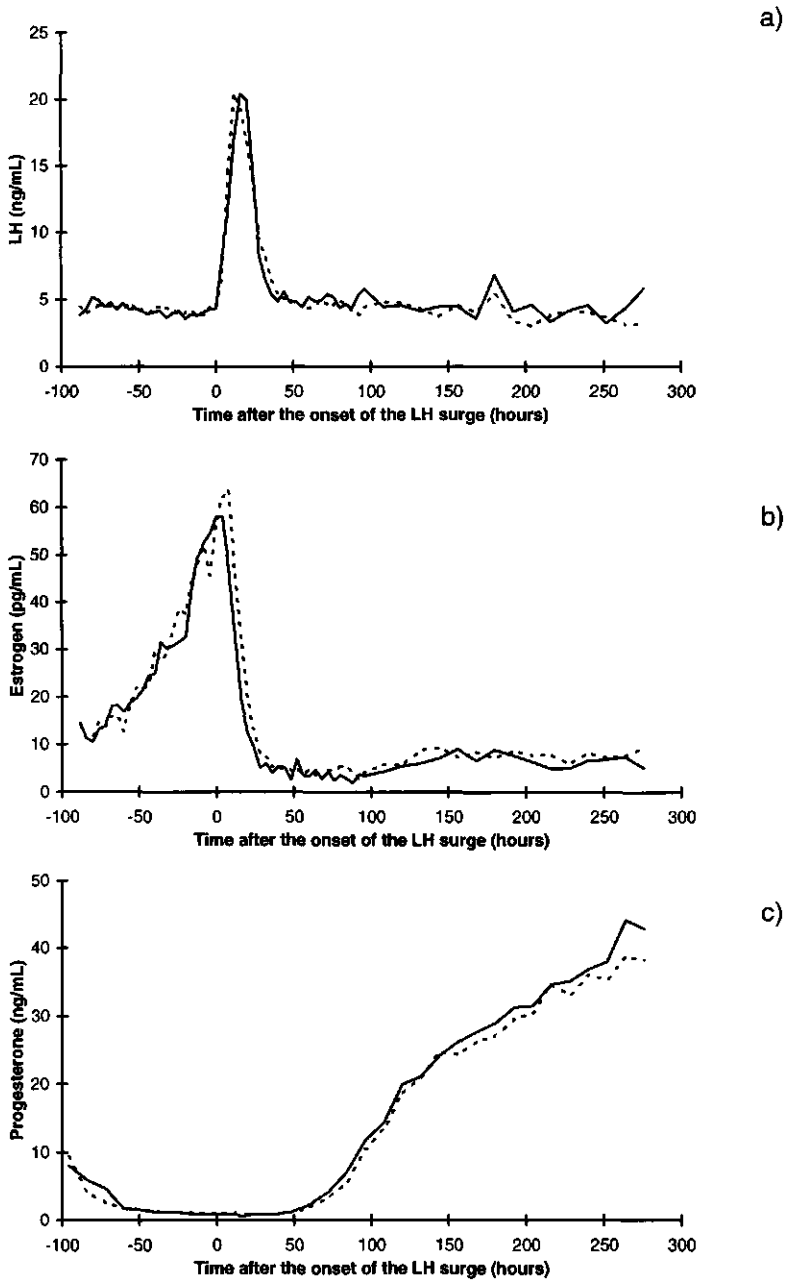

Figure 1. *Average LH, estrogen and progesterone profiles (a, b, c) for AA gilts (——) and BB gilts (----)*

Periovulatory Concentrations of LH, Estradiol and Progesterone

The average LH, estradiol and progesterone profiles for AA and BB gilts are shown in Figure 1. Because there were no differences (P>0.3) between the baseline before and after LH-peak, an average baseline was calculated. There were no significant differences (P>0.5) between genotypes for average basal LH concentration (4.6±0.3 versus 4.4±0.2 ng/mL for AA and BB gilts, respectively), duration of the LH surge (42.2±3.2 versus 40.7±2.4 h for AA and BB gilts, respectively), LH peak value (24.1±2.4 versus 25.4±1.8 ng/mL for AA and BB gilts, respectively), or the area under the LH curve (294±30 versus 317±22 h ng/mL for AA and BB gilts respectively; Figure 1a).

The same held for the estradiol profile: There were no significant differences (P>0.3) between the genotypes in terms of average basal estradiol concentration (6.1±1.4 versus 7.7±1.1 pg/mL for AA and BB gilts, respectively), duration of the estradiol surge (99.5±6.6 versus 105.0±5.4 h for AA and BB gilts, respectively), estradiol peak value (67.3±6.6 versus 72.2±5.1 pg/mL for AA and BB gilts, respectively), or the area under the estradiol curve (2319±372 versus 2556±304 h pg/mL for AA and BB gilts, respectively). Ascending and descending slopes of estradiol curves were the same (P>0.5) for both genotypes (ascending slopes 0.64±0.07 and 0.65±0.05 and descending slopes -2.87±0.43 and -2.70±0.33 pg/mL/h for AA and BB gilts, respectively; Figure 1b).

Average basal progesterone concentration (1.0±0.1 versus 1.1±0.1 ng/mL for AA and BB gilts, respectively), duration of basal period (103.9±4.3 versus 101.9±3.5 h for AA and BB gilts, respectively), and slope of the ascending part of the profile (0.28±0.02 versus 0.30±0.02 ng/mL/h for AA and BB gilts, respectively) were also not significantly different (P>0.5) between both genotypes (Figure 1c).

Genotype did not affect the mean interval from the onset of LH surge to the onset of estrus (P>0.5). For both genotypes estrus started after the onset of LH surge. The mean interval from the onset of LH surge to the onset of estrus was 5.5±4.7 h (with a range of -12 to +36 h) for the AA gilts and 4.3±3.5 h (with a range of -12 to +24 h) for the BB gilts.

Although the mean interval from the peak concentration of estradiol until the time of onset of estrus did not differ (P>0.3) between both genotypes (6.1±5.2 versus

Table 1. Components of litter size in AA (n = 17) and BB (n = 39) Meishan synthetic gilts at Day 35/36 of pregnancy (least squares means ± SEM)

	ESR genotype		
Components	AA	BB	p value
Corpora lutea (n)	19.3 ± 0.8	20.1 ± 0.5	0.359
Vital embryos (n)	15.1 ± 1.0	14.2 ± 0.6	0.456
Embryonic survival[a] (%)	78.1 ± 4.1	71.1 ± 2.7	0.157
Total embryos (n)	16.0 ± 1.0	14.7 ± 0.7	0.281
Total embryos[a] (%)	82.8 ± 4.1	73.6 ± 2.7	0.064
Implantation sites (n)	16.2 ± 1.0	14.9 ± 0.6	0.266
Implantation sites[a] (%)	84.0 ± 4.1	74.5 ± 2.7	0.055
Ovaries			
Total weight (g)	22.6 ± 1.0	22.8 ± 0.7	0.877
Corpora lutea			
Total weight (g)	8.0 ± 0.4	8.0 ± 0.3	0.994
Average weight (g)	0.42 ± 0.02	0.40 ± 0.01	0.378
Embryos[b]			
Weight (g)	4.41 ± 0.15	4.33 ± 0.10	0.695
Length (cm)	3.84 ± 0.05	3.84 ± 0.03	0.931
Implantation sites[b]			
Width (cm)	10.2 ± 0.3	10.0 ± 0.2	0.420
Length (cm)	25.0 ± 1.2	27.6 ± 0.8	0.083
Placentae[b]			
Weight (g)	41.2 ± 2.6	45.7 ± 1.7	0.156
Length (cm)	41.4 ± 1.6	46.2 ± 1.1	0.017
Uteri (filled)			
Weight (g)	4447.4 ± 377.7	4616.4 ± 247.8	0.710
Length (cm)	532.9 ± 21.3	541.8 ± 14.0	0.728
Uteri (empty)			
Weight (g)	1987.5 ± 106.2	2008.2 ± 69.7	0.872
Length (cm)	582.9 ± 22.7	596.2 ± 14.9	0.626

[a] Percentage is calculated as percentage of the number of corpora lutea.

[b] Results represent only vital embryos (i.e., morphologically normal, not degenerating embryos).

0.6±4.0 h for AA and BB gilts, respectively), the interval from onset of LH surge and peak concentration of estradiol tended to be different (P=0.068) between genotypes (-0.6±1.8 versus 3.9±1.4 h for AA and BB gilts, respectively).

Components of Litter Size

Number of corpora lutea, number of implantation sites, number of vital embryos and embryonic survival did not differ (P>0.15) between genotypes (Table 1). Furthermore, genotype neither affected the total weight of the ovaries, total weight of the corpora lutea, average weight of the corpora lutea, nor weight or length of the uterus (before or after dissection of embryos; Table1; P>0.3). While width of implantation sites was similar for both genotypes (P>0.4), length of implantation sites tended to be longer for the BB gilts (P=0.083). Furthermore, placentae of BB gilts were longer (P=0.017) than those of AA gilts. However, the length of the placenta divided by the length of implantation site was the same (P>0.5) for both genotypes (1.71±0.05 versus 1.74±0.03 for AA and BB gilts, respectively). Genotype did not affect (P>0.15) the weight of the placentae (Table 1).

Including total number of implantation sites as a covariable in the statistical analysis did not change the effect of genotype on placental length (42.0±1.5 versus 45.9±1.0 cm for AA and BB gilts, respectively; P=0.036), placental weight (41.8±2.6 versus 45.4±1.7 g for AA and BB gilts, respectively; P=0.240), placental length divided by length of implantation site (1.70±0.05 versus 1.74±0.03 for AA and BB gilts, respectively; P=0.486), or uterine length measurements (filled 525.4±20.1 versus 545.2±13.2 cm and empty 574.6±21.3 versus 600.0±13.9 cm for AA and BB gilts, respectively; P=0.417 and P=0.326). However, after correction for the total number of implantation sites found, the length of implantation sites were similar for both genotypes (25.4±1.2 versus 27.4±0.8 cm for AA and BB gilts, respectively; P=0.166).

While the average distance between embryos tended to be longer (P=0.054) for BB gilts (30.6±2.2 versus 35.8±1.5 cm for AA and BB gilts, respectively), correction for the total number of implantation sites in the statistical analysis, resulted in a comparable distance (32.3±1.7 versus 35.3±1.1 cm for AA and BB gilts, respectively; P=0.140). Furthermore, the average interval between implantation sites were comparable (P=0.120) for both genotypes (9.7±1.9 versus 13.4±1.3 cm for AA and BB gilts, respectively).

DISCUSSION

Although the B allele in the line that was used is associated with a larger litter size (14), the number of corpora lutea was the same for both genotypes. Furthermore, no differences in number and percentage of vital Day 35/36 embryos were found in this experiment. This indicates that the difference in litter size is not due to differences in oocyte maturation, fertilization, implantation or embryonic survival, but is probably due to a difference in fetal survival. Thus uterine capacity, defined as the maximum number of fetuses that can be maintained to term when the number of vital embryos is not a constraint (1,2), might be different for both genotypes.

The average numbers of vital embryos detected in this study for AA and BB gilts were 15.1 and 14.2, respectively. For gilts of the same line Southwood et al. (14) found a total number born of 10.5 and 13.4, respectively. Assuming that the gilts in this study are representative for the population studied by Southwood et al. (14), this implies that for AA gilts fetal mortality will be more than 5 times as high as that of the BB gilts.

Mechanisms that might lead to a difference in uterine capacity are: differences in placental efficiency (i.e., differences in the potential surface area of contact of endometrium with placental membranes, thickness of the placental membranes and vascularization of placenta and endometrium; 3) and differences in the use of available uterine space (i.e., differences in potential of adaptation of uterine length to litter size; 16).

Embryos of BB gilts had significantly longer placentae than embryos of AA gilts, also after correction for the total number of implantation sites. At the moment of slaughter the vital embryos from AA and BB gilts were at the same developmental stage, as their length and weight did not differ. A limited placental development is accompanied with a decrease in fetal development (5). Thus, being shorter, the placentae of embryos of AA gilts might be less efficient in supporting further development of all embryos, thus leading to higher fetal mortality.

The length of the placenta divided by the length of implantation site was the same for both genotypes. This suggests that the AA and BB gilts had a similar endometrial folding of the uterine surface area.

As the average length of the filled uterus was the same for both genotypes, the potential available uterine space for the vital embryos was the same for AA and BB

gilts. However, while number of corpora lutea as well as embryonic survival was the same for both genotypes, the percentage of visible implantation sites tended to be higher for the AA gilts at Day 35/36. This suggests a difference in the timing of embryonic mortality between AA and BB gilts: in BB gilts probably more embryos died before implantation, while in AA gilts more embryos died after implantation. As the implantation sites of these degenerated embryos still occupy part of uterine space, the remaining space will be smaller for vital embryos of AA gilts at Day 35/36 than for vital embryos of BB gilts. This is confirmed by the fact that the average distance between embryos tended to be longer for BB gilts, while correction for the total number of implantation sites resulted in a similar distance between embryos for AA and BB gilts. The same held for the uteri after removal of embryos: uterine length was the same for both genotypes, interval between adjacent implantation sites was the same for both genotypes and length of implantation sites tended to be longer for embryos of BB gilts, but was the same for both genotypes after correction for total number of implantation sites. Thus, the shorter placentae of embryos of AA gilts might be explained by a stronger competition for space during the first 35/36 d of pregnancy compared with embryos of BB gilts, which might ultimately lead to a higher fetal mortality.

In summary, the difference in litter size found in gilts with different ESR genotypes is not due to differences in ovulation rate and/or embryonic survival and thus must be due to a difference in fetal survival. Being shorter, the placentae of AA gilts might be less efficient in supporting further development of all embryos, thus leading to the expected higher fetal mortality. The difference in placental length between AA and BB gilts might have been related to a difference in the timing of embryonic mortality.

REFERENCES

1. Christenson RK, Leymaster KA, Young LD (1987). Justification of unilateral hysterectomy-ovariectomy as a model to evaluate uterine capacity in swine. J Anim Sci 65:738-744.
2. Christenson RK, Vallet JL, Leymaster KA, Young LD (1993). Uterine function in Meishan pigs. J Reprod Fertil Suppl 48:279-289.
3. Ford SP (1997). Embryonic and fetal development in different genotypes in pigs. J Reprod Fertil Suppl 52:165-176.
4. Helmond FA, Simons PA, Hein PR (1980). The effects of progesterone on estrogens-induced luteinizing hormone and follicle-stimulating hormone release in the female rhesus monkey. Endocrinology 107:478-485.

5. Knight JW, Bazer FW, Thatcher WW, Franke DE, Wallace HD (1977). Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 44:620-637.
6. Niswender GD, Reichert LE, Zimmerman DR (1970). Radioimmunoassay of serum levels of luteinizing hormone throughout the estrous cycle in pigs. Endocrinology 87:576-580.
7. Rothschild MF, Jacobson C, Vaske DA, Tuggle CK, Short TH, Sasaki S, Eckardt GR, McLaren DG (1994). A major gene for litter size in pigs. Proc World Congr Gen Appl Livest Prod 21:225-228.
8. Rothschild M, Jacobson C, Vaske D, Tuggle C, Wang L, Short T, Eckardt G, Sasaki S, Vincent A, Mc. Laren D, Southwood O, Van der Steen H, Mileham A, Plastow G (1996). The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc Natl Acad Sci USA 93:201-205.
9. Rothschild MF, Larson RG, Jacobson C, Pearson P (1991). Pvu II polymorphisms at the porcine oestrogen receptor locus (ESR). Anim Genet 22:448.
10. SAS/STAT (1990). User's Guide. Cary NC, SAS Institute.
11. Short TH, Rothschild MF, Southwood OI, McLaren DG, De Vries A, Van der Steen H, Eckardt GR, Tuggle CK, Helm J, Vaske DA, Mileham AJ, Plastow G (1997). Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. J Anim Sci 75:3138-3142.
12. Soede NM, Helmond FA, Kemp B (1994). Periovulatory profiles of oestradiol, LH and progesterone in relation to oestrus and embryo mortality in multiparous sows using transrectal ultrasonography to detect ovulation. J Reprod Fertil 101:633-641.
13. Southwood OI, Hoste S, Short TH, Mileham AJ, Cuthbert-Heavens D (1996). Evaluation of genetic markers for litter size in Meishan synthetic and Large White pigs. Anim Sci 62:625 (abst.)
14. Southwood OI, Van der Steen HAM, Mileham AJ, Plastow, GS, Cuthbert-Heavens D (1995). Evaluation of the oestrogen receptor (ESR) gene in Meishan synthetic pigs. 46th Ann Mtg Eur Assoc Anim Prod; 53 (abst.)
15. Van der Lende T, Hazeleger W, De Jager D (1990). Weight distribution within litters at the early foetal stage and at birth in relation to embryonic mortality in the pig. Livest Prod Sci 26:53-65.
16. Wu MC, Hentzel MD, Dziuk PJ (1987). Relationships between uterine length and number of fetuses and prenatal mortality in pigs. J Anim Sci 65:762-770.

Chapter 3

Fetal and placental traits at Day 35 of pregnancy in relation to the estrogen receptor genotype in pigs

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Published in: Theriogenology 54 (2000) 843-858
Erratum in: Theriogenology 55 (2001) 1029-1032

ABSTRACT

Fetuses from gilts with estrogen receptor (ESR) genotype AA (AA-AA and AA-AB) and BB (BB-AB and BB-BB) were compared at Day 35/36 of pregnancy, to examine whether fetal ESR genotype nested within maternal ESR genotype would affect fetal traits. Furthermore the relation of fetal body weight and fetal heart weight to various placental traits were evaluated relative to ESR genotype. Fetal and placental weight and length, and implantation surface area were not affected by fetal ESR genotype nested within maternal ESR genotype. Fetal weight was related similarly to placental length, placental weight, and implantation surface area: up to a certain threshold value (40 cm, 40 g and 250 cm^2, respectively), an increase in the trait was associated with an increase of fetal weight. Thereafter, fetal weight did not change anymore. Thus, at Day 35/36 of pregnancy porcine fetuses seem to have a maximum growth potential. The percentage of AA-AA fetuses that had not reached this maximum growth potential was larger than of the other three genotype combinations studied, and therefore a higher subsequent fetal mortality may be expected in this group. Hearts of AA-AB fetuses were significantly heavier than those of BB-AB and BB-BB fetuses and tended to be heavier than those of AA-AA fetuses. The reason for this hypertrophy is unclear, but might be related to a difference in placental vascularity. Heart weight of fetuses from BB gilts increased with fetal weight, while heart weights of fetuses from AA gilts did not. Heart weight increased with an increase of placental length and implantation surface area up to 51 cm and 437 cm^2, respectively, and thereafter decreased again. For BB-AB fetuses a similar relation was found between heart weight and placental weight, while heart weight of the other three genotype combinations remained unaffected as placental weight increased. The fetus and placenta are continuously changing during early pregnancy, and therefore different mechanisms may change the demands for cardiac output. However, keeping in mind that placental size and blood volume are relatively large, placental vascularity and vascular development may play a major role. Therefore, further research on heart size, placental size and vascularity, relative to ESR genotype, is recommended.

Key words: pigs, estrogen receptor gene, placenta, heart, embryonic growth

Acknowledgments

The authors thank Frans Vermeulen for his assistance and Richard Wales (PIC International Group) for genotyping the fetuses.

INTRODUCTION

Using the candidate gene approach, Rothschild et al. (7) suggested that the estrogen receptor (ESR) gene in pigs is a major gene for litter size. Two ESR alleles (A and B) were described, of which the B allele, discovered initially in Chinese pigs (9), is significantly associated with a higher litter size (7, 8, 12, 14, 15). A physiological study of gilts with different ESR genotypes showed that the litter size difference associated with ESR genotype is probably due to a difference in uterine capacity, since there were no differences in number of ovulations and number of fetuses at Day 35/36 of pregnancy. Furthermore, placentae of fetuses from BB gilts were significantly longer than those of fetuses from AA gilts (16).

Until now, all research concerning ESR genotype in pigs has been restricted to the maternal ESR genotype. Fetal ESR genotype however, might very well be a factor to consider also. It is not known, for instance, whether the observed differences in placental size are due only to the maternal, or also the fetal ESR genotype. Therefore, in the present study, the effect of fetal ESR genotype on fetal traits was compared within different maternal ESR genotypes.

Several authors have examined placental and fetal development throughout pregnancy (3, 6, 17). However, possible relations between fetal and placental traits at Day 35 of pregnancy, and possible variation in these relations, have not been reported before. Our dataset was obtained from samples collected earlier (16), and contained information about fetal weight, fetal length, placental weight, placental length, and implantation surface area at Day 35 of pregnancy. Furthermore, information was also available for heart weight. Thus, relations between fetal weight and different placental traits as well as relations between fetal heart weight and different placental traits could be evaluated relative to fetal and maternal ESR genotype.

The objectives of the present study were 1) to examine whether fetal ESR genotype within maternal ESR genotype affects fetal traits at Day 35/36 of pregnancy and 2) to examine how fetal weight and fetal heart weight are related to other fetal traits at Day 35/36 of pregnancy, and whether found relations are affected by fetal and/or maternal ESR genotype.

MATERIALS AND METHODS

The data used came from an experiment that had been designed to compare several physiological traits for gilts with different ESR genotypes. The design of the experiment was described extensively by Van Rens et al. (16). Briefly, 56 pregnant gilts (AA, n = 17 and BB, n = 39) were slaughtered in 4 batches on Days 35 or 36 of pregnancy (Day 0 = day of first insemination), after which components of litter size were determined and several uterine and fetal morphological traits were measured. Immediately after morphometry and weighing, fetuses were frozen individually in liquid nitrogen and stored at –80 °C until DNA isolation.

Each gilt had been inseminated twice with pooled semen from 3 Great Yorkshire sire line boars. The ESR genotypes of these boars were not known.

Only data from fetuses that were morphologically normal and not degenerating (referred to as vital fetuses) were used. Individual fetal and placental weights and lengths and implantation surface areas of all vital fetuses were analyzed.

Dissection, DNA Isolation and Genotyping

For DNA isolation the fetuses were thawed on ice. With 2 pairs of tweezers the chest was opened and the heart was isolated and stored in a humid petri dish until weighing (1 h after isolation). Subsequently a small sample of the inside of the liver was removed for DNA isolation. DNA was isolated using a PurGene Genomic DNA isolation kit for human and mammalian whole blood and bone marrow cells (D-5000, Gentra Systems Inc., Minneapolis, USA). DNA was then analysed for the ESR marker at PIC's Research Center (Cambridge, UK) according to Short et al. (12).

Fetuses from AA mothers were genotyped as ESR AA or AB, and are referred to as AA-AA and AA-AB fetuses, respectively. Fetuses from BB mothers were genotyped as ESR AB or BB and are referred to as BB-AB and BB-BB fetuses, respectively.

Statistics

All data were analyzed with SAS (10), using the procedure GLM. When fetal ESR genotype nested within maternal ESR genotype had a significant effect, multiple comparisons were performed with adjustments according to Tukey-Kramer (11).

Effects of fetal ESR genotypes nested within maternal ESR genotype. Fetal data analyses involved measurements obtained on each individual fetus, i.e. fetal and placental weight and length, and implantation surface area. Data of 806 fetuses (AA-AA n=107, AA-AB n=143, BB-AB n=248 and BB-BB n=308) were analyzed for the effect of fetal genotype nested within maternal genotype according to following model:

$$y_{ijklm}=\mu+batch_i+ESRm_j+batch*ESRm_{ij}+sow_k(batch*ESRm_{ij})+ESRft_l(ESRm_j)+e_{ijklm}$$

[Model 1]

in which 'batch' is the batch in which the gilts were handled, 'ESRm' the maternal ESR genotype and 'ESRft' the fetal genotype.

The same model was used for analyzing the effect of fetal genotype nested within maternal genotype on heart weight. Only weights of hearts that were still intact after isolation from the fetus were used for analyses. In total, weights of 751 hearts (AA-AA *n*=98, AA-AB n=129, BB-AB *n*=240 and BB-BB *n*=284) were available.

Relations among fetal traits. The relations of fetal or heart weight to placental and fetal measurements were assumed to be polynomial. To determine the order of the polynomial model, a stepwise forward procedure was used. In every step a subsequent order and its interaction with ESRft nested within ESRm was included in the model, but only if the last included order or its interaction was significant. The forward procedure was continued until the highest included order and its interaction was not significant, or until the fourth order was reached. At each next step all lower order interactions were included in the model. Once the highest order was reached, interactions which were not significant were eliminated stepwise, removing in each step the most not significant interaction.

The most complex model that could be reached was:

$$y_{ijklm} = \mu + batch_i + ESRm_j + batch*ESRm_{ij} + sow_k(batch*ESRm_{ij}) + ESRft_l(ESRm_j) + b1*X^1_{ijklm} + b1_{lj}*X^1_{ijklm}*[ESRft_l(ESRm_j)] \ldots\ldots + b4*X^4_{ijklm} + b4_{lj}*X^4_{ijklm}*[ESRft_l(ESRm_j)] + e_{ijklm}$$

[Model 2]

in which 'batch' is the batch in which the gilts were handled, 'ESRm' the maternal genotype, 'ESRft' the fetal genotype, 'X' the variable to be tested for its relation to fetal or heart weight (thus, 'X' is fetal length, placental length, placental weight or implantation surface area), and 'b1,....,b4' and '$b1_{lj}$,....,$b4_{lj}$' are the regression coefficients.

For the relation of heart weight to fetal weight an allometric relation was assumed. Thus, following model was used to determine the relation between the two variables:

$$\log(heartg)_{ijklm} = \mu + batch_i + ESRm_j + batch*ESRm_{ij} + sow_k(batch*ESRm_{ij}) + ESRft_l(ESRm_j) + b*\log(ftg)_{ijklm} + b_{lj}\ \log(ftgr)_{ijklm}*ESRft_l(ESRm_j) + e_{ijklm}$$

[Model 3]

in which 'heartg' is the heart weight, 'ftg' is fetal weight, 'batch' the batch in which the gilts were handled, 'ESRm' the maternal genotype, 'ESRft' the fetal genotype, and 'b' and 'b_{lj}' are the regression coefficients.

For drawing the figures, the intercepts of the equations of the relations were calculated by combined use of the least squares means calculated with Model 1 and the slopes calculated with Models 2 or 3.

If a relation differed significantly between fetal ESR genotypes nested within maternal ESR genotypes and the accompanying figure showed genotype combinations that seemed to cause this significance, the options "solutions" and "contrast" of SAS were used to compare genotype combinations.

RESULTS

Effects of Fetal ESR Genotypes Nested within Maternal ESR Genotype

Fetal ESR genotype nested within maternal ESR genotype had a significant effect on fetal heart weight ($P < 0.05$), but not on any of the other variables studied (Table 1). The heart weights of AB fetuses in AA gilts were significantly heavier ($P < 0.05$) than those of AB and BB fetuses in BB gilts and tended to be heavier ($P < 0.1$) than those of AA fetuses in AA gilts (Table 1).

Relations among Fetal Traits

Fetal and placental weight. The relation of fetal weight to fetal length was best described by a fourth order equation and differed significantly ($P < 0.05$) between fetal ESR genotypes nested within maternal ESR genotypes (Figure 1a, Table 2). Over a large range of values for fetal length (90% of the observations), fetal weight increased essentially linear with fetal length (Figure 1a).

The relation of placental weight to placental length was best described by a second order equation and differed significantly ($P < 0.05$) between fetal ESR genotypes within maternal ESR genotypes (Figure 1b, Table 3). The relation of placental weight to placental length of AA-AA fetuses differed significantly from AA-AB, BB-AB and BB-BB fetuses ($P < 0.05$, Figure 1b).

The relations of fetal weight to the three extra-embryonic traits (placental length, placental weight, and implantation surface area) were best described by a third order equation. Except for the relation between fetal weight and placental length, the others differed significantly between fetal ESR genotypes nested within maternal ESR genotypes (Figure 2, Table 2).

Fetal weight increased with placental length and weight, but only for placental lengths and weights of less than 40 cm and 40 g, respectively (Figure 2a, Figure 2b). For longer and heavier placentae (i.e., 63 and 55% of all observations, respectively), fetal weight remained on average constant.

Likewise, fetal weight increased with implantation surface area, when the latter was less than 250 cm^2. For implantation surface areas of 250 to 600 cm^2 (i.e., 50% of

Table 1. Effect of fetal ESR genotype nested within maternal ESR genotype on fetal traits (least squares means ± SEM)[1]

y	AA-AA	AA-AB	BB-AB	BB-BB	pESRft(ESRm)	n
Placental length (cm)	40.98 ± 1.03	41.65 ± 0.94	46.29 ± 0.71	46.50 ± 0.64	0.8739	806
Placental weight (g)	40.31 ± 1.33	41.44 ± 1.22	45.04 ± 0.92	46.08 ± 0.82	0.5855	806
Implantation surface area (cm^2)	250.02 ± 7.33	257.41 ± 6.73	268.44 ± 5.06	278.64 ± 4.54	0.2600	806
Fetal weight (g)	4.36 ± 0.05	4.40 ± 0.04	4.29 ± 0.03	4.32 ± 0.03	0.5960	806
Fetal length (cm)	3.83 ± 0.02	3.84 ± 0.01	3.82 ± 0.01	3.84 ± 0.01	0.4554	802
Heart weight (mg)	38.5^{a} ± 0.7	40.8^{b} ± 0.7	37.9^{ac} ± 0.5	38.5^{ac} ± 0.4	0.0498	750

[1] According to Model 1

[a,b] Different superscripts in the same row differ with $P < 0.1$

[b,c] Different superscripts in the same row differ with $P < 0.05$

all observations), fetal weight remained on average constant (Figure 2c). Within this range for implantation surface area there was a tendency for the heterozygous fetuses (AA-AB and BB-AB) to be slightly heavier than homozygous fetuses (AA-AA and BB-BB; Figure 2c).

To get a better impression about how the different fetal genotypes were distributed, placental length and weight were divided into four classes each and frequencies per fetal genotype were calculated (Table 4). These classes were based upon the relation of fetal weight to both traits (Figure 2a, 2b). The classes differed in the way in which fetal weight changed as placental weight or length increased. Thus a 'fast changing' (the steep part of the slope in the figures, i.e., for placental weight from 0 to 30 g ([0,30] g) and for placental length from 0 to 30 cm ([0,30] cm)), a 'slow changing' (<30,40] g and <30,40] cm), a 'not changing' (<40,70] g and <40,80] cm), and a 'rest group' (<70,→> g and <80,→> cm) were created. The frequencies were compared per class using the Chi-square test. For placental weight, the frequencies in the 'not changing' and the 'slow changing' classes significantly differed from the expected values ($P < 0.05$), clearly due to the frequencies for the AA-AA fetuses. The percentage of AA-AA fetuses that belonged to the 'not changing' group was lower than the other three genotypes. On the other hand, the percentage of AA-AA fetuses that belonged to the 'slow changing' group was higher than the other three genotypes (Table 4). For placental length the same tendency was observed, although it was not significant (Table 4).

Heart weight. The allometric relation of heart weight to fetal weight ($R^2 = 0.496$) differed significantly ($P = 0.0232$) between fetal ESR genotypes nested within maternal ESR genotypes (Figure 1c). The relation appeared to depend on the ESR genotype of the mother. Heart weight of fetuses from AA mothers did not increase as fetal weight increased, while heart weight of fetuses from BB mothers increased as fetal weight increased ($P < 0.05$; Figure 1c).

The relations of heart weight to the three extra-embryonic traits (placental length and weight and implantation surface area) all were best described by a second order equation (Table 5). Only the relation of heart weight to placental weight differed significantly between fetal ESR genotypes nested within maternal ESR genotypes ($P < 0.05$; Table 5).

Table 2. Relation of fetal weight (Y) to other fetal traits (X) [1]

	X = length fetus	X = length placenta	X = weight placenta	X = implantation surface area
X^4*ESRft(ESRm)	0.0170	NI	NI	NI
X^3*ESRft(ESRm)	NI	NI	NI	NI
X^2*ESRft(ESRm)	NI	NI	NI	NI
X^1*ESRft(ESRm)	NI	NI	0.0075	0.0141
X^4	0.0001	NI	NI	NI
X^3	0.0001	0.0001	0.0001	0.0001
X^2	0.0001	0.0001	0.0001	0.0001
X^1	0.0001	0.0001	0.0001	0.0001
ESRft(ESRm)	0.0180	0.3593	0.0156	0.0282
R^2	0.9123	0.8522	0.8580	0.8256

[1] According to Model 2

NI: P > 0.05 and thus not included in the model

Table 3. Relation of placental weight (Y) to placental length [1]

	X= length placenta
X^4*ESRft(ESRm)	NI
X^3*ESRft(ESRm)	NI
X^2*ESRft(ESRm)	0.0286
X^1*ESRft(ESRm)	NI
X^4	NI
X^3	NI
X^2	0.001
X^1	0.001
ESRft(ESRm)	0.0638
R^2	0.7955

[1] According to Model 2

NI: P > 0.05 and thus not included in the model

Table 4. Frequency distribution per genotype combination for 4 placental length and weight classes (for definition of classes, see text)[1]

Placental length (cm)	[0,30]	<30,40]	<40,70]	<70,→>	Total #
AA-AA	13.1	33.6	52.3	0.9	107
AA-AB	11.9	26.6	61.5	0.0	143
BB-AB	15.7	21.8	61.3	1.2	248
BB-BB	9.7	22.4	67.5	0.3	308
total	12.4	24.5	62.5	0.6	806

Placental weight (g)	[0,30]	<30,40][2]	<40,80][3]	<80,→>	Total #
AA-AA	17.8	45.8	35.5	0.9	107
AA-AB	16.8	25.2	56.6	1.4	143
BB-AB	20.6	18.6	58.5	2.4	248
BB-BB	16.9	22.7	58.8	1.6	308
total	18.1	25.0	55.2	1.7	806

[1] Categories were based upon figures 2a (placental length) and 2b (placental weight).

[2,3] Frequencies in the column differed significantly from the expected value (Chi square test, $P < 0.05$).

Table 5. Relation of fetal heart weight (Y) to placental traits (X) [1]

	X= length placenta	X= weight placenta	X= implantation surface area
X^4*ESRft(ESRm)	NI	NI	NI
X^3*ESRft(ESRm)	NI	NI	NI
X^2*ESRft(ESRm)	NI	0.0140	NI
X^1*ESRft(ESRm)	NI	0.0103	NI
X^4	NI	NI	NI
X^3	NI	NI	NI
X^2	0.0001	0.2770	0.0103
X^1	0.0001	0.0615	0.0002
ESRft(ESRm)	0.0526	0.0229	0.0914
R^2	0.5003	0.5047	0.4951

[1] accoding to model 2.

NI: $P > 0.05$ and thus not included in the model.

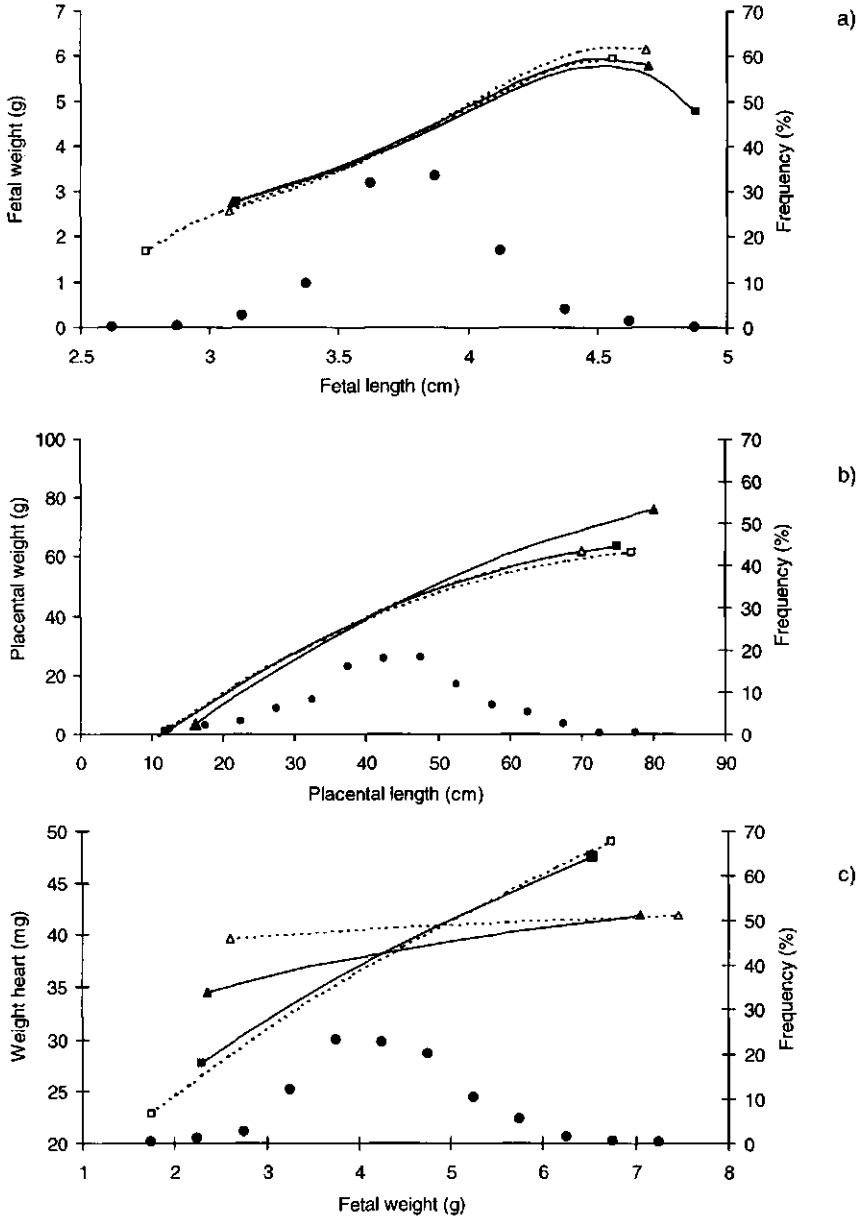

Figure 1. *Relations among fetal traits at Day 35/36 of pregnancy, including frequency distributions (●) for fetal length (a), placental length (b) and fetal weight (c).*
▲—▲ = *AA-AA* △·····△ = *AA-AB* ■—■ = *BB-BB* □·····□ = *BB-AB*

Fetal heart weight increased with placental length up to a placental length of 51.26 cm. For longer placentae (24% of the population), fetal heart weight decreased with placental length (Figure 3a). Within this relation AA-AB fetuses had significantly ($P < 0.05$) heavier hearts than BB-AB and BB-BB fetuses and tended to have ($P < 0.01$) heavier hearts than AA-AA fetuses (Figure 3a).

Similarly, fetal heart weight increased with implantation surface area up to an implantation surface area of 437 cm^2, and hereafter (4% of the population) decreased again (Figure 3c). Within this relation AA-AB fetuses had significantly ($P < 0.05$) heavier hearts than BB-AB and BB-BB fetuses (Figure 3c).

Heart weight of BB-AB fetuses increased as placental weight increased up to 55.9 g, and then decreased as placental weight increased (22.6% of all BB-AB fetuses; Figure 3b). For the other three genotype combinations, the relation of heart weight to placental weight was not significant, i.e. heart weight remained constant with increasing placental weight ($P > 0.15$; Figure 3b). Similar to the relations of heart weight to placental length and implantation surface area, AA-AB fetuses tended to have heavier heart weights compared to the other three genotype combinations (Figure 3b).

DISCUSSION

Fetal weight and length, placental weight and length and implantation surface area were not affected by fetal ESR genotype within maternal ESR genotype at Day 35/36 of pregnancy. These results imply that the difference in average placental length between AA and BB gilts found by Van Rens et al. (16) is entirely controlled by the genotype of the gilt. This seems to be in contrast with results of Wilson et al. (18) who showed that placental size and vascularity are determined by fetal and not maternal genotype. Wilson et al. (18) however compared two different breeds, while our study compared gilts within the same breed differing in ESR genotype only. Furthermore, fetal breed affected placental size and vascularity only after Day 90 of pregnancy (1,18).

Fetal ESR genotype nested within maternal ESR genotype significantly affected the relation between fetal weight and fetal length, fetal weight and placental weight and fetal weight and implantation surface area. For all three relations however, this effect did not result in striking differences in the curves for the different genotype combinations.

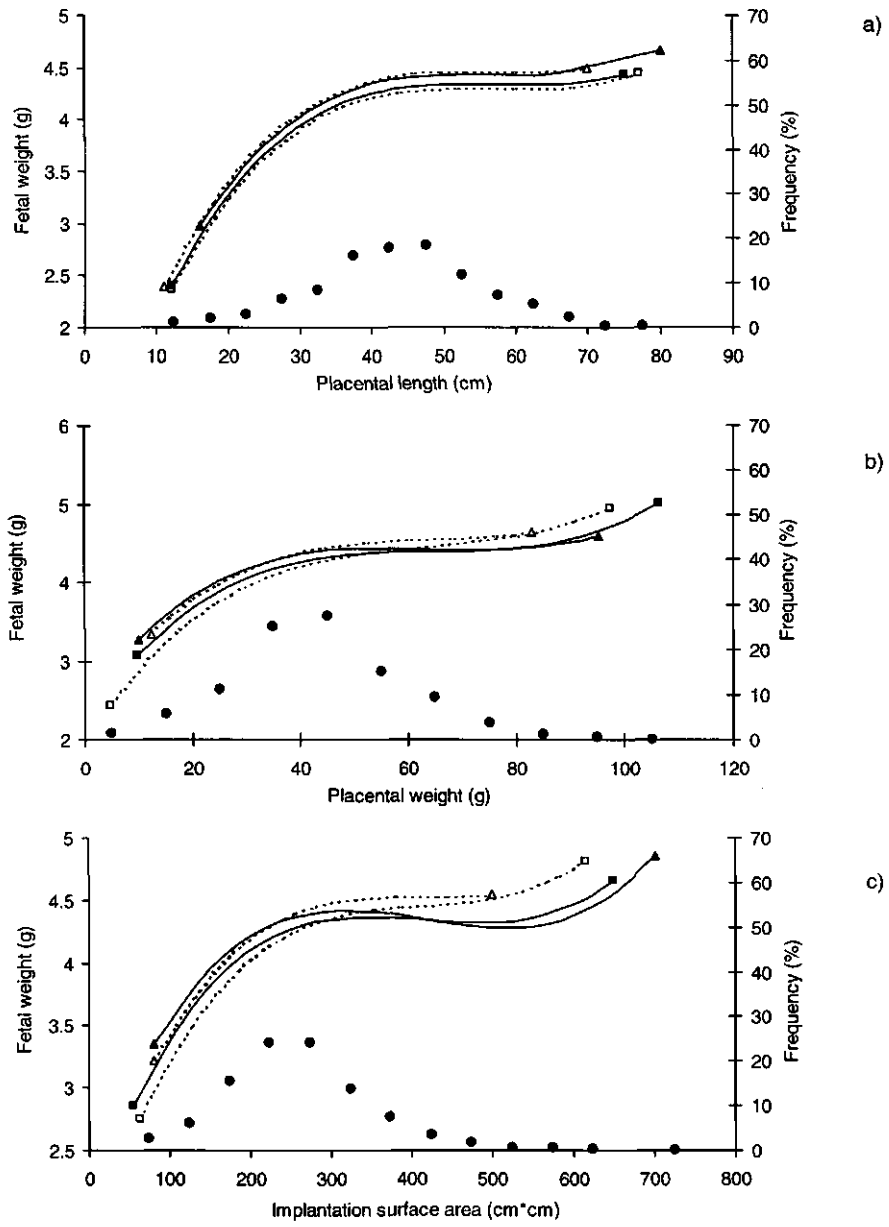

Figure 2. *Relations of fetal weight to different extra-embryonic traits at Day 35/36 of pregnancy, including frequency distributions (●) for placental length (a), placental weight (b) and implantation surface area (c).*

▲—▲ = *AA-AA* △·····△ = *AA-AB* ■—■ = *BB-BB* □·····□ = *BB-AB*

Fetal weight was related to placental length, placental weight and implantation surface area in an unexpected way. A larger placenta and a larger implantation surface area imply a larger potential surface area of contact with the endometrium and thus a better transport of nutrients from uterus to fetus. Therefore, fetuses with larger, heavier placentae and larger implantation surface areas were expected to be heavier than fetuses with shorter, lighter placentae and smaller implantation surface areas. This indeed was the case for the fetuses with placentae smaller than 40 cm or 40 g and implantation surface areas smaller than 250 cm^2 (37 to 50% of the fetuses studied). For the remaining fetuses (at least 50% of the fetuses studied) however, placental length and weight ranged from 40 to 70 cm and 40 to 80 g, respectively, and implantation surface area ranged from 250 to 450 cm^2, without affecting fetal weight. Sterle et al. (13) described a comparable relation between fetal weight and implantation length of 44 d old porcine fetuses. Thus, it seems that already in early pregnancy porcine fetuses have a maximum growth potential. Whether fetuses will be able to reach this maximum growth potential depends on available nutrients, supplied by the placenta. Apparently the placenta has to grow to at least 40 cm and 40 g during the first 35/36 days of pregnancy, to be able to nurture the fetus sufficiently so it can reach its maximum growth potential. This leads to the conclusion that fetuses with a smaller placenta are to some extent growth retarded.

Placentae that already had reached the 40 cm and 40 g at an earlier stage of pregnancy apparently have been able to continue growing. This lead of placental growth may lead to advantages later in pregnancy when uterine space may become limiting for further placental growth. Studies in which placental and fetal growth have been examined throughout pregnancy confirm latter statement: In pigs, placental length increases until Day 60 of pregnancy, with little change thereafter (3, 17). The increase in placental length precedes the increase in placental weight (3), which also appears to have reached more or less a maximum by about Day 65 (3, 6, 17) and changes relatively little thereafter, until it takes an upward turn at about Day 100 (6). The increase in placental length is most rapid between Days 20 and 30. It is suggested that the extent to which placental development occurs between Days 20 and 30 of gestation has a significant influence on subsequent fetal growth and survival (3). Fetal growth appears to be correlated with placental development (3, 17). The most rapid increase in fetal weight occurred after Day 50 (3, 6, 17).

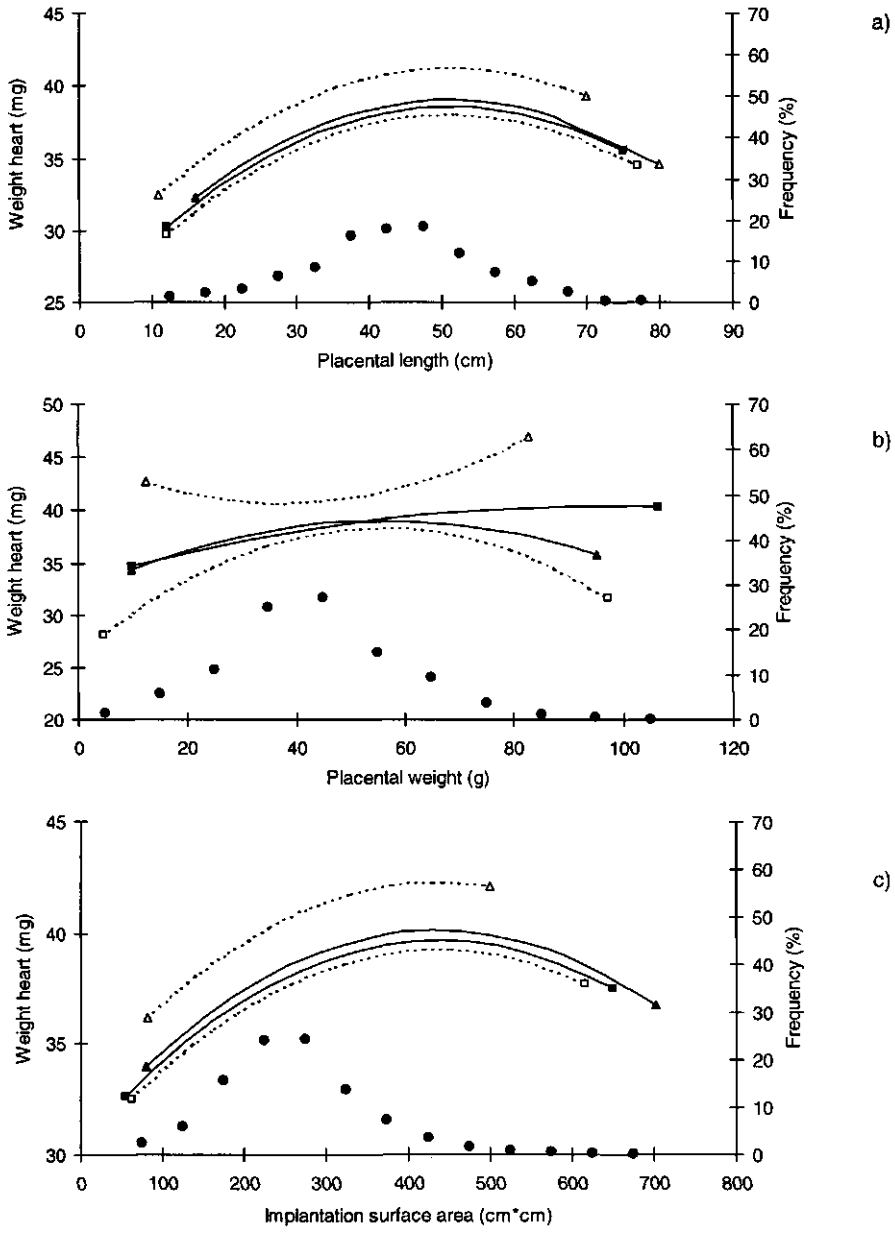

Figure 3. *Relations of fetal heart weight to different extra-embryonic traits at Day 35/36 of pregnancy, including frequency distributions (●) for placental length (a), placental weight (b) and implantation surface area (c).*

▲—▲ = *AA-AA* △·····△ = *AA-AB* ■—■ = *BB-BB* □·····□ = *BB-AB*

The percentage of AA-AA fetuses that had reached the maximum growth potential was significantly lower than of the other three genotype classes. A larger percentage was growth retarded, which, dependent on uterine capacity of the sow, might lead to a higher fetal mortality. These results indicate it would be worthwhile to analyze litter size results at piglet level, to examine whether the lower litter sizes of AA gilts reported elsewhere (7, 8, 12, 14, 15) are associated with an unexpected ratio of AA and AB piglets that were born.

In the absence of stored food in the form of yolk, the porcine embryo draws upon the uterine circulation of the mother. The nutrients absorbed from the maternal blood by the placenta have to be transported to the body of the growing embryo by its own blood stream. Continued growth of fetus as well as placenta can not progress unless the developing tissues are supplied with oxygen and nutrients, and their waste products are removed. Thus circulation is necessary very early in embryonic development. The heart which distributes the blood to the developing organs will start pumping as a tube at the 13 somite stage (about Day 15 of pregnancy) and will differentiate into a four-chambered organ from that stage to the 6 to 9 mm stage (about Day 18 to 20 of pregnancy) (5). Thus, at Day 35 of pregnancy the heart is well developed.

In the present study the relation of heart weight to fetal weight depended on the ESR genotype of the mother. Heart weight of fetuses from AA mothers were relatively independent of fetal weight, while heart weight of fetuses from BB mothers increased as fetal weight increased. Especially in the first half of pregnancy, a smaller, lighter embryo is assumed to be less developed than a larger one (4, 6, 17). Thus, assumed that a small fetus in present study was less developed than a larger one, the heart of fetuses of AA mothers seemed to develop earlier compared to the heart of fetuses of BB mothers. However, once developed, they did not increase in weight anymore. Fetuses of BB mothers appeared to take more time to develop their heart (since small fetuses had lighter hearts than fetuses of a similar developmental stage of AA mothers), but then seemed to spend more energy in growth of the heart than fetuses of AA mothers (since large fetuses of BB mothers had heavier hearts than fetuses of a similar developmental stage of AA mothers). The earlier development of fetal heart in AA mothers was accompanied by less developed placentae (16).

AA-AB fetuses had significantly heavier hearts than BB-AB and BB-BB fetuses. Furthermore they tended to have heavier hearts than AA-AA fetuses. A heavier

heart implies a higher cardiac output, and thus, for some reason AA-AB needed a higher cardiac output compared to the other three genotype classes. In adults, cardiac output and thus cardiac size can change as a reaction to for instance a chronical change in vascular resistance, a change in nutrient and oxygen demands (e.g. endurance exersize; 19) or a change in bodysize (i.e., growth; 20).

Fetal cardiovascular control is affected by an interaction of the fetal somatic and placental circulations (2). At Day 35/36 of pregnancy, porcine placental weight and length are on average ten times higher than fetal length and weight (16). Thus, blood volume of placental circulation will be enormous, compared to blood volume of systemic and pulmonary circulation, and thus placental circulation probably plays a major role in fetal cardiovascular control at that moment. Therefore, a difference in placental circulation might very well be reflected in a difference in fetal heart weight. The present data set does not contain information about placental vascularity. Nevertheless, the fact that the relation of heart weight to placental weight of AA-AB fetuses also differs from the other genotype classes, points to a placental factor affecting heart size. The continous growth and continous changes of both placenta and fetus at this stage of early pregnancy however, might change the demands for a higher cardiac output continuously through other pathways.

Obviously, the results of present study do not give enough information to explain why AA-AB fetuses have heavier hearts. However they give some challenges for further research on porcine fetal heart size, placentation and placental vascularity in combination with ESR genotype around Day 35/36 of pregnancy.

REFERENCES

1. Biensen NJ, Wilson ME, and Ford SP (1998). The impact of either a Meishan or Yorkshire uterus on Meishan or Yorkshire fetal and placental development to Days 70, 90, and 110 of gestation. J Anim Sci 76:2169-2176.
2. Faber JJ, and Anderson, DF (1997). Angiotensin mediated interaction of fetal kidney and placenta in the control of fetal arterial pressure and its role in hydrops fetalis. Placenta 18:313-326.
3. Knight JW, Bazer FW, Thatcher WW, Franke DE and Wallace HD (1977). Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 44:620-637.
4. Marrable AW and Ashdown RR (1967). Quantitative observations on pig embryos of known ages. J Agric Sci 69:443-447.
5. Patten BM (1948). Embryology of the pig. 3rd edition. McGraw-Hill book company inc. New York, Toronto, London.

6. Pomeroy RW (1960). Infertility and neonatal mortality in the sow. III Neonatal mortality and foetal development. J Agric Sci 54:31-56.
7. Rothschild MF, Jacobson C, Vaske DA, Tuggle CK, Short TH, Sasaki S, Eckardt GR and McLaren DG (1994). A major gene for litter size in pigs. Proc World Congr Genet Appl Livest Prod 21:225-228.
8. Rothschild M, Jacobson C, Vaske D, Tuggle C, Wang L, Short T, Eckardt G, Sasaki S, Vincent A, McLaren D, Southwood O, Van der Steen H, Mileham A and Plastow G (1996). The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc Natl Acad Sci USA 93:201-205.
9. Rothschild MF, Larson RG, Jacobson C, and Pearson P (1991). Pvu II polymorphisms at the porcine oestrogen receptor locus (ESR). Anim Genet 22:448.
10. SAS/STAT (1990). User's Guide. Cary NC, SAS Institute.
11. SAS/STAT (1996). Changes and enhancements through release 6.11. Cary NC, SAS Institute.
12. Short TH, Rothschild MF, Southwood OI, McLaren DG, De Vries A, Van der Steen H, Eckardt GR, Tuggle CK, Helm J, Vaske DA, Mileham AJ and Plastow GS (1997). Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. J Anim Sci 75:3138-3142.
13. Sterle JA, Cantley TC, Lamberson WR, Lucy MC, Gerrard DE, Matteri RL, and Day BN (1995). Effects of recombinant procine somatotropin on placental size, fetal growth, and IGF-I and IGF-II concentrations in pigs. J Anim Sci 73:2980-2985.
14. Southwood OI, Hoste S, Short TH, Mileham AJ and Cuthbert-Heavens D (1996). Evaluation of genetic markers for litter size in Meishan synthetic and Large White pigs. Anim Sci 62:625 (abst.)
15. Southwood OI, Van der Steen HAM, Mileham AJ, Plastow GS, Cuthbert-Heavens D (1995). Evaluation of the oestrogen receptor (ESR) gene in Meishan synthetic pigs. 46th Ann Mtg Eur Assoc Anim Prod; 53 (abst.)
16. Van Rens BTTM, Hazeleger W and Van der Lende T (2000). Periovulatory hormone profiles and components of litter size in gilts with different estrogen receptor (ESR) genotypes. Theriogenology 53:1375-1387.
17. Warwick BL (1928). Prenatal growth of swine. J Morphol Physiol 46:59-84.
18. Wilson ME, Biensen NJ, Youngs CR, and Ford SP (1998). Development of Meishan and Yorkshire littermate conceptuses in either a Meishan or Yorkshire uterine environment to day 90 of gestation and to term. Biol Reprod 58:905-910.
19. Wolfe, LA, Cunningham, DA, Boughner, DR (1986). Physical conditioning effects on cardiac dimensions: a review of echocardiographic studies. Can J Applied Sport Sci 11;66-79.
20. Yang, TS and Lin, JH (1997). Variation of heart size and its correlation with growth performance and vascular space in domestic pigs. Anim Sci 64;523-528.

Chapter 4

The effect of estrogen receptor genotype on litter size and placental traits at term in F2 crossbred gilts

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Submitted

ABSTRACT

The effect of estrogen receptor (ESR) genotype (two alleles, A and B) on litter size of 275 Large White x Meishan F2 crossbred gilts (73 AA, 126 AB and 76 BB gilts) was tested. In addition, for 63 of these gilts (18 AA, 24 AB, and 21 BB) the effect of ESR genotype on average placental traits at term was tested, since individual placental information was available for 88% of the 628 liveborn piglets. Without affecting average birthweight of the piglets, ESR genotype significantly affected litter size, i.e. AB gilts had larger litters than BB gilts ($P<0.05$). Total number born was 11.38±0.38, 11.88±0.28, and 10.68±0.35, while number born alive was 10.45±0.39, 11.07±0.29, and 9.85±0.36 for AA, AB and BB gilts, respectively. Since the B allele in previous research was associated with largest litters, the hypothesis that ESR is a marker rather than the major gene itself is discussed. Average placental length, surface area, and weight including and excluding amnion were not affected by ESR genotype. However, placentae of AB gilts had a significantly lower number of areolae per placenta than BB gilts and had a lower number of areolae per cm^2 placenta than AA and BB gilts. Number of areolae was 8945±663, 7240±619, and 9694±633, for AA, AB and BB gilts, respectively. Although the reason for the low number of areolae on placentae in AB gilts is not yet known, the results suggest that the ESR linked major gene for litter size might be involved in the development and activity of endometrial glands.

Keywords: pig, estrogen receptor gene, litter size, placenta, Meishan, areolae

Acknowledgments

The authors like to acknowledge Jet de Hoog, Jascha Leenhouwers, Nicole Ng-A-Tham and Henk Vos for assisting in labeling, Bart Spee and Frans Vermeulen for isolating the DNA, Richard Wales (PIC International Group) for genotyping the animals, and professor S.P. Ford (Iowa State University, US) for his advice.

INTRODUCTION

Using the candidate gene approach, Rothschild et al. (18) suggested that the estrogen receptor (ESR) gene is a major gene for litter size in pigs. Two ESR alleles (A and B) were described, of which the B allele appeared to be associated with a larger litter size in several lines studied (19, 24). A physiological study of gilts from one of those lines indicated that the litter size difference must be due to a difference in fetal survival, since there were no differences in number of ovulations and in number of fetuses at Day 35 of pregnancy, while placentae of Day 35 fetuses of BB gilts were significantly longer than those of AA gilts (26). At present, there is no information about placental traits at term for gilts differing in ESR genotype. Furthermore, it is not known whether the ESR gene polymorphism itself causes differences in litter size or whether this polymorphism provides a marker for a closely linked major gene for litter size. Therefore, the objectives of this research were to evaluate the effect of the ESR genotype on litter size in Large White x Meishan F2 crossbred gilts and to examine the effects of ESR genotype on placental traits at term.

MATERIALS AND METHODS

Animals

All pigs used in this experiment were bred and raised at the experimental farm of Wageningen University. Two half sib Large White Boars (ESR genotype AA) and 8 Meishan (2 BB and 6 AB) sows were used as parents (boar 1 was mated to 5 sows and boar 2 was mated to 3 sows). From the F1 offspring, 6 AB boars and 21 AB gilts were selected to produce the F2 population. To avoid inbreeding as much as possible, the F1 offspring of boar 1 was only mated to the F1 offspring of boar 2.

Females of the second, third, fourth and fifth litter of the F1 crossbred sows were used as the experimental animals in this research. Because of a lack of space, a random selection of 31 AB gilts was excluded from the experiment. Thus a total number of 334 Large White x Meishan F2 crossbred gilts (94 AA, 154 AB and 86 BB) were included in experiment 1. Of these gilts, 63 (18 AA, 24 AB, and 21 BB) were also included in experiment 2.

Experiment 1: Farrowing Experiment

At an average age of 8 weeks, the gilts were housed in groups of 4. By preference, groups consisted of littermates with similar bodyweights and different ESR genotypes.

Once housed in groups, the animals were checked once daily (1600) for estrus by the back pressure test in presence of a vasectomized adult boar. When the gilts showed their fourth estrus, they were artificially inseminated twice with an interval of 24 hours with semen of a Great York-S boar ($3*10^9$ sperm cells per dose). A different boar was used for first and second insemination. ESR genotype of the boars was not known. Animals that returned to estrus, were inseminated again, following the same protocol. Animals that returned to estrus for a second time were excluded from the experiment.

Animals that did not return to estrus, remained in the original group until not later than day 107 after insemination. They were then transferred to individual farrowing pens (Danish Farrowing Pen 220*380 cm^2, farrowing crate 265x66 cm^2, concrete floor covered with saw dust).

Within 16 h after farrowing, live born, stillborn and non-fresh stillborn piglets were distinguished, and sex, birthweight and teat number of the piglets was determined. All expelled placentae were meticulously investigated to recover all mummified fetuses.

Pregnant gilts were fed 1.25 running up to 1.5 kg commercial sow ration (12.56 MJ metabolizable energy/kg, crude protein 144 g/kg, ileal digestible lysine 7.5 g/kg) twice daily at 0830 and 1630. Water was always available ad libitum.

The gilts were weighed at 17 days after showing their third estrus. Gilts which had returned to estrus and thus had been inseminated again, were weighed just preceding insemination.

Experiment 2: Placentae at Term

The placentae of 63 F2 females (18 AA, 24 AB, and 21 BB) from experiment 1 were labeled during farrowing as described below. The experimental animals were a random sample of the second litter of 19 Large White x Meishan F1 sows.

Labeling of the placentae. Placentae were labeled as described by Wilson et al. (28), with slight modifications. The gilts were observed every 1h beginning at 1800 on Day 112 of gestation for signs of impending parturition. Once milk let-down or vulvar swelling and mucous secretion was observed, the gilts were monitored continuously until farrowing and placental expulsion was completed. As a piglet was expelled, it was caught, and the umbilical cord was ligated with surgical silk (35165, Linnen Braun EP-5 USP 2, Instruvet, Amerongen, The Netherlands) containing a specific code. The umbilical cord was then cut between piglet and tag, allowing the placental end of the cord with its tag to retract into the vagina. Subsequently the piglet was earmarked with a number corresponding to the specific code on the tag.

Immediately after expulsion, the placentae were identified and stored at 4 °C until morphometric analysis.

Morphometry of placentae. Within 24 hours after expulsion, the placentae were identified and carefully isolated from each other. Their length was measured, excluding eventual necrotic tips. The allantochorion was opened over the whole length at the anti-mesometrial side and the umbilical cord was removed. Subsequently, placental weight was determined before and after removal of amniotic membranes.

The remaining chorio-allantois was then spread on a surface containing parallel black bands of 1 cm, which were positioned at 4 cm from each other. The longest axis of the chorio-allantois was positioned perpendicular to the black bands. All areolae visible on the black bands were counted. Total number of areolae was calculated by multiplying the result by five. Subsequently placental surface area was determined.

Statistical Analysis

All data were analysed with SAS (22). Unless mentioned differently, the procedure GLM was used for analysing linear models.

Farrowing experiment (experiment 1). In total 275 gilts (at successful insemination on average(±sd) 106±22 kg, 257±53 d old, and estrus number 4.6±1.3) had farrowed. The effect of ESR was tested on following traits: Total number born (TNB, in this paper defined as the sum of the number of live born, stillborn and non-fresh stillborn piglets), number born alive (NBA), sum of mummies and non-fresh stillborn piglets (mumdnf), sum of TNB and mummies (TNB+mum), number of mummies (mum), gestation length (GL), birthweight (BW), i.e. the average birthweight of live born piglets per gilt, and teat number (TN), i.e., the average number of teats of the live born piglets per gilt.

Results are presented as least squares means and standard errors of least squares means, estimated after stepwise elimination of non-significant effects (except for 'ESR'), i.e. effects with $P > 0.05$, using the following model:

$$y_{ijklmn} = m+ESR_i+fam_j+batch_k+season_l+enr_m+kgins+ageins+ \\ +fam*ESR_{ij}+batch*ESR_{ik}+season*ESR_{il}+enr*ESR_{im}+ \\ kgins*ESR+ageins*ESR+e_{ijklmn} \qquad \text{[model 1]}$$

in which 'fam' (j=1-27) is the family the F2 gilt belonged to (i.e., the combination F1 boar x F1 sow the F2 gilt descended from), 'batch' (k=1-4) is the parity of the F1 where the F2 gilt descended from (i.e. the second until fifth litter), 'season' (l=1-4) is the season in which the gilt was inseminated (autumn, winter, spring and summer), 'enr' (m=1,2) is the estrus number at insemination (4, or >4), 'kgins' is the weight at insemination, and 'ageins' is the age at insemination.

When ESR genotype had a significant effect (i.e. $P<0.05$), multiple comparisons were performed with adjustments according to Tukey-Kramer (23). In addition, for some of the traits tested, TNB has been included in the model as a covariable in order to examine whether the eventual ESR effect . In this case, of all possible interactions, only the interactions of ESR with the continuous variables (kgins and ageins) and TNB have been included in the model. Again, least squares means and standard errors of least squares means were estimated after stepwise

elimination of non-significant effects (except for 'ESR' and 'TNB'), i.e. effects with $P > 0.05$.

The effect of ESR genotype on the number of teats of the gilts themselves was also tested, using a part of model 1. Only 'fam' and its interaction with ESR were in the initial model. The stepwize elimination strategy was as described above.

The effect of ESR genotype on the number of mummies (mum) and on the sum of mummies and non-fresh stillborn piglets (mumdnf) both as a ratio of TNB+mum has been tested using the GENMOD procedure of SAS (binomial distribution), which included all effects that remained significant after stepwise elimination of the non-significant effects using model 1. Least squares means and standard errors of least squares means presented in the results, however, are estimates resulting from GLM analysis using model 1.

Placentae at term (experiment 2). As far as placental traits were concerned, all statistical analyses were performed on average values per gilt. These values represent only the liveborn piglets. Results are presented as least squares means and standard errors of least squares means, estimated after stepwise elimination of non-significant effects (except for 'ESR'), i.e. effects with $P > 0.1$ (since the numbers are smaller than in experiment 1), using the following model:

$$y_{ijklm}=m+ESR_i+fam_j+season_k+enr_l+kgins+ageins+ESR*kgins+ESR*ageins+e_{ijklm}$$

[model 2]

in which 'fam' (j=1-18) is the family the F2 gilt belonged to (i.e., the combination F1boar x F1 sow the F2 gilt descended from), 'season' (k=1,2) is the season in which the gilt was inseminated (autumn and winter), 'enr' (l=1,2) is the estrus number at insemination (4, or >4), 'kgins' is the weight at insemination, and 'ageins' is the age at insemination.

When ESR genotype had a significant effect (i.e. $P<0.05$), multiple comparisons were performed with adjustments according to Tukey-Kramer (23).

Traits that were examined were: Placental length, surface area, and weight with and without amnion, number of areolae and number of areolae per cm^2. For latter

Table 1. Reasons for culling experimental animals before farrowing[1]

	AA	AB	BB
available gilts	94 (100%)	154 (100%)	86 (100%)
not inseminated[2]	2 (2.13%)	5 (3.25%)	3 (3.49%)
returned to estrus twice	6 (6.38%)	6 (3.90%)	2 (2.33%)
not pregnant	3 (3.19%)	0 (0.00%)	0 (0.00%)
illness	10 (10.64%)	17 (11.04%)	5 (5.81%)
total	21 (22.34%)	28 (18.18%)	10 (11.63%)

[1] numbers of gilts that were culled (between brackets the percentage per genotype)
[2] animals that did not reach their fourth estrus within the experimental period

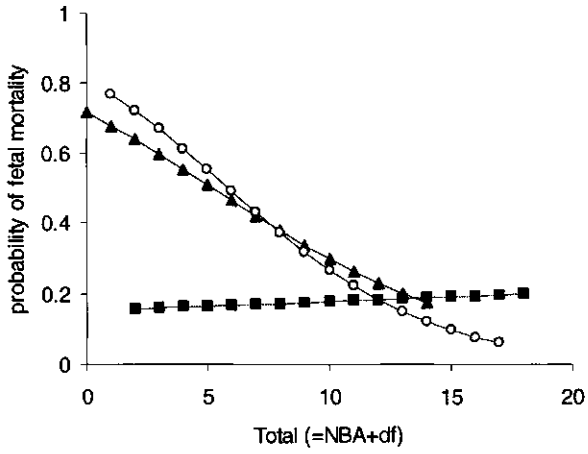

Figure 1. Relation of the probability of fetal mortality to number of piglets born (i.e. number born alive plus fresh stillborn piglets) for the three different ESR genotypes
▲ = AA ○ = AB ■ = BB

trait, the number of areolae per cm^2 was calculated for each placenta individually, after which the average value per gilt was used for examining the effect of ESR genotype.

Furthermore, for each liveborn piglet of which own placental information was known (since its placenta was labeled successfully), placental efficiencies were calculated by dividing birthweight by placental length, surface area, weight or number of areolae. The average efficiencies per gilt were then used for examining the effect of ESR genotype.

In addition, for some of the traits tested, TNB and its interaction with ESR had been included in the model as a covariable, after which least squares means and standard errors of least squares means were estimated after stepwise elimination of non-significant effects (except for 'ESR' and 'TNB'), i.e. effects with $P > 0.1$. Since the numbers are smaller than for experiment 1, a different P value is used to retain effects.

RESULTS

Experiment 1

In total, 59 gilts (21 AA, 28 AB, and 10 BB) were excluded from the experiment for different reasons (Table 1), and 275 gilts (73 AA, 126 AB and 76 BB) farrowed.

Litter size results are presented in Table 2. ESR genotype significantly affected TNB+mum, TNB and NBA, i.e. AB gilts had larger litters than BB gilts. ESR genotype did not affect mumdnf or mum (Table 2). The probability for fetal mortality (i.e. mumdnf/(TNB+mum)), however decreased significantly with an increase in the sum of NBA and fresh still born piglets for AA and AB gilts, while the probability for fetal mortality did not change with an increase in the sum of NBA and fresh still born piglets for BB gilts (Figure 1).

Teat number and gestation length of the F2 crossbred gilts was not affected by ESR genotype (Table 2). Furthermore, average birthweight and teat number of liveborn piglets was similar for the three genotypes (Table 2).

Table 2. Litter traits[1] of Large White x Meishan F2 crossbred gilts with different estrogen receptor (ESR) genotype (lsmeans±sem)

	n	AA	AB	BB	P
TNB + mum	268	12.31±0.40^{ab}	12.55±0.30^{a}	11.21±0.38^{b}	0.0196
TNB	275	11.38±0.38^{ab}	11.88±0.28^{a}	10.68±0.35^{b}	0.0307
NBA	267	10.45±0.39^{ab}	11.07±0.29^{a}	9.85±0.36^{b}	0.0307
mum + dnf	268	1.29±0.20	1.04±0.15	0.73±0.19	0.1410[2]
mum	268	0.95±0.15	0.71±0.11	0.57±0.15	0.1780[2]
gestation length (d)	268	113.8±0.2	114.1±0.2	114.0±0.2	0.3845
gestation length (d)^{TNB}	199	----------[3]	114.3±0.2	114.1±0.2	0.2881
teat number	275	15.67±0.14	15.59±0.11	15.49±0.14	0.6544
average piglet traits[4]					
birthweight (g)	266	1228±28	1218±22	1190±25	0.4689
teat number	265	14.36±0.07	14.38±0.05	14.26±0.07	0.3373
birthweight (g)^{TNB}	266	1244±23^{a}	1248±19^{a}	1171±21^{b}	0.0072
teat number^{TNB}	265	14.36±0.07	14.39±0.05	14.25±0.07	0.2736

1 TNB=total number born (the sum of number of liveborn, stillborn and non-fresh stillborn), NBA=number born alive, mum=mummies, dnf=non-fresh stillborn piglets

2 P value after using proc GENMOD (binomial distribution)

3 an interaction of ESR with TNB was found (for explanation: see text)

4 Results represent live born piglets

a,b Means in the same row with different superscripts differ significantly (pdiff<0.05)

TNB TNB was included as a covariable in the model

Including TNB in the model did not change the effect of ESR genotype on average teat number of liveborn piglets (Table 2). For gestation length, however, an interaction between TNB and ESR was found (P=0.031), resulting in a regression line with a slope that differed from zero for AA gilts but did not differ from zero for AB and BB gilts. The slopes of the regression lines were 0.14 (P=0.017), -0.06 (P=0.300) and 0.001 (P=0.980) for AA, AB and BB gilts, respectively (R^2=0.024), indicating a slight increase in gestation length with an increase in litter size for AA gilts. When AA animals were excluded from the analysis, there was no difference between gestation length of AB and BB gilts after including TNB in the model (Table 2).

Including TNB in the model changed the effect of ESR genotype on birthweight. Piglets of BB gilts had on average a significant lower birthweight than piglets of AA and AB gilts (Table 2). The relation of the average birthweight to TNB was similar for the three genotypes, i.e. the average birthweight of the piglets decreased with 33 g with each additional piglet (P=0.001). Correction for gestation length did not change the effect of ESR genotype on birthweight, neither before, nor after including TNB in the model.

Experiment 2

The placentae of 63 gilts (18 AA, 24 AB and 21 BB) had been labeled. For these gilts, TNB was 10.67, 10.46, and 9.48, and NBA was 10.56, 10.21, and 9.24 for AA, AB and BB gilts, respectively. Total number of piglets born was 642. For 88% of all 628 liveborn piglets, own placental information was available.

Results of placental morphometry and efficiency are presented in Table 3. The average placental length, surface area, and weight per gilt were not affected by ESR genotype, neither was birthweight. The average number of areolae, however, was lower for AB gilts compared to BB gilts, and the average number of areolae per cm^2 was lower for AB gilts compared to both AA and BB gilts (Table 3). Birthweight divided by the number of areolae was higher for AB gilts compared to AA and BB gilts (Table 3).

Including TNB in the model did not change the effect of ESR genotype on placental weight (including and excluding amnion), birthweight, or number of areolae. However, the number of areolae of AB gilts now differed from AA gilts (Table 3).

Table 3. The effect of estrogen receptor (ESR) genotype of Large White x Meishan F2 crossbred gilts on average placental traits of their liveborn piglets

	TNB not included in the model[1]					TNB included in the model[1]				
	n	AA	AB	BB	P	n	AA	AB	BB	P
length (cm)	63	74.9±2.4	73.6±2.1	74.1±2.3	0.9149	42	75.3±1.1^{a}	71.3±1.1^{b}	----------[2]	0.0229
surface area (cm^2)	63	1629±54	1612±48	1586±51	0.8447	63	1654±38	1645±35	1535±36	0.0564
weight incl. amnion (g)	63	203.6±10.9	202.0±10.2	207.2±10.4	0.9379	63	207.4±9.8	207.1±9.2	200.5±9.5	0.8564
weight excl. amnion (g)	62	177.9±9.2	176.7±8.5	181.5±9.0	0.9258	62	180.7±8.1	181.1±7.5	176.2±8.0	0.8976
number of areolae	63	8945±663^{ab}	7240±619^{a}	9694±633^{b}	0.0299	63	9317±439^{a}	7749±413^{b}	9034±426^{ab}	0.0291
number of areolae per cm^2	63	5.48±0.27^{a}	4.68±0.25^{b}	5.85±0.26^{a}	0.0092	42	5.6±0.2^{a}	4.9±0.2^{b}	----------[3]	0.0195
birthweight (g)	63	1249±42	1228±38	1242±40	0.9314	63	1267±35	1240±33	1219±34	0.6146
Efficiencies (birthweight/placental trait)										
birthweight/length	63	17.46±0.39	17.41±0.34	17.21±0.36	0.8742	63	17.18±0.40	17.20±0.35	17.16±0.36	0.9971
birthweight/surface area	63	0.78±0.02	0.77±0.02	0.79±0.02	0.7819	63	0.78±0.02	0.77±0.02	0.79±0.02	0.7569
birthweight/weight excl. amn	62	7.33±0.20	7.16±0.19	7.21±0.20	0.8142	62	7.32±0.21	7.13±0.19	7.24±0.20	0.8041
birthweight/areolae	63	0.145±0.007^{a}	0.170±0.007^{b}	0.145±0.007^{a}	0.0201	63	0.142±0.007^{a}	0.167±0.006^{b}	0.150±0.006^{ab}	0.0268

1 ^{a,b} Means in the same row with different superscripts differ significantly (pdiff<0.05)

2 an interaction of ESR with age at successful insemination was found (for explanation: see text)

3 an interaction of ESR with TNB was found (for explanation: see text)

Placental surface area tended to be affected by ESR genotype after including TNB in the model. Thus, BB gilts tended to have smaller placentae than AA (P=0.08) and AB (P=0.09) gilts. For the number of areolae per cm^2 placenta, an interaction between TNB and ESR genotype was found (P=0.035). The slope of the regression line of BB gilts (-0.29) significantly differed from zero (P<0.0001), while the slopes of AA (-0.09) and AB (-0.09) gilts did not (P=0.15 and P=0.21, respectively; R^2=0.227). When BB gilts were excluded from the analysis, number of areolae per cm^2 was significantly larger for AA gilts compared to AB gilts (Table 3).

For placental length, an interaction between age at insemination and ESR genotype (P=0.079) was found after including TNB in the model. Slopes of the three regression lines were 0.076 (P=0.23), 0.003 (P=0.94) and 0.15 (P=0.0037) for AA, AB and BB gilts, respectively (R^2=0.052). When BB gilts were excluded from the analysis, liveborn piglets of AA gilts had significantly longer placentae than of AB gilts (Table 3). When the interaction between age at insemination and ESR genotype was eliminated from the model, ESR genotype did not affect placental length (P=0.377) after including TNB in the model. Least squares means were then 74.9±1.6, 73.6±1.5, and 71.7±1.6 cm for AA, AB and BB gilts, respectively.

DISCUSSION

In the present study, ESR genotype significantly affected litter size of Large White x Meishan F2 crossbred gilts. AB gilts had 1.2, and AA gilts 0.6 more live born piglets than BB gilts. This result is not in agreement with any other published results. A summary of known published experiments in which the effect of ESR genotype on litter size has been tested is presented in Table 4. If in gilts differences in litter size were found, they were always in favor of the B allele (Table 4). These differences did not always hold in higher parities though (Table 4). In the gilts of the present study however, the A allele was favorable, showing complete dominance for TNB as well as NBA. Hence, it can be hypothesized that the ESR gene polymorphism provides a marker for either another mutation in the ESR gene, or a mutation in a closely linked gene affecting litter size. In the following discussion the phrase "major gene" will be used to indicate both. If the ESR gene polymorphism used in the present study is merely a marker for litter size, the favorable allele of the actual major gene for litter size is linked to the A allele instead of the B allele in our experimental animals. The polymorphism of the presumed closely linked major gene for litter size might very well consist of more than two (favorable) alleles.

Table 4 Summary of published experiments in which the effect of estrogen receptor (ESR) genotype on litter size has been tested

	# obs			TNB[1]			NBA[1]				
Line	AA	AB	BB	AA	AB	BB	AA	AB	BB	parity	Authors
50% US//50% MS synthetic	32	41	12	10.9^{c}	12.4^{d}	12.2^{cd}	10.4^{g}	11.4^{h}	11.8^{h}	1	Rothschild et al. (18)
50% UK or US// 50% MS synthetic	75	94	38	11.2^{a}	12.5^{b}	13.5^{b}	10.0^{a}	11.1^{b}	12.3^{b}	1	Rothschild et al. (21)
50% UK or US// 50% MS synthetic	50	80	35	11.8	12.6	12.7	11.3	11.6	11.3	> 1	Rothschild et al. (21)
Large White	674	652	425	9.7^{c}	10.1^{d}	10.4^{d}	8.9^{e}	9.2^{f}	9.5^{f}	1	Rothschild et al. (21)
Large White	541	595	405	10.2^{a}	11.0^{b}	10.8^{ab}	9.0^{a}	9.9^{b}	9.9^{ab}	>1	Rothschild et al. (21)
L93 50% UK//50% MS synthetic	20	24	18	10.53^{c}	12.64^{d}	13.39^{d}	9.28^{c}	10.97^{cd}	11.88^{d}	1	Southwood et al. (25)
L93 50% UK//50% MS synthetic	25	35	17	11.97	12.63	12.95	10.70	11.10	9.69	>1	Southwood et al. (25)
LW Hyper and LW Temoin	82	267	109	13.53	13.68	13.84	12.28	12.12	12.75	>0	Legault et al. (16)
MS synthetic female line	62	73	26	10.1^{a}	11.4^{b}	12.4^{b}	9.1^{a}	10.5^{b}	11.4^{b}	1	Rothschild et al. (19)
MS synthetic female line	98	125	53	11.0^{a}	12.4^{b}	12.5^{b}	10.2^{a}	11.5^{b}	11.7^{b}	>0	Rothschild et al. (19)
Large White lines	444	391	244	9.5^{c}	9.9^{cd}	10.7^{d}	8.7^{c}	9.2^{cd}	9.9^{d}	1	Rothschild et al. (19)
Large White lines	759	677	476	9.8^{a}	10.4^{b}	10.7^{b}	9.0^{a}	9.5^{b}	9.9^{b}	>0	Rothschild et al. (19)
LW and LW synthetic	------	------	------	10.14^{a}	10.59^{b}	10.97^{c}	9.42^{a}	9.87^{b}	10.22^{c}	1	Short et al. (24)
LW and LW synthetic	------	------	------	11.36^{a}	11.86^{b}	12.04^{b}	10.03^{a}	10.51^{b}	10.71^{b}	>0	Short et al. (24)
Zlotnicka Spotted x Polish Large White	------	------	------	8.95	8.22	------	------	------	------	>0	Korwin-Kossakowska et al. (15)

[1] ^{a,b,c} different letters in the same row, $P < 0.01$; ^{c,d} different letters in the same row, $P < 0.05$; ^{e,f} different letters in the same row, $P < 0.1$; ^{g,h} different letters in the same row, $P < 0.2$

In our study, the B allele always originated from the Meishan, and the A allele from the Large White grandparents. Thus, the ESR A allele in our population might be linked to a completely different favorable allele of the major gene than the B allele in other studies.

Since the experiment was performed with gilts of a segregating F2 cross of Large White and Meishan pigs, a large genetic variation for all other characteristics will exist, at least compared to a pure line or to the F1. Thus, the chance of a certain combination of background genes blurring out the effect of ESR genotype is low. However, since the whole population descended from two Large White half sib boars and eight Meishan sows only, the previously observed effect of the favorable B allele (Table 4) might have been overruled by the presence of a different unknown major gene for litter size. This however, is very unlikely, since the effect of another major gene is expected to be expressed equally in all three ESR genotypes, unless its physiological effect is coupled to or dependent on the ESR effect.

The mutation causing the ESR gene polymorphism was in an intron (M.F. Rothschild, personal communication), which makes a difference in expression or in structure of ESR relatively unlikely, and thus ESR being a marker for litter size more likely.

The ESR gene tested in this and all other studies (Table 4), is located on the p region of chromosome 1 (19). Its polymorphism was identified using a cDNA insert of a human ESR gene probe (20). This human ESR gene, located on chromosome 6 (12), encodes for human ESRα, and not for the later discovered human ESRβ, whose gene is located on chromosome 14 (10).

Besides the effect of ESR genotype on litter size, its effect on average teat number and average birthweight of the piglets has also been examined in the present study. Rothschild et al. (19) suggested that the B allele was associated with increased teat number in Meishan synthetic pigs. Short et al. (24) did not confirm this result in Large White lines, but described a slight but significant negative effect of the B allele. In the present study, no significant effect of ESR genotype on own teat number or on teat number of the piglets was found. Least squares mean estimates of both traits however, showed an association of the A allele with increased teat number, rather than the B allele. A similar tendency was found by Rothschild et al. (19) in Large White synthetics. Functional teat number of the

ESR / at term / gilt

number of glands, leading to comparable numbers of areolae on their placentae, provided that endometrial glands are distributed equally in the uterus. Despite the fact that AA and AB gilts had comparable litter sizes, and also comparable placental sizes for their piglets, the density of areolae differed. Piglets of AB gilts had less areolae per cm^2 placenta than of AA gilts, and thus very probably have had access to less endometrial glands. The reason for this is not known, but might be related to a difference in density of endometrial glands, a difference in length of implantation sites, and/or to a difference in uterine length, possibly combined with a difference in size of unoccupied parts of the uterus. Assuming that the total number of uterine glands in both genotypes is the same, the difference in density of these uterine glands (concluded from the difference in density of areolae), might be the result of an already initially longer uterus in AB gilts (leading to larger unoccupied uterine sites for AB gilts), or the result of a more stretched uterus at the end of pregnancy in AB gilts. Furthermore, the density of uterine glands might have been similar for both genotypes, while the endometrial folding in AB gilts might have been higher, also leading to a lower density of areolae on the placentae. On the other hand, the total number of uterine glands might have differed for both genotypes, which, dependent on the degree of stretching of the uterine horns might have lead to a lower density of uterine glands resulting in a lower density of areolae in AB gilts. The lower density of areolae in AB gilts has not affected average birthweight, however, suggesting a relatively higher activity of the endometrial glands connected to placentae of piglets of AB gilts, or a lack of any association of number of areolae with birthweight. The latter is probably not the case, since the average birthweight in this study increased both with an increase in the average number of areolae ($P=0.0001$) and the average number of areolae per cm^2 placenta ($P=0.0580$; results not shown). The possibility of uterine crowding for AB gilts seems unlikely, since uterine crowding does not only seem to be related with lower numbers of areolae per placenta, but also with smaller placental sizes (14).

Including TNB in the model did not dramatically change the effect of ESR on number of areolae, but indicated a different effect of TNB on the number of areolae per cm^2 for the BB gilts compared to the AA and AB gilts. The number of areolae per cm^2 placenta of piglets from BB gilts significantly decreased with an increase of TNB, suggesting that uteri of this genotype have less capacity compared to uteri of AA and AB gilts.

Uterine length, distribution and size of implantation sites, and number and distribution of endometrial glands were not known. Further reseach in this area might explain the difference of number of areolae between the different ESR genotypes, and might indirectly help in the search for the proposed linked major gene for litter size.

Implications

Results of the present study in comparison to published data make it plausible that porcine ESR α gene is a marker rather than the actual major gene for litter size. Therefore, for each genetic line, the favorable ESR allele should be distinguished before selecting on ESR.

Except for the number of areolae, average placental characteristics at farrowing were not affected by ESR genotype of the mother. In contrast to the effect on litter size, the effect on number of areolae per cm^2 was overdominant. The reason for this is not yet clear. The ESR linked major gene for litter size however, might therefore very well be involved in the development and activity of endometrial glands.

REFERENCES

1. Abromavich CE (1926). The morphology and distribution of the rosettes on the foetal placenta of the pig. Anat Rec 39:69-72.
2. Bal HS, and Getty R (1970). Postnatal growth of the swine uterus from birth to six months. Growth 34:15-30.
3. Bielańska-Osuchowska Z (1995). Ultrastructure of mucous areolae in the pig placenta. Folia Histochemica et Cytobiologica 33:103-110.
4. Bielańska-Osuchowska Z, and Kuńska A (1995). A new appproach to the areolar structures in the pig placenta: Histochemistry and development of mucous areolae. Folia Histochemica et Cytobiologica 33:95-101.
5. Brambel CE (1933). Allantochorionic differentiations of the pig studied morphologically and histochemically. Am J Anat 52:397-459.
6. Chen TT, Bazer FW, Gebhardt BM, and Roberts RM (1975). Uterine secretion in mammals: synthesis and placental transport of a purple acid phosphatase in pigs. Biol Reprod 13:304-313.
7. Chen ZY, and Dziuk PJ (1993). Influence of initial length of uterus per embryo and gestation stage on prenatal survival, development, and sex ratio in the pig. J Anim Sci 71:1895-1901.
8. Dantzer V (1984). Scanning electron microscopy of exposed surfaces of the porcine placenta. Acta Anat 118:96-106.
9. Dantzer V, and Leiser R (1993). Microvasculature of regular and irregular areolae of the areola-gland subunit of the porcine placenta: structural and functional aspects. Anat Embryol 188:257-267.

10. Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjöld M, and Gustafsson J-Å (1997). Human estrogen receptor β-gene structure, chromosomal localization, and expression pattern. J Clin Endocr Metab 82:4258-4265.
11. Friess AE, Sinowatz F, Skolek-Winnisch R, and Träutner W (1981). The placenta of the pig. II The ultrastructure of the areolae. Anat Embryol 163:43-53.
12. Green S, Walter P, Kumar V, Krust A, Bornert J-M, Argos P, and Chambon P (1986). Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature 320:134-139.
13. Haley CS, Lee GJ and Ritchie M (1995). Comparative reproductive performance in Meishan and Large White pigs and their crosses. Anim Sci 60:259-267.
14. Knight JW, Bazer FW, Thatcher WW, Franke DE, and Wallace HD (1977). Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 44:620-637.
15. Korwin-Kossakowska A, Kuryl J, and Pierzchala M (1999). An analysis of relations between the polymorphism of estrogene receptor gene and some reproduction traits in Zlotnicka Spotted x Polish Large White pigs. Animal Science Papers and Reports 17:155-161.
16. Legault C, Gruand J, Lebost J, Garreau H, Ollivier L, Messer LA, and Rothschild MF (1996). Fréquence et effet sur la prolificité du gène ESR dans deux lignées Large White en France. Journées Rech Porcine en France 28:9-14.
17. Perry JS, and Rowlands IW (1962). Early pregnancy in the pig. J Reprod Fertil 4:175-188.
18. Rothschild MF, Jacobson C, Vaske DA, Tuggle CK, Short TH, Sasaki S, Eckardt GR, and McLaren DG (1994). A major gene for litter size in pigs. Proc World Congr Genet Appl Livest Prod 21:225-228.
19. Rothschild M, Jacobson C, Vaske D, Tuggle C, Wang L, Short T, Eckardt G, Sasaki S, Vincent A, Mc Laren D, Southwood O, Van der Steen H, Mileham A, and Plastow G (1996). The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc Natl Acad Sci USA 93:201-205.
20. Rothschild MF, Larson RG, Jacobson C, and Pearson P (1991). Pvu II polymorphisms at the porcine oestrogen receptor locus (ESR). Anim Genet 22:448.
21. Rothschild MF, Vaske DA, Tuggle CK, Messer LA, Mc Laren DG, Short TH, Eckardt GR, Mileham AJ, Plastow GS, in collaboration with Southwood OI and Van der Steen HAM (1995). Estrogen receptor locus is a major gene for litter size in the pig. 46th Ann Mtg Eur Assoc Anim Prod 1995:5 pp.
22. SAS/STAT (1990). User's Guide. Cary NC, SAS Institute.
23. SAS/STAT (1996). Changes and enhancements through release 6.11. Cary NC, SAS Institute.
24. Short TH, Rothschild MF, Southwood OI, McLaren DG, De Vries A, Van der Steen H, Eckardt GR, Tuggle CK, Helm J, Vaske DA, Mileham AJ, and Plastow GS (1997). Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. J Anim Sci 75:3138-3142.
25. Southwood OI, Van der Steen HAM, Mileham AJ, Plastow GS, and Cuthbert-Heavens D (1995). Evaluation of the oestrogen receptor (ESR) gene in Meishan synthetic pigs. 46th Ann Mtg Eur Assoc Anim Prod 1995;1:53 abstr.
26. Van Rens BTTM, Hazeleger W, and Van der Lende T (2000). Periovulatory hormone profiles and components of litter size in gilts with different estrogen receptor (ESR) genotypes. Theriogenology 53:1375-1387.
27. Van Rens BTTM, and Van der Lende T (2000). Fetal and placental traits at Day 35 of pregnancy and their relationship with the estrogen receptor genotype in pigs. Theriogenology 54:843-858 (Erratum in Theriogenology 2001;55:1029-1032).

28. Wilson ME, Biensen NJ, Youngs CR, and Ford SP (1998). Development of Meishan and Yorkshire littermate conceptuses in either a Meishan or Yorkshire uterine environment to day 90 of gestation and to term. Biol Reprod 58:905-910.
29. Wu MC, Hentzel MD, and Dziuk PJ (1987). Relationships between uterine length and number of fetuses and prenatal mortality in pigs. J Anim Sci 65:762-770.

Chapter 5

Piglet and placental traits at term in relation to the estrogen receptor genotype in gilts

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Submitted

ABSTRACT

Liveborn piglets from gilts with estrogen receptor (ESR) genotype AA (95 AA-AA and 91 AA-AB piglets), AB (88 AB-AA, 118 AB-AB, and 37 AB-BB piglets), and BB (97 BB-AB and 89 BB-BB piglets) were compared after farrowing, to examine whether piglet ESR genotype (ESRp) nested within maternal ESR genotype (ESRm) affected placental traits at term, piglet birthweight and growth until weaning. Furthermore the relation of birthweight to various placental traits and the relations between placental traits were evaluated relative to ESR genotype. For this study, 62 Large White x Meishan F2 crossbred gilts (18 AA, 24 AB, and 20 BB) were used. The gilts belonged to a population in which the A allele is favorable for litter size. ESRp nested within ESRm did not affect placental length, weight, surface area and number of areolae. ESRp nested within ESRm affected amnion weight (AA-AA amnions were heavier than AA-AB, AB-AA and BB-AB amnions), placental weight after including placental surface area in the model (AA-AB placentae were lighter than AA-AA, AB-BB and BB-AB placentae), placental efficiency calculated as birthweight divided by placental weight (AB-AA placentae were less efficient than AA-AB placentae), and the relations of birthweight to placental weight and birthweight to number of areolae. The found differences imply an interaction of maternal and fetal ESR genotype on placental traits (especially weight and number of areolae) during fetal development. Furthermore, the found effects on placental and amnion weight might be the result of a difference in thickness and/or vascularisation. The favorable ESR allele for litter size, i.e. the A allele, appears to be the unfavorable allele for pre-weaning piglet growth. Therefore, further research on ESR in relation to vascularisation, weight and thickness of placentae, uterine size, endometrial gland development, and piglet growth is recommended.

Key words: pigs, estrogen receptor gene, placenta, growth, areolae

Acknowledgments

The authors thank Bart Spee, Piet de Groot, Jascha Leenhouwers, and Henk Vos for their assistance and Alan Mileham and Kerry Harvey (PIC International Group) for genotyping the piglets.

INTRODUCTION

Using the candidate gene approach, Rothschild et al. (11) suggested that the estrogen receptor (ESR) gene in pigs is a major gene for litter size. Two ESR alleles (A and B) were described, of which the B allele, discovered initially in Chinese pigs (12), is significantly associated with a higher first litter size for several lines studied (reviewed by Van Rens et al. 16). Litter sizes of Large White x Meishan F2 crossbred gilts, however, were affected by ESR genotype in a different way, i.e. AB gilts had largest and BB gilts had smallest litters (16). Thus, Van Rens et al. (16) suggested that the ESR polymorphism is a marker rather than a major gene for litter size. The actual major gene can be another mutation in the ESR gene or a mutation in a closely linked gene.

A physiological study of gilts belonging to a genetic line in which the B allele is favorable for litter size, showed that the litter size difference associated with ESR genotype is probably due to a difference in fetal survival, since there were no differences in number of ovulations and number of fetuses at Day 35 of pregnancy (17). Furthermore, placentae of fetuses from BB gilts were significantly longer than those of fetuses from AA gilts (17). The difference found appeared to be entirely due to the maternal ESR genotype, since fetal ESR genotype nested within maternal ESR genotype did not affect fetal and placental weight and length and implantation surface area at Day 35 of pregnancy (18).

The effect of maternal ESR genotype on placental traits at term has only been studied in Large White x Meishan F2 crossbred gilts belonging to a population in which the A allele is favorable for litter size. This study showed complete dominance for total number born and number born alive (16). In these gilts, average placental length, surface area, and weight were not affected by maternal ESR genotype. The number of areolae per cm^2 placenta, however, was significantly lower for AB gilts compared to AA and BB gilts, despite the fact that litter sizes of AA and AB gilts were comparable. It is not known whether the observed difference is only due to maternal, or also to the piglet ESR genotype. Therefore, in the present study, the effect of piglet ESR genotype on piglet and placental traits at term was compared within the different maternal ESR genotypes.

The dataset used for this study is the same as used by Van Rens et al. (16). It contains information about individual piglet birthweight and growth until weaning,

as well as placental weight, length and surface area and number of areolae per placenta at term.

Although the morphology of porcine placental areolae has been examined thoroughly by several authors (e.g. 1-7), information about development and/or distribution of the areolae throughout gestation is hardly available (4,8). Furthermore, information of the number of areolae at term and its relation to other placental characteristics at term has not been reported before. Therefore, in the present study individual placental and piglet traits were studied in relation to the number of areolae. Furthermore, the effect of piglet ESR genotype nested within maternal ESR genotype on the relations was studied.

The objectives of the present study were 1) to examine whether piglet ESR genotype nested within maternal ESR genotype affects piglet or placental traits at term or piglet growth before weaning and 2) to examine how birthweight is related to placental traits at term, how placental traits at term are related to each other, and whether found relations are affected by piglet ESR genotype nested within maternal ESR genotype.

MATERIALS AND METHODS

The data used came from an experiment that had been designed to compare placental traits at term for gilts with different ESR genotypes. The design of the experiment was described extensively by Van Rens et al. (16). Briefly, the placentae of 62 pregnant Large White x Meishan F2 crossbred gilts (18 AA, 24 AB, and 20 BB) were labeled and piglets were earnotched during farrowing according to Wilson et al. (20) with slight modifications (16), in order to match individual piglets with their placentae. Immediately after expulsion, the placentae were identified (the unlabeled ones were provided with a code) and stored at 4 °C. Within 24 hours after expulsion several placental traits were measured, and a part of the umbilical cord of each placenta was stored at –80 °C.

Piglets were weighed individually immediately after all piglets were born and the majority of the placentae were expelled, i.e. 1 to 12 h after birth. Furthermore, the piglets were weighed at days 7, 14 and 21 after birth.

Pregnant gilts were fed 1.25 running up to 1.50 kg commercial sow ration (12.56 MJ metabolizable energy/kg, 144 g/kg crude protein, 7.5 g/kg ileal digestible lysine) twice daily at 0830 and 1630. On the day of farrowing they were not fed, and on days 1 and 2 and during the remaining lactation period they were fed 0.5 kg, 1.0 kg and 1.5 kg, respectively, twice daily at 0830 and 1630. Water was always available at libitum. For the piglets, water and creep feed was available ad libitum, from birth until the end of the experimental period, i.e. three weeks after birth.

From each piglet (at a minimum age of 2 weeks) a 10 ml blood sample was taken and stored at –80 °C for DNA isolation. For the same purpose, a piece of liver of all piglets that had died before blood sampling was stored at –80 °C.

DNA Isolation and Genotyping

To determine the ESR genotype of the piglets, DNA was isolated either from the blood or liver sample. To determine the ESR genotype of the placentae which had not been labeled successfully, DNA was isolated from the inside of the umbilical cord.

DNA was isolated using a PurGene Genomic DNA isolation kit for human and mammalian whole blood and bone marrow cells (D-5000, Gentra Systems Inc., Minneapolis, USA). DNA was then analysed for the ESR marker at PIC's Research Center (Cambridge, UK) according to Short et al. (15).

Piglets or placentae from AA mothers were genotyped as ESR AA or AB, and are referred to as AA-AA and AA-AB, respectively. Piglets or placentae from AB mothers were genotyped as ESR AA, AB or BB, and are referred to as AB-AA, AB-AB and AB-BB, respectively. Piglets or placentae from BB mothers were genotyped as ESR AB or BB, and are referred to as BB-AB and BB-BB, respectively.

Statistics

All data were analyzed with SAS (13), using the procedure GLM. When piglet ESR genotype nested within maternal ESR genotype had a significant effect, multiple comparisons were performed with adjustments according to Tukey-Kramer (14).

Effects of piglet ESR genotype nested within maternal ESR genotype on piglet and placental traits. Analyses of placenta and piglet data involved measurements obtained on individual placentae or piglets, i.e. placental weight, length, surface area, number of areolae, number of areolae per cm^2 placenta, amnion weight and piglet birthweight, weight at Days 7, 14 and 21, and growth rate. Only data from live born piglets were analysed. Data of 615 piglets and 603 placentae (12 were not found or incomplete) were analyzed for the effect of placental or piglet ESR genotype nested within maternal ESR genotype according to following model:

$$y_{ijkl}=\mu+ESRm_i + sow_j(ESRm_i) + ESRp_k(ESRm_i) + e_{ijkl}$$

[Model 1]

in which 'ESRm' is the maternal ESR genotype and 'ESRp' is the ESR genotype of the piglet or placenta.

Relations among traits. The relations of birthweight to placental traits and the relations between placental traits were assumed to be polynomial. To determine the order of the polynomial model, a stepwise forward procedure was used. In every step a subsequent order and its interaction with ESRp nested within ESRm was included in the model, but only if the last included order or its interaction was significant. The forward procedure was continued until the highest included order and its interaction was not significant, or until the fourth order was reached. At each next step all lower orders and their interactions were included in the model. Once the highest order was reached, interactions which were not significant were eliminated stepwise, removing in each step the non-significant interaction of the highest order.

The most complex model that could be reached was:

$$y_{ijkl} = \mu + ESRm_i + sow_j(ESRm_i) + ESRp_k(ESRm_i) + b1*X^1_{ijkl} + b1_{ik}*X^1_{ijkl}*[ESRp_k(ESRm_i)] + \ldots\ldots + b4*X^4_{ijkl} + b4_{ik}*X^4_{ijkl}*[ESRp_k(ESRm_i)] + e_{ijkl}$$

[Model 2]

in which 'ESRm' is the maternal ESR genotype, 'ESRp' is the ESR genotype of the piglet or placenta, 'X' the variable to be tested for its relation to y, and 'b1,....,b4' and '$b1_{ik}$,....,$b4_{ik}$' are the regression coefficients

For drawing the figures, the intercepts of the equations of the relations were calculated by combined use of the least squares means calculated with Model 1 and the slopes calculated with Model 2.

If a relation differed significantly between piglet or placenta ESR genotypes nested within maternal ESR genotype, the options 'solutions' and 'contrast' of SAS were used to compare genotype combinations.

RESULTS

Effects of Piglet ESR Genotype nested within Maternal ESR Genotype on Piglet and Placental Traits

Results are presented in Table 1. Piglet ESR genotype nested within maternal ESR genotype did not affect birthweight or weight of the piglets at Days 7 and 14, but tended to affect their weight at Day 21, i.e. BB-AB piglets were heavier than piglets from AA and AB mothers ($P<0.05$, except for the AB-BB piglets, $P<0.1$), while BB-BB piglets were heavier than AB piglets from AA and AB mothers ($P<0.05$). Piglet ESR genotype nested within maternal ESR genotype did not affect growth during the first week, but tended to affect piglet growth during the first three weeks, i.e. BB-AB piglets had grown faster compared to piglets from AA and AB mothers ($P<0.05$), while BB-BB piglets had grown faster compared to AB piglets from AA and AB mothers ($P<0.05$).

Piglet ESR genotype nested within maternal ESR genotype did not affect number of areolae, placental weight, length, or surface area (Table 1). However, it tended to affect number of areolae per cm^2 placenta, i.e. both AB-AA and AB-AB placentae had less areolae per cm^2 compared to placentae from AA and BB mothers, while AB-BB placentae had only less areolae per cm^2 compared to placentae from BB mothers. Furthermore, amnion weight was affected significantly by piglet ESR genotype nested within maternal ESR genotype. AA-AA amnions were heavier than AA-AB, AB-AA and BB-AB amnions (Table 1) and tended to be heavier than AB-AB amnions ($P=0.06$).

Placental efficiencies, defined as birthweight divided by placental length, placental surface area, or number of areolae were not affected by piglet ESR genotype nested within maternal ESR genotype. Placental efficiency, defined as birthweight

Table 1. Effect of piglet ESR genotype nested within maternal ESR genotype on piglet and traits (least squares means ± SEM)[1]

y		AA-AA	AA-AB	AB-AA	AB-AB	AB-BB	BB-AB	BB-BB	P	n
Piglet	n_{max}	95	91	88	118	37	97	89		615
Birthweight (g)		1294±26	1274±25	1267±25	1255±20	1241±40	1224±26	1256±28	0.884	615
Weight day 7 (g)		2393±51	2328±47	2383±49	2268±39	2291±79	2425±53	2401±57	0.390	559
Weight day 14 (g)		3938±90	3754±85	3836±86	3618±69	3903±141	4169±92	4125±100	0.113	539
Weight day 21 (g)		5057±119^{ac}	4827±112^{a}	5051±113^{ac}	4734±91^{a}	5028±186^{ab}	5631±121^{b}	5454±132^{bc}	0.098	536
Growth day 0-7 (g/d)		155±5	150±5	157±5	144±4	151±8	167±5	161±6	0.232	559
Growth day 0-21 (g/d)		178±5^{ac}	169±5^{a}	179±5^{ac}	165±4^{a}	179±8^{ac}	209±5^{b}	198±6^{bc}	0.071	536
Placenta										
Placental length (cm)		75.8±1.5	73.9±1.4	74.5±1.4	72.6±1.1	74.5±2.2	73.2±1.5	75.7±1.6	0.514	603
Placental weight (g)		194±5	177±5	191±5	184±4	190±8	183±5	181±6	0.215	599
Weight amnion (g)		32.8±1.6^{a}	23.6±1.5^{b}	26.2±1.4^{b}	27.2±1.2^{ab}	25.9±2.3^{ab}	25.9±1.6^{b}	27.2±1.7^{ab}	0.004	599
Placental surface area (cm^2)		1692±36	1665±35	1690±33	1644±27	1613±54	1564±36	1632±39	0.496	603
Number of areolae		9564±232	9101±223	8299±254	7828±208	8279±361	9168±226	9366±245	0.319	567
Number of areolae/cm^2		5.603±0.089^{ac}	5.426±0.086^{ac}	4.957±0.097^{b}	4.785±0.079^{b}	5.159±0.138^{bc}	5.677±0.088^{a}	5.659±0.095^{a}	0.099	563
BW/length		17.6±0.3	17.7±0.3	17.1±0.3	17.2±0.2	17.2±0.5	16.8±0.3	17.1±0.4	0.989	528
BW/weight		6.96±0.15^{ab}	7.44±0.13^{a}	6.80±0.13^{b}	7.10±0.11^{ab}	6.77±0.22^{ab}	6.99±0.15^{ab}	7.32±0.16^{ab}	0.034	524
BW/surface area		0.791±0.014	0.779±0.012	0.759±0.012	0.765±0.010	0.800±0.020	0.786±0.013	0.790±0.014	0.472	528
BW/number of areolae		0.148±0.004	0.148±0.003	0.158±0.004	0.168±0.003	0.155±0.006	0.147±0.004	0.150±0.004	0.171	495

^{a,b,c} different superscripts in the same row differ, $P<0.05$

[1] according to Model 1

divided by placental weight, however, was affected by piglet ESR genotype nested within maternal ESR genotype, i.e. AB-AA piglets had a less efficient placenta than AA-AB piglets (Table 1).

Relations among Piglet and Placental Traits

Birthweight. The relations of birthweight to placental length, surface area and number of areolae per cm^2 did not differ between piglet ESR genotypes nested within maternal ESR genotypes (Table 2). Birthweight was significantly related to all three covariables ($P<0.05$, Table 2). This did not change the effect of piglet ESR genotype nested within maternal ESR genotype on birthweight. The three covariables explained 44, 48, and 9%, of total R^2, respectively (Table 2).

The relation of birthweight to placental length was best described by a fourth order equation (Figure 1a, Table 2). For placentae shorter than 75 cm, birthweight increased with placental length (Figure 1a). For longer placentae (i.e. 50% of all observations), birthweight remained on average constant, except for the longest placenta (i.e. 0.2% of all observations). The relation of birthweight to placental surface area was best described by a second order equation (Figure 1b, Table 2), and the relation of birthweight to number of areolae per cm^2 was best described by a linear equation (Figure 1c, Table 2)

The relation of birthweight to placental weight, which was significantly affected by piglet ESR genotype nested within maternal ESR genotype ($P<0.05$, Table 2), was best described by a second order polynomial (Table 2, Figure 2). On average, birthweight increased with placental weight. The slope of increase was highest for placentae with lowest weights and became less as placental weight increased (Figure 2). BB-AB piglets differed from AB-AB, AB-BB and BB-BB piglets for their relation of birthweight to placental weight (Table 2, Figure 2). The covariable placental weight explained 48% of total R^2.

The relation of birthweight to number of areolae, which was significantly affected by piglet ESR genotype nested within maternal ESR genotype ($P<0.05$, Table 2), was best described by a third order polynomial (Table 2, Figure 3). On average, birthweight increased with number of areolae until 7500 areolae (i.e. 50% of all observations). For higher numbers of areolae, the slope of increase decreased until zero. BB-AB piglets differed from AB-AA and AB-AB piglets for their relation of birthweight to number of areolae (Table 2, Figure 3a). Furthermore AB-AB piglets

Table 2. Relations of birthweight (=Y) to placental traits (=X) at term

Y	X	equation	interaction X^n* ESRp(ESRm)		ESRp(ESRm)		X^n	R^2	differences between regression coefficients
			n	P	P	n	P		
birthweight (g) (R^2=0.409[1])	length	poly 4	1	ns	ns (p=0.95)	1	0.5312	0.735	
			2	ns		2	0.0699		
			3	ns		3	0.0176		-----
			4	ns		4	0.0088		
	weight	poly 2	1	0.0313	---	1	---	0.789	BB-AB & BB-BB (p=0.0014)
			2	ns		2	---		AB-AB (p=0.0082)
									AB-BB (p=0.0399)
									BB-AB & AA-AA (p=0.0644)
									BB-BB & AA-AB (p=0.0887)
	surface area	poly 2	1	ns	ns (p=0.57)	1	0.0001	0.781	-----
			2	ns		2	0.0001		
	number of areolae	poly 3	1	0.0395	---	1	---	0.756	BB-AB & AB-AA (p=0.0485)
			2	ns		2	---		AB-AB (p=0.0015)
			3	ns		3	---		AB-BB (p=0.0580)
									BB-BB (p=0.0553)
									AB-AB & AA-AA (p=0.0858)
									AA-AB (p=0.0103)
	number of areolae per cm^2	linear	1	ns	ns (p=0.86)	1	0.0041	0.447	-----

[1] R^2 of the model without covariable (X), i.e. R^2 of Model 1.

ns effect was not significant (P>0.05)

differed from AA-AB and BB-AB piglets in their relation of birthweight to number of areolae (Table 2, Figure 3b).

Placental weight. The relations of placental weight to placental length, surface area and number of areolae did not differ between piglet ESR genotypes nested within maternal ESR genotypes (Table 3). Placental weight was significantly related to all three covariables ($P<0.05$, Table 3). The covariables placental length, surface area and number of areolae explained 35, 39, and 39% of total R^2, respectively.

Although the relations of placental weight to placental length and number of areolae both were best described by a second order equation (Table 3), over a large range of placental length as well as number of areolae, the placental weight increased essentially linear with both traits (Figure 4a, Figure 4c).

The relation of placental weight to placental surface area was best described by a linear equation, resulting in a significant increase of placental weight with an increase of placental surface area (Table 3, Figure 4b). After correction for surface area, placentae of AA-AB piglets were lighter ($P<0.05$) than placentae of AA-AA, AB-BB and BB-AB piglets. Placental weights after correction for surface area were 187±4, 170±4, 183±4, 179±3, 194±6, 186±4, and 178±4 for AA-AA, AA-AB, AB-AA, AB-AB, AB-BB, BB-AB and BB-BB piglets, respectively.

Number of areolae. The relations of number of areolae to placental length, weight and surface area did not differ between piglet ESR genotypes nested within maternal ESR genotypes (Table 3). Number of areolae was significantly related to all three covariables ($P<0.05$, Table 3). The covariables, which explained 27, 23, and 25% of total R^2, respectively, did not change the effect of piglet ESR genotype nested within maternal ESR genotype on the number of areolae.

The relations of number of areolae to placental length and placental surface area were best described by a linear equation (Table 3). Number of areolae increased with an increase of placental length (Figure 5a) and placental surface area (Figure 5c). The relation of number of areolae to placental weight was best described by a second order equation (Table 3, Figure 5b).

Table 3. Relations between placental traits at term

Y	X	equation	interaction X^n* ESRp(ESRm)		ESRp(ESRm)	X^n		R^2
			n	P	P	n	P	
placental weight (R^2=0.467[1])	placental length	poly 2	1	ns	ns (p=0.17)	1	0.0001	0.719
			2	ns		2	0.0001	
	surface area	linear	1	ns	0.0030	1	0.0001	0.761
	number of areolae	poly 2	1	ns	ns (p=0.13)	1	0.0001	0.762
			2	ns		2	0.0001	
number of areolae (R^2=0.628[1])	placental length	linear	1	ns	ns (p=0.67)	1	0.0001	0.862
	placental weight	poly 2	1	ns	ns (p=0.77)	1	0.0001	0.819
			2	ns		2	0.0001	
	placental surface area	linear	1	ns	ns (p=0.29)	1	0.0001	0.841

[1] R^2 of the model without covariable (X), i.e. R^2 of Model 1.

ns effect was not significant (P>0.05)

DISCUSSION

In the present study, placental length, weight and surface area at term were not affected by piglet ESR genotype nested within maternal ESR genotype. Neither was number of areolae. This implies that the difference in average number of areolae between AB and BB gilts found by Van Rens et al. (16) in the same experiment is entirely due to the ESR genotype of the gilt. The weight of the amnion however, was affected by piglet ESR genotype nested within maternal ESR genotype. AA piglets from AA gilts had heavier amions than their AB littermates, and than AB-AA and BB-AB piglets. This implies that the amnion of AA-AA piglets probably was larger or thicker than amnions of the other mentioned genotype combinations. A larger amnion might be associated with a larger amniotic cavity and thus a better protection to mechanical injury, while a thicker amnion might be associated with a better materno-fetal barrier.

It seems surprising that amniotic weight is affected by piglet ESR genotype nested within maternal ESR genotype, while the number of areolae is only affected by maternal ESR genotype, since both amnion and chorioallantois are tissues of fetal origin (e.g. 9). The amnion, however, is fully surrounded by fetal tissue, while the chorioallantois is in close contact with maternal tissue. Furthermore, development of the areolae is entirely dependent on presence of endometrial glands, which is a maternal trait only.

Although most placental traits were not affected signficantly by piglet ESR genotype nested within maternal ESR genotype, the lsmean estimates showed some remarkable similarities in ranking. AA-AA piglets had the largest values for all placental traits, i.e. length, weight, surface area, number of areolae and amnion weight. Furthermore, the lowest values always belonged to AB piglets. Within maternal genotypes, the lsmean estimates also showed a special ranking. In both homozygous mothers, the homozygous piglets had the highest values. (Except for placental weight in BB mothers, which was highest for the AB piglets). In the heterozygous mothers, the values for placental traits were highest for the AA piglets (except for amnion weight, which was highest for the AB piglets). At least a part of the mentioned similarities in ranking might be due to the fact that the placental traits are correlated. Correlations between placental weight, length, surface area and number of areolae in the current study are between 0.6 and 0.8. Amnion weight was less correlated with placental surface area, length and number

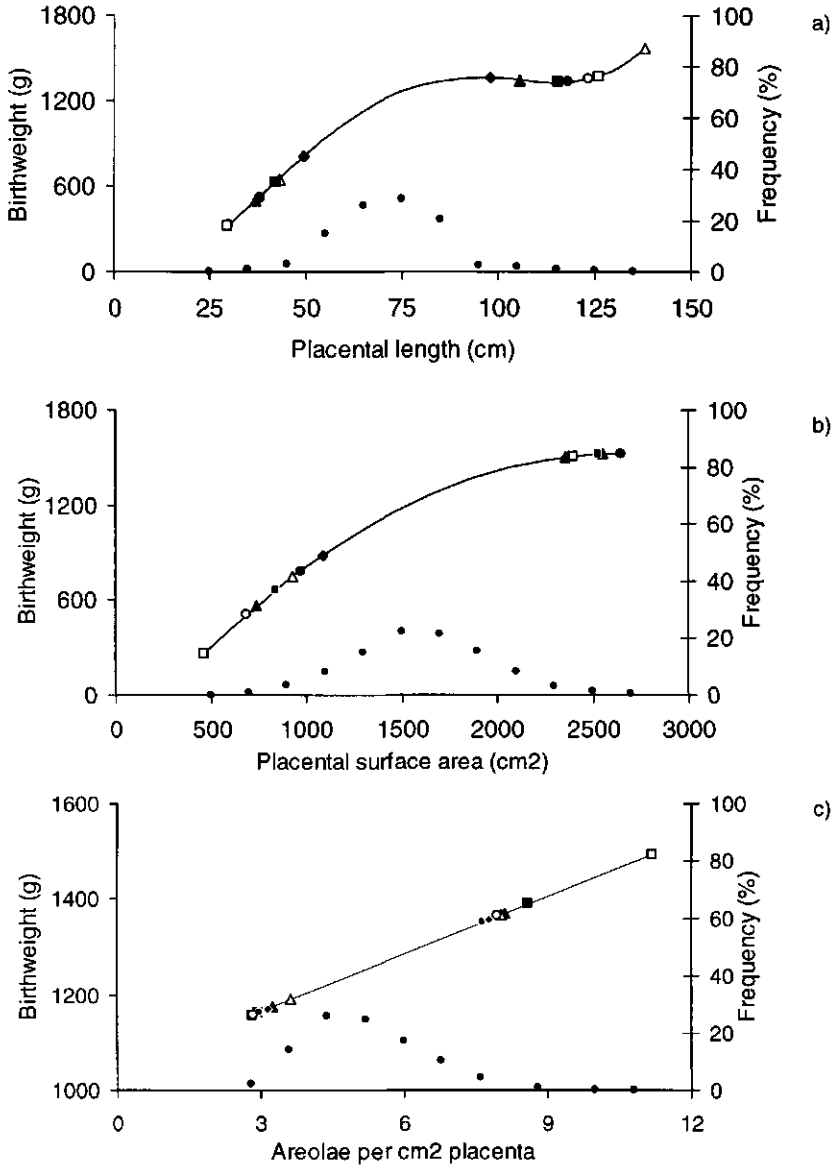


Figure 1. *Relations of birthweight to placental traits at term, including frequency distributions (●) for placental length (a), surface area (b) and number of areolae per cm² (c). Markers represent min. and max. value for each group:*

▲ = *AA-AA* △ = *AA-AB* ■ = *BB-BB* □ = *BB-AB*

● = *AB-AA* ○ = *AB-AB* ◆ = *AB-BB*

of areolae (R = 0.3), while the correlation between amnion weight and placental weight was 0.5.

Number of areolae per cm^2, which was affected by maternal ESR genotype in this experiment (16), tended to be affected by piglet ESR genotype nested within maternal ESR genotype as well. Within each maternal ESR genotype, piglet ESR genotypes did not differ from each other. However, it seems that the lower average numbers of areolae per cm^2 in AB mothers compared to the AA and BB mothers (16) is a consequence of the lower numbers of areolae per cm^2 of the AA and AB piglets rather than of the BB piglets of AB mothers.

At maternal level, placental efficiencies calculated as birthweight divided by placental length, surface area or weight were not affected by ESR genotype (16). At a fetal level however, placental efficiency calculated as birthweight divided by placental weight was affected by piglet ESR genotype nested within maternal ESR genotype, i.e. AA piglets from AB mothers had less efficient placentae than AB piglets from AA mothers. The difference in placental efficiency, calculated as birthweight divided by number of areolae, however, appears to be entirely due to maternal ESR genotype (16), since ESR genotype of the piglet nested within ESR genotype of the mother did not affect this trait.

After including surface area in the model, placental weight was affected by ESR genotype of the piglet nested within ESR genotype of the mother. This implies that differences in weight existed between placentae with a similar surface area. These differences must be due to a difference in thickness of the placenta, which might e.g. be the result of a difference in vascularisation. Placentae of AA-AB piglets thus were thinner and perhaps less vascularized than placentae of AA-AA, AB-BB and BB-AB piglets. The A allele was the favorable allele for litter size in the present study (16). A study of Day 35 fetuses of AA and BB gilts of a line in which the B allele was the favorable allele for litter size, showed that AA-AB fetuses had the heaviest and BB-AB fetuses had the lightest hearts (18). In that study, placental vascularity relative to ESR genotype was recommended for further research. Results of present study confirm this recommendation.

All relations of birthweight to the placental traits at term, except the linear relation to areolar density, had a more or less similar course, which started with an increase of birthweight with an increase of the placental trait, until a certain threshold value of the placental trait, whereafter birthweight did not change anymore. Dependent

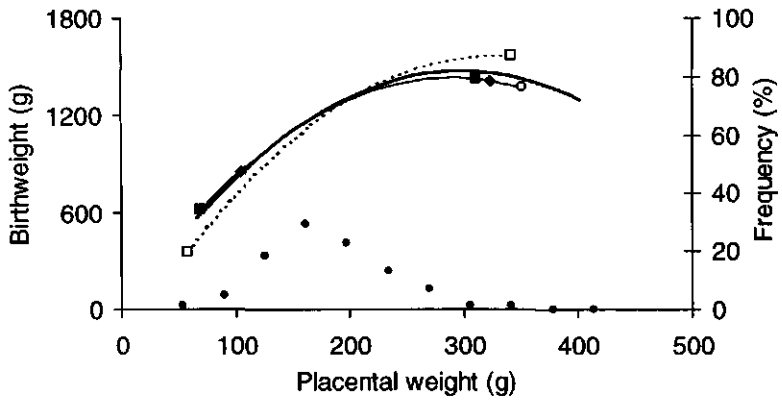


Figure 2. *Relation birthweight to placental weight at term, including frequency distribution (●) for placental weight.*
Markers represent min. and max. value for each group:
□····□ *= BB-AB* ○◆■—■◆○ *= average of AB-AB, AB-BB and BB-BB*
= average of all genotype combinations except for BB-AB

on the placental trait, this threshold value could not always be distinguished accurately. Warwick (19), Pomeroy (10) and Knight et al. (8) already reported a direct relationship of fetal size to size of the fetal membranes at different stages of pregnancy, but assumed that the relationship was linear. Furthermore they did not present equations of the relationship of birthweight to placental size at term. Van Rens and Van der Lende (18) did report equations of the relation of fetal weight to placental length and weight, but only for the relation at Day 35 of gestation. Placental length and weight both explained 14% of R^2 then (18, results not shown). At term, however, placental length and weight explained 44 and 48%, of R^2, respectively. This is in agreement with results of Warwick (19), Pomeroy (10) and Knight et al. (8), which all imply a stronger relation between placental weight and fetal weight at the later stages of pregnancy compared to earlier stages. A larger, heavier placenta implies a larger potential surface area of contact with the endometrium, and thus a better transport of nutrients from uterus to fetus. Therefore, piglets with a larger placenta were expected to be heavier. Apparently, the piglets with placentae larger than a certain threshold value have reached their maximum growth potential and do not benefit from the extra placental capacity anymore.

Information about development and/or distribution of the areolae throughout gestation is hardly available (4, 8). Furthermore, information on the number of areolae at term and its relation to other placental characteristics at term has not been reported before. In the present study, birthweight was related to number of areolae in a similar way as birthweight was related to placental size, i.e. piglets with placentae with more areolae were heavier, until a certain threshold value, whereafter birthweight did not increase anymore with an increase in number of areolae. Number of areolae explained a similar percentage of R^2 in its relation to birthweight as placental length, weight or surface area did, i.e. 46% of R^2. Areolar density, however, explained only 9% of R^2. Still birthweight had a significant linear relation to areolar density, i.e. increased with an increase of areolar density.

Number of areolae was related to placental length and placental surface area in a similar way, i.e. larger placentae had more areolae. Larger placentae have more contact with uterine epithelium and thus have contact with more uterine glands, resulting in a higher number of areolae on the placenta. The relation of areola to placental weight is comparable to the other two relations, i.e. the number of areolae increases with an increase in placental weight. The degree of increase of the number of areolae, however, decreases as placental weight increases.

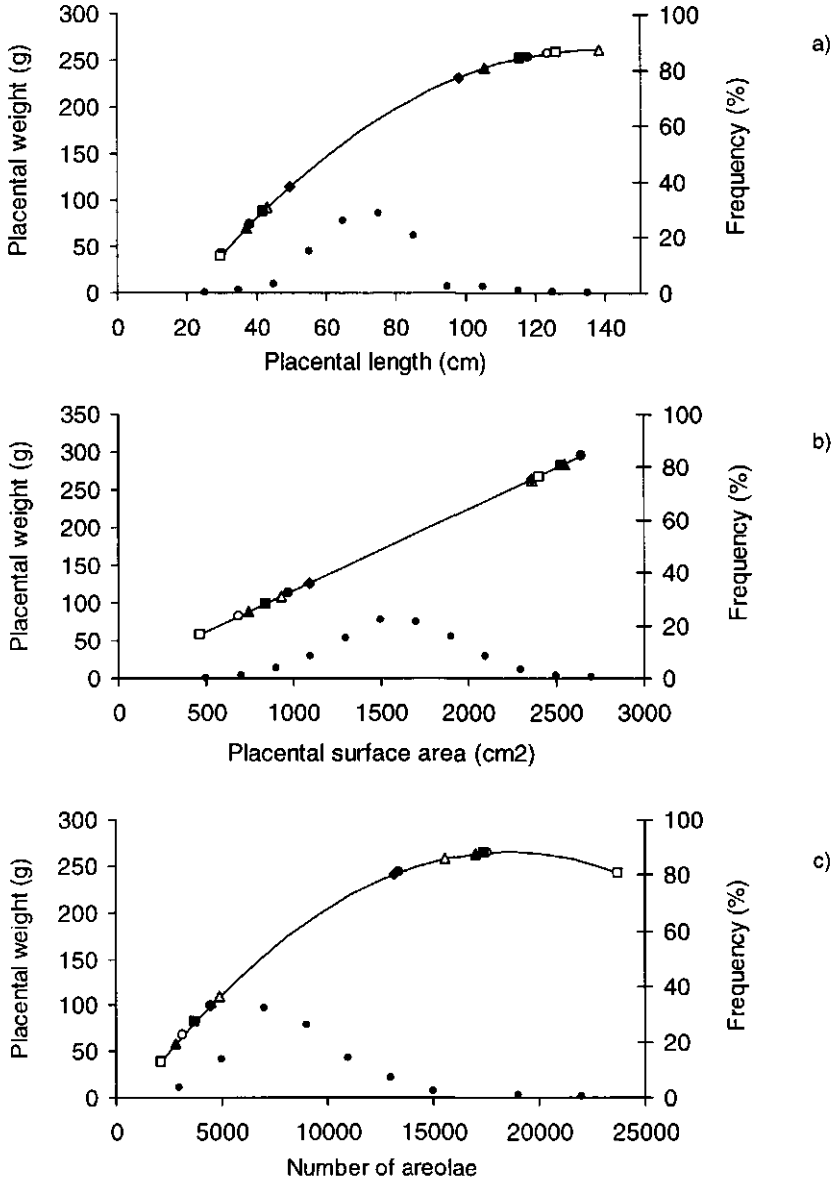


Figure 4. *Relations of placental weight to other placental traits at term, including frequency distributions (●) for placental length (a), surface area (b) and number of areolae (c). Markers represent min. and max. value for each group:*

▲ = *AA-AA* △ = *AA-AB* ■ = *BB-BB* □ = *BB-AB*

● = *AB-AA* ○ = *AB-AB* ◆ = *AB-BB*

difference in competition for milk, as a result of a difference in litter size, since no cross fostering has been applied. Growth of the piglets in relation to the ESR genotype (of mother or piglet) has not been examined before. Results of the present study, however, point to the importance of such research, since the favorable allele for litter size might be the unfavorable allele for piglet growth.

Summarized, piglet ESR genotype nested within maternal ESR genotype did not affect length, weight, surface area and number of areolae of placentae of piglets from the Large White x Meishan F2 crossbred gilts examined in the present study. Piglet ESR genotype nested within maternal ESR genotype, however, did affect amnion weight, placental weight after including surface area in the model, placental efficiency calculated as birthweight divided by placental weight, and the relations of birthweight to placental weight and birthweight to number of areolae. The found differences and tendencies imply an interaction of maternal and fetal ESR genotype on placental traits (especially weight and number of areolae) during fetal development. The observed effects on placental and amnion weight might be the result of a difference in thickness (amnion and placenta), which might be the result of a difference in vascularisation (placenta only). The favorable ESR allele for litter size, i.e. the A allele, appears to be the unfavorable allele for piglet growth in the Large White x Meishan F2 crossbred gilts studied. Therefore, further research on ESR in relation to vascularisation and thickness of placentae, uterine size, endometrial gland development, and piglet growth is recommended.

REFERENCES

1. Abromavich CE (1926). The morphology and distribution of the rosettes on the foetal placenta of the pig. Anat Rec 39:69-72.
2. Bielańska-Osuchowska Z (1995). Ultrastructure of mucous areolae in the pig placenta. Folia Histochemica et Cytobiologica 33:103-110.
3. Bielańska-Osuchowska Z and Kuńska A (1995). A new appproach to the areolar structures in the pig placenta: Histochemistry and development of mucous areolae. Folia Histochemica et Cytobiologica 33:95-101.
4. Brambel CE (1933). Allantochorionic differentiations of the pig studied morphologically and histochemically. Am J Anat 52: 397-459.
5. Dantzer V (1984). Scanning electron microscopy of exposed surfaces of the porcine placenta. Acta Anat 118:96-106.
6. Dantzer V and Leiser R (1993). Microvasculature of regular and irregular areolae of the areola-gland subunit of the porcine placenta: structural and functional aspects. Anat Embryol 188: 257-267.

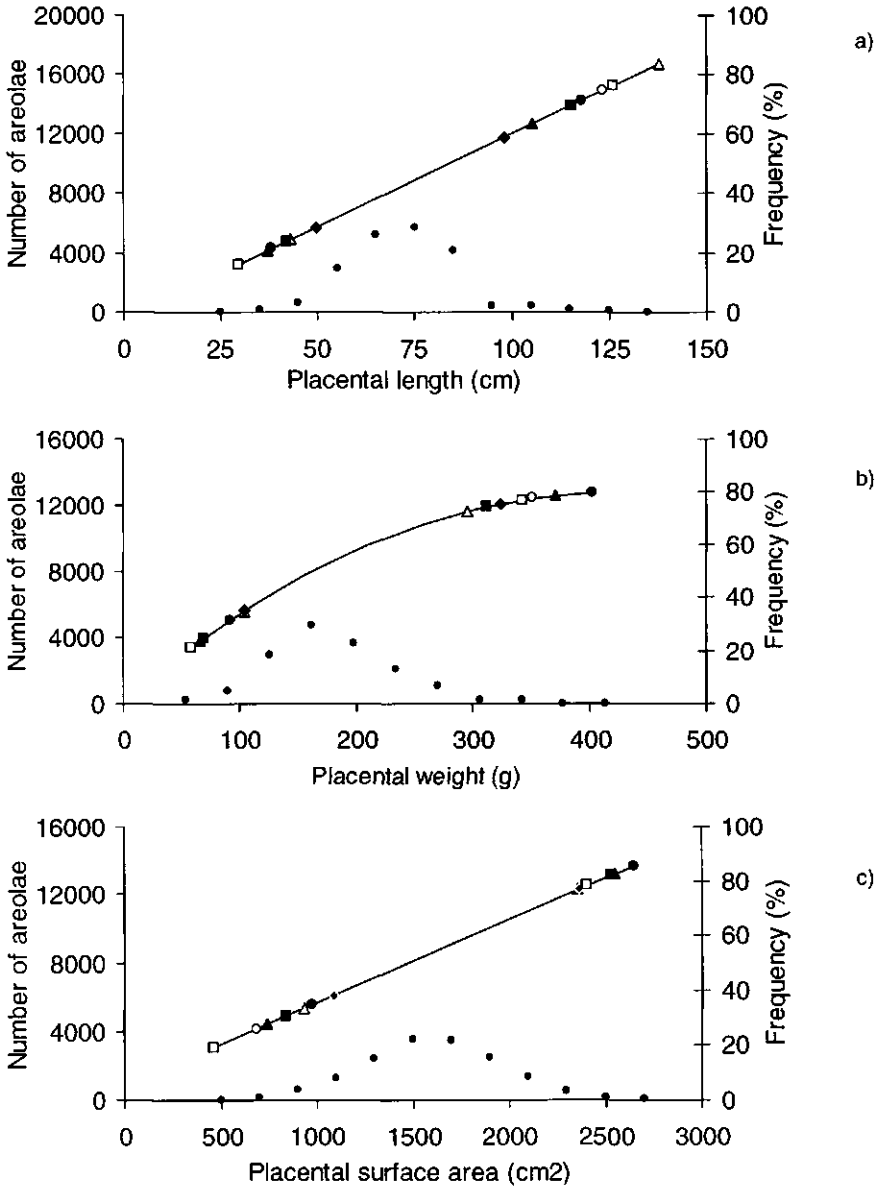


Figure 5. *Relations of number of areolae to placental traits at term, including frequency distributions (●) for placental length (a), weight (b) and surface area (c). Markers represent min. and max. value for each group:*

▲ = *AA-AA* △ = *AA-AB* ■ = *BB-BB* □ = *BB-AB*
● = *AB-AA* ○ = *AB-AB* ◆ = *AB-BB*

7. Friess AE, Sinowatz F, Skolek-Winnisch R, and Träutner W (1981). The placenta of the pig. II The ultrastructure of the areolae. Anat Embryol 163:43-53.
8. Knight JW, Bazer FW, Thatcher WW, Franke DE and Wallace HD (1977). Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 44:620-637.
9. Patten BM (1948). Embryology of the pig. 3rd edition. McGraw-Hill book company inc. New York, Toronto, London.
10. Pomeroy RW (1960). Infertility and neonatal mortality in the sow. III Neonatal mortality and foetal development. J Agric Sci 54:31-56.
11. Rothschild MF, Jacobson C, Vaske DA, Tuggle CK, Short TH, Sasaki S, Eckardt GR and McLaren DG (1994). A major gene for litter size in pigs. Proc World Congr Genet Appl Livest Prod 21:225-228.
12. Rothschild MF, Larson RG, Jacobson C, and Pearson P (1991). Pvu II polymorphisms at the porcine oestrogen receptor locus (ESR). Anim Genet 22:448.
13. SAS/STAT (1990). User's Guide. Cary NC, SAS Institute.
14. SAS/STAT (1996). Changes and enhancements through release 6.11. Cary NC, SAS Institute.
15. Short TH, Rothschild MF, Southwood OI, McLaren DG, De Vries A, Van der Steen H, Eckardt GR, Tuggle CK, Helm J, Vaske DA, Mileham AJ and Plastow GS (1997). Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. J Anim Sci 75:3138-3142.
16. Van Rens BTTM, De Groot, PN and Van der Lende T (2001). The effect of estrogen receptor genotype on litter size and placental traits at term in F2 crossbred gilts. Submitted.
17. Van Rens BTTM, Hazeleger W and Van der Lende T (2000). Periovulatory hormone profiles and components of litter size in gilts with different estrogen receptor (ESR) genotypes. Theriogenology 53:1375-1387.
18. Van Rens BTTM and Van der Lende T (2000). Fetal and placental traits at Day 35 of pregnancy in relation to the estrogen receptor genotype in pigs. Theriogenology 54:843-858. Erratum in Theriogenology 2001;55:1029-1032.
19. Warwick BL (1928). Prenatal growth of swine. J Morphol Physiol 46:59-84.
20. Wilson ME, Biensen NJ, Youngs CR, and Ford SP (1998). Development of Meishan and Yorkshire littermate conceptuses in either a Meishan or Yorkshire uterine environment to day 90 of gestation and to term. Biol Reprod 58:905-910.

Chapter 6

Components of litter size in gilts with different prolactin receptor genotypes

B.T.T.M. van Rens, G.J. Evans, and T. van der Lende

ABSTRACT

Behavioral estrus and components of litter size at Day 35/36 of pregnancy were studied in gilts with prolactin receptor (PRLR) genotype AA (n=9), AB (n=25) and BB (n=22). This polymorphism has been associated with litter size. Estrus length was not affected by genotype, but estrous cycle length tended to be longer for AA gilts compared to AB and BB gilts. AA gilts had a significantly higher ovulation rate (21.5±0.9) than BB gilts (18.7±0.6), resulting in a higher number of vital fetuses at Day 35/36 which may lead to a subsequent difference in litter size. Ovulation rate of AB gilts (20.0±0.5) was intermediate. Genotype affected the total weight of the ovaries. Even after subtraction of the total weight of corpora lutea, ovarian weight of AA gilts was highest (16.6±1.0 g), of BB lowest (13.4±0.6 g) and of AB gilts intermediate (15.0±0.6 g). Unlike AB gilts, AA and BB gilts were able to adapt uterine length to litter size, which led to longer uteri for AA gilts compared to BB. This was accompanied by heavier placentae and larger implantation surface areas for embryos of AA gilts compared to embryos of BB or AB gilts. Results of this experiment do not exclude the possibility that prolactin receptor gene itself is the major gene for litter size.

Keywords: pigs, prolactin receptor gene, uterus, placenta, ovaries

Acknowledgements

The authors like to acknowledge Piet de Groot, Kerry Harvey, Wouter Hazeleger, Frans Vermeulen and Henk Vos for their assistance. Furthermore they like to thank Graham Plastow and Hein van der Steen for reading this manuscript.

INTRODUCTION

In various vertebrates, more than 300 separate actions of prolactin (PRL) have been reported, including effects on water and salt balance, growth and development, endocrinology and metabolism, brain and behavior, and immune regulation and protection (reviewed by Bole-Feysot et al., 1998). A large proportion of these actions are directly or indirectly associated with the process of reproduction, e.g. actions related to nurturing of the young and ovarian and uterine actions. All these actions of PRL are mediated by its receptor, PRLR.

In the pig, the prolactin receptor gene has been recently mapped to chromosome 16 (Vincent et al., 1997). A polymorphism has been detected and two alleles, A and B, were described. This polymorphism was significantly associated with total number of piglets born and(or) number born alive in some genetic lines tested (Rothschild et al., 1998, Vincent et al., 1998, Van Rens and Van der Lende, 2000). The mechanism through which this gene affects litter size is not yet known. Furthermore, it is not known whether the PRLR polymorphism itself causes differences in litter size or whether this polymorphism provides a marker for a closely linked major gene for litter size.

In this study, ovulation rate, embryonic survival and embryonic, placental and uterine development in gilts with PRLR genotype AA, AB and BB were compared. The data used came from an experiment which had been designed to compare these traits for gilts with different estrogen receptor (ESR) genotypes (Van Rens et al., 2000).

MATERIALS AND METHODS

Animals

Seventy-nine gilts (50% Landrace/ 50% Meishan synthetic) were provided in four batches at 7 to 8 week intervals by Pig Improvement Company (PIC Europe, UK). Animals of each batch were treated equally, except for additional cannulation of animals in batches 2 and 3.

At the day of arrival, the gilts were randomly housed in groups of three. Each gilt was given 1.25 kg of a normal sow ration (metabolizable energy 12.5 MJ/kg, crude

protein 141 g/kg, ileal digestible lysine 5.8 g/kg) twice daily at 8:30 and 16:30 h. Water was available ad libitum.

Experimental design

The design of the experiment was described extensively by Van Rens et al. (2000). Starting at the day after arrival, the gilts were checked for estrus by the back pressure test in presence of a vasectomized adult boar twice daily (at 8:00 and 16:00 h). No information on estrus prior to arrival at the experimental farm was available. Animals that did not show estrus within the first three weeks after arrival were removed from the experiment. One day after the second estrus shown, the gilts were housed individually. The animals from batches 2 and 3 were cannulated then, for frequent collection of blood samples to determine periovulatory LH, estradiol and progesterone profiles as described by van Rens et al. (2000; Results not shown, see Discussion).

At third estrus the gilts were artificially inseminated twice with an interval of 24 hours with pooled semen of three Great Yorkshire sire line boars ($3*10^9$ sperm cells per dose). When the first signs of estrus were detected in the morning, first insemination took place on the same day (at 15:30 h); when estrus was detected in the afternoon, first insemination took place on the next day (at 15:30 h).

On Day 35 or 36 after the first insemination the animals that did not return to estrus were slaughtered to study reproductive parameters. Reproductive tracts and blood samples were collected immediately after slaughter and transported to the laboratory on ice. The reproductive tracts were analyzed immediately; the blood samples were stored at –80 °C until DNA isolation.
All gilts were weighed on the day of arrival at the experimental farm, the day of individual housing and the day before slaughter.

Collection of data after slaughter

Morphometry and weight analyses were performed according to Van der Lende *et al.* (1990). One person performed the collection of all data which consisted of crown-rump length and weight of the embryos, length and weight of both uterine horns (filled as well as empty), length and weight of the extra-embryonic membranes, length and width of the implantation sites, their distance from the

utero-cervical junction, weight of the ovaries and individual weight and number of corpora lutea after dissection (Van Rens et al., 2000).

Embryos that were morphologically normal and not degenerating are referred to as 'vital embryos' in this paper.

DNA isolation and genotyping

Only gilts that were pregnant at slaughter were typed for PRLR.
DNA was isolated using a PurGene Genomic DNA isolation kit for human and mammalian whole blood and bone marrow cells (D-5000, Gentra Systems Inc.,Minneapolis, USA). DNA was then genotyped for the PRLR marker at PIC's Research Center (Cambridge, UK).

The region of the PRLR gene containing the polymorphic *Alu* I site was amplified using primers GTGTCTGCAGTGGCCCG and CTCGAAACGTGGCTCCG in a 10 µl PCR containing 1x PCR Buffer II (Perkin Elmer), 0.2 mM dATP, dTTP, dGTP and dCTP, 1.5 mM $MgCl_2$ and 0.5 units AmpliTaq Gold (Perkin Elmer). Primers were used at a concentration of 0.25 µM with a thermal cycling regime of 94 °C for 12 minutes followed by 35 cycles of 94 °C for 1 minute, 60 °C for 1 minute and 72 °C for 1 minute and ending with a final step of 72 °C for 4 minutes. To each reaction 1.5 µl of 10x Alu I Buffer (Stratagene), 2.5 units of *Alu* I and H_2O to make up to 15 µl was added. Reactions were incubated at 37 °C for 2 hours. Following digestion, 5 µl of loading buffer was added and the products subject to electrophoresis on a 4% agarose gel composed of 3% NuSieve GTG agarose (FMC BioProducts) and 1% Seakem ME agarose (FMC BioProducts). The region of the gene amplified contained one *Alu* I site that was present in both of the PRLR alleles and acted as a positive control for digestion giving fragments of 60 and 50 base pairs in length. In the presence of the polymorphic *Alu* I site the 50 bp fragment is digested to fragments of 31 and 19 bp. The 50 bp fragment was designated the B allele and the 31 bp fragment was designated the A allele.

Statistical analysis

All data were analyzed with SAS/STAT (1990). The procedure GLM was used for analyzing linear models.

Data from 56 gilts were available. Estrous cycle length was defined as the time interval between the first day of estrus and the first day of the subsequent estrus. The time of onset of estrus was defined as the first time the gilt showed estrus minus half the time interval since the previous estrus detection. The end of estrus was defined as the last time the gilt showed estrus plus half the time interval to the subsequent estrus detection. As far as parameters for conceptus development are concerned, all statistical analyses were performed on average values per gilt. These values represent only the vital embryos. Results are expressed as least squares means and standard errors of least squares means, estimated after stepwise elimination of non-significant effects (except for 'genotype' and 'batch'), using the following model:

$$y_{ijkl} = m+genotype_i+day_j+batch_k+genotype*day_{ij}+genotype*batch_{ik}+day*batch_{jk}+ bw+bw*genotype+e_{ijkl}$$

in which 'day'(j=1,2) is day after first insemination on which the pig has been slaughtered, 'batch' (k=1,4) is the batch in which the pig has been obtained from PIC and 'bw' is the covariable bodyweight at the day of individual housing. When genotype had or tended to have a significant effect (i.e. $P < 0.10$), multiple comparisons were performed with adjustments according to Tukey-Kramer (SAS/STAT, 1996). In addition, for uterine and placental properties the total number of implantation sites has been included in the model as a covariable.

RESULTS

Gilts

In total, 63 gilts were inseminated, of which 56 gilts (9AA, 25 AB and 22 BB) became pregnant. The experiment had initially been designed to compare gilts with different estrogen receptor genotypes, which explains the unequal numbers for the different PRLR genotypes. Because only the pregnant gilts were typed for PRLR, information about pregnancy rates of different PRLR genotypes was not available.

Bodyweight at relocation to individual cages and age at insemination were 129 ± 6, 117 ± 3 and 115 ± 4 kg and 247 ± 6, 252 ± 4 and 240 ± 4 days for AA, AB and BB gilts, respectively ($P = 0.118$ and $P = 0.109$). Bodyweight and age at slaughter

were 161 ± 6, 151 ± 4 and 152 ± 4 kg and 282 ± 6, 287 ± 4 and 276 ± 4 days for AA, AB and BB gilts, respectively (P = 0.334 and P = 0.117).

Estrus

In total three estruses were detected for each gilt. Genotype did not affect the length of estrus (P > 0.50). The first estrus lasted on average 56 ± 7, 59 ± 4 and 58 ± 4 hours, the second 66 ± 4, 63 ± 3 and 63 ± 3 hours and the third 55 ± 5, 53 ± 3 and 57 ± 3 hours for AA, AB and BB gilts, respectively. The cannulated gilts had a significantly longer third oestrus (i.e. the oestrus after cannulation) (P = 0.0001) than the non-cannulated gilts (63±3 h and 46±3 h, respectively).

The first estrous cycle length was significantly affected (P = 0.03) and the second tended to be affected by genotype (P = 0.07): The length of the first estrous cycle was 21.6 ± 0.5, 20.3 ± 0.3 and 20.0 ± 0.3 days for AA, AB and BB gilts, respectively (the cycle length of AA gilts was longer than of BB gilts (P = 0.03) and tended to be longer than of AB gilts (P = 0.06)). The length of the second cycle was 22.8 ± 0.7, 21.0 ± 0.4 and 20.8 ± 0.5 days for AA, AB and BB gilts respectively (the cycle length of AA gilts tended to be longer than of BB gilts (P = 0.07) and of AB gilts (P = 0.09)).

Components of litter size

Results are presented in Tables 1 to 3.
PRLR genotype significantly affected the number of corpora lutea, and tended to affect the total number of implantation sites. AA gilts had more ovulations than BB gilts, while AB gilts were in between (Table 1). Genotype did not affect total number of embryos and number of vital embryos found at Day 35/36, although the trend found for number of corpora lutea and implantation sites was still present in the least squares mean estimates (Table 1). Percentage of implantation sites, embryos and vital embryos was similar for all three genotypes (Table 1).

Neither length nor weight of the vital embryos was affected by PRLR genotype (Table 2). Placental weight was significantly affected by PRLR genotype, i.e. AA gilts had heavier placentae than BB gilts and tended to have heavier placentae than AB gilts (Table 2).

Table 1. Components of litter size in gilts with PRLR genotype AA, AB and BB at Day 35/36 of pregnancy (ls means ± sem)

Component	AA (n=9)	AB (n=25)	BB (n=22)	p value
# CL[G]	21.5 ± 0.9[a]	20.0 ± 0.5[ab]	18.7 ± 0.6[b]	0.048
# Impl. sites	17.3 ± 1.3	15.7 ± 0.8	13.9 ± 0.9	0.095
# Embryos	17.0 ± 1.3	15.6 ± 0.8	13.7 ± 0.9	0.111
# Vital embryos	16.3 ± 1.3	14.7 ± 0.8	13.4 ± 0.9	0.188
% Impl. sites[G]	80.8 ± 5.9	79.3 ± 3.4	74.5 ± 3.7	0.552
% Embryos[G]	79.2 ± 5.9	78.6 ± 3.4	73.4 ± 3.7	0.537
% Vital embr.[G]	75.8 ± 6.0	74.4 ± 3.4	71.5 ± 3.8	0.791

[a,b] Means in the same row with different superscripts differ significantly (pdiff < 0.05)
[G] Bodyweight was included as a covariable in the model

For length of placentae and length of implantation sites, a significant interaction between bodyweight and genotype was found (P = 0.03 and P = 0.04, respectively). For both components, the regression line for BB gilts differed from that for AA and AB gilts (Figure 1). The slopes of the regression lines were 0.031 (P = 0.79), -0.0002 (P = 0.998) and 0.286 (P = 0.0006) for placental length and 0.071 (P = 0.39), 0.002 (P = 0.97) and 0.213 (P = 0.0005) for length of implantation sites for AA, AB and BB gilts, respectively. When BB gilts were excluded from the analysis, AA gilts had significantly longer implantation sites and tended to have longer placentae than AB gilts (Table 2).

Both width and surface area of the implantation sites (i.e. length*width) were significantly affected by PRLR genotype, i.e. AA gilts had wider implantation sites than BB gilts and had a larger surface area compared to BB gilts and AB gilts (Table 2).

The ratio between placental length and length of implantation sites was similar (P = 0.88) for the three PRLR genotypes (1.70 ± 0.07, 1.74 ± 0.04 and 1.73 ± 0.04, for AA, AB and BB gilts, respectively).

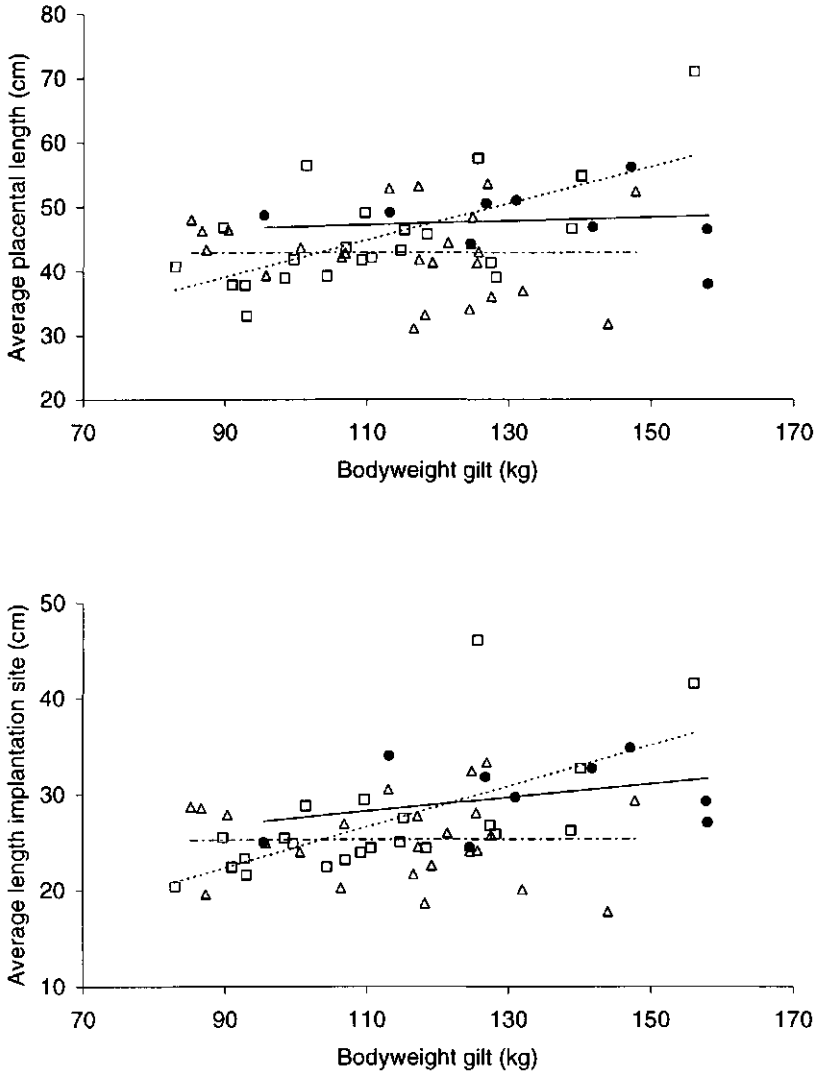


Figure 1. Relation average placental length (a) or average length implantation site (b) of vital embryos to bodyweight gilt
(● —— = AA ; △ -·-·- = AB ; □ ····· = BB)

Table 2. Embryonic and placental development in gilts with PRLR genotype AA, AB and BB at Day 35/36 of pregnancy (ls means ± sem)

Component[1,2]	AA (n=9)	AB (n=25)	BB (n=22)	p value
Embryos				
Bodyweight (g)	4.25 ± 0.26	4.44 ± 0.15	4.22 ± 0.17	0.623
Length (cm)	3.85 ± 0.08	3.88 ± 0.05	3.79 ± 0.05	0.421
Placentae				
Weight (g)	52.5 ± 3.4^{a}	43.2 ± 2.0^{c}	42.0 ± 2.3^{bc}	0.044
Corrected weight (g)	54.8 ± 3.2^{a}	43.7 ± 1.9^{b}	40.4 ± 2.1^{b}	0.004
Length (cm)[3]	47.7 ± 2.1^{a}	43.1 ± 1.2^{c}	----------	0.070
Corrected length^{G} (cm)	47.8 ± 2.0	43.7 ± 1.1	43.9 ± 1.3	0.212
Implantation site				
Length (cm)[3]	29.4 ± 1.4^{a}	25.3 ± 0.9^{b}	--------	0.026
Corrected length^{G}(cm)	29.4 ± 1.5	25.8 ± 0.8	26.3 ± 0.9	0.125
Width (cm)	11.0 ± 0.4^{a}	10.2 ± 0.2^{ab}	9.5 ± 0.3^{b}	0.010
Corrected width (cm)	10.8 ± 0.4^{a}	10.1 ± 0.2^{ab}	9.6 ± 0.2^{b}	0.058
Length*width^{G} (cm^{2})	309 ± 19^{a}	257 ± 11^{bc}	256 ± 12^{c}	0.049
Corrected length*width^{G} (cm^{2})	314 ± 18^{a}	257 ± 10^{b}	253 ± 12^{b}	0.025

1 Results represent only vital embryos (i.e. morphologically normal, not degenerating embryos)

2 Corrected length, weight or width: corrected for total number of implantation sites

3 BB not included in the analysis (see text for explanation)

a,b Means in the same row with different superscripts differ significantly (pdiff < 0.05)

a,c Means in the same row with different superscripts differ significantly (pdiff < 0.1)

G Bodyweight was included as a covariable in the model

Length and weight of empty uteri were significantly affected by PRLR genotype (Table 3), i.e. empty uteri of AA gilts were significantly longer and heavier than those of BB gilts, and were significantly longer and tended to be heavier than those of AB gilts. Furthermore AB gilts tended to have heavier empty uteri than BB gilts. Length and weight of filled uteri were affected in the same way, or at least showed the same tendencies (Table 3).

PRLR genotype significantly affected the total weight of ovaries: Ovaries of BB gilts tended to be lighter than ovaries of AA gilts and AB gilts (Table 3). After correction for number of corpora lutea, PRLR genotype did not affect ($P = 0.272$) ovarian weights anymore (23.3 ± 1.2, 23.2 ± 0.7 and 21.6 ± 0.8 g for AA, AB and BB gilts, respectively). The total weight of the corpora lutea however, was not affected by PRLR genotype. Neither was the average corpus luteum weight (Table 3). The weight of ovaries after subtraction of weight of corpora lutea was significantly affected by PRLR genotype, i.e. the weight was higher for AA gilts compared to BB gilts (Table 3). Again, after correction for number of corpora lutea, the effect of PRLR genotype lost its significance ($P = 0.15$) although the tendency remained the same (16.0 ± 1.0, 15.0 ± 0.6 and 13.8 ± 0.6 g for AA, AB and BB gilts, respectively).

Including total number of implantation sites as a covariable in the statistical analysis resulted in a more pronounced significant effect of PRLR genotype on placental weight, as the P value decreased (Table 2). The effect on implantation width became less pronounced, as the P value increased, but still tended to be significant. For placental length and length of implantation sites there was no longer an interaction between bodyweight and genotype after including the total number of implantation sites in the model. Thus all three genotypes could be included in the analysis, which resulted in the tendency of AA gilts to have longer placentae and implantation sites compared to AB and BB gilts (Table 2). The effect of PRLR genotype on implantation surface area remained significant after correction for number of implantation sites (more pronounced) (Table 2).

Including the total number of implantation sites as a covariable in the statistical analysis reduced the effects of PRLR genotype on uterine weight properties, shown as an increase in P values which are no longer significant (Table 3). The effect on empty uterine weight however, remained partly the same in a way that AA gilts still had heavier uteri than BB gilts.

Table 3. Reproductive tracts and ovaries of Meishan synthetics with PRLR genotype AA, AB and BB at Day 35/36 of pregnancy (ls means ± sem)

Component[1]	AA (n=9)	AB (n=25)	BB (n=22)	p value
Uteri (filled)				
Length[G] (cm)	599 ± 27[a]	526 ± 15[c]	520 ± 17[bc]	0.045
Corrected length[G] (cm)[·]	567 ± 23	--------	535 ± 14	0.282
Weight[G] (g)	5493 ± 489[a]	4509 ± 281[ac]	4094 ± 308[c]	0.075
Corrected weight[G] (g)	5118 ± 408	4430 ± 231	4381 ± 259	0.306
Uteri (empty)				
Length[G] (cm)	669 ± 28[a]	578 ± 16[b]	566 ± 18[b]	0.012
Corrected length[G](cm)[·]	640 ± 26[a]	--------	578 ± 16[c]	0.077
Weight[G](g)	2371 ± 130[a]	2019 ± 74[c]	1792 ± 82[bc]	0.003
Corrected weight[G] (g)	2264 ± 119[a]	1989 ± 68[ab]	1874 ± 76[b]	0.041
Ovaries				
Total weight[G] (g)	24.4 ± 1.3[a]	23.4 ± 0.7[a]	20.9 ± 0.8[c]	0.032
Minus weight cl[G] (g)	16.6 ± 1.0[a]	15.0 ± 0.6[ab]	13.4 ± 0.6[b]	0.031
Corpora lutea				
Total weight[G] (g)	7.73 ± 0.49	8.35 ± 0.28	7.58 ± 0.31	0.170
Average weight[G] (g)	0.36 ± 0.03	0.42 ± 0.01	0.41 ± 0.02	0.140

[1] Corrected length or weight: corrected for total number of implantation sites.

[·] AB not included in the analysis (see text for explanation)

[a,b] Means in the same row with different superscripts differ significantly (pdiff < 0.05)

[a,c] Means in the same row with different superscripts differ significantly (pdiff < 0.1)

[G] Bodyweight was included as a covariable in the model

For uterine length, an interaction between genotype and number of implantation sites was found (P = 0.02 and P = 0.02 for filled and empty uterus, respectively). Both for filled and empty uterus, the slopes of the regression lines of AA and BB gilts significantly differed from zero, while AB gilts had a slope that did not differ from zero (Figure 2). The slopes of the regression lines were 13.9 (P = 0.02), -3.8 (P = 0.37) and 8.9 (P = 0.02) for filled uterus and 10.4 (P = 0.08), -4.4 (P = 0.33) and 11.3 (P = 0.004) for empty uterus for AA, AB and BB gilts, respectively. When AB gilts were excluded from the analysis, AA gilts still tended to have longer empty uteri than BB gilts after correction for number of implantation sites. Length of filled uteri however, showed different least squares mean estimates only (Table 3).

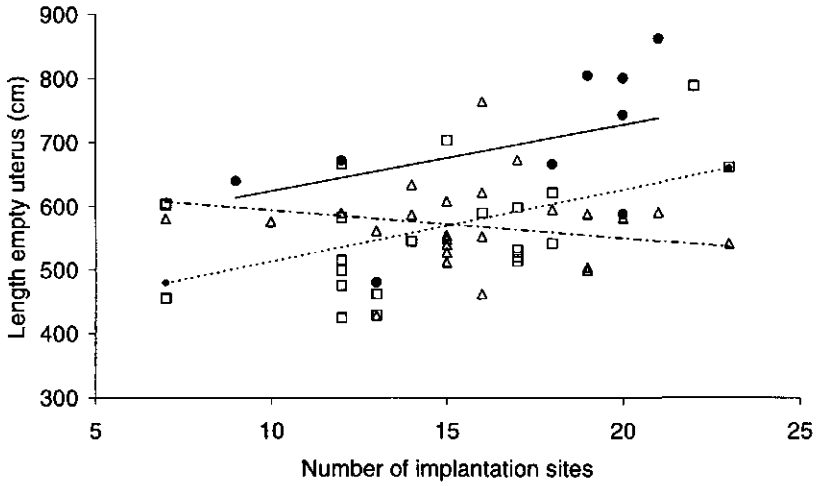
DISCUSSION

The present study compares gilts with PRLR genotype AA (AA gilts), AB (AB gilts) and BB (BB gilts) of a 50% Landrace/50% Meishan synthetic line. In various pig breeds PRLR genotype seems to affect litter size (Rothschild et al., 1998; Vincent et al., 1998. Van Rens and Van der Lende, 2000). Because no physiological studies on the effect of PRLR genotype on prolificacy of pigs have been performed before, estrus behavior and periovulatory hormone profiles as well as ovulation rate, embryonic survival and placental, embryonic and uterine development were examined for the three genotypes.

Estrus length was similar for the three genotypes. However, estrous cycle tended to be longer for AA gilts compared to AB and BB gilts.

Since only 5 AA, 11 AB and 9 BB gilts had been cannulated, periovulatory hormone profiles have not been shown in the results. However, based on these restricted numbers, the periovulatory LH and progesterone profiles appeared to be similar for the three genotypes tested. The same held for the estradiol profile, except for the duration of the estradiol surge, which was longer only for AB gilts (n=9) compared to BB gilts (n=7), probably caused by a difference in onset of estradiol surge.

Ovulation rate was significantly affected by PRLR genotype. The number of implantation sites, total number of embryos and number of vital embryos however were not. Nevertheless, they all showed a similar trend as the number of corpora lutea, i.e. AA gilts had the highest numbers, BB gilts the lowest and AB gilts were



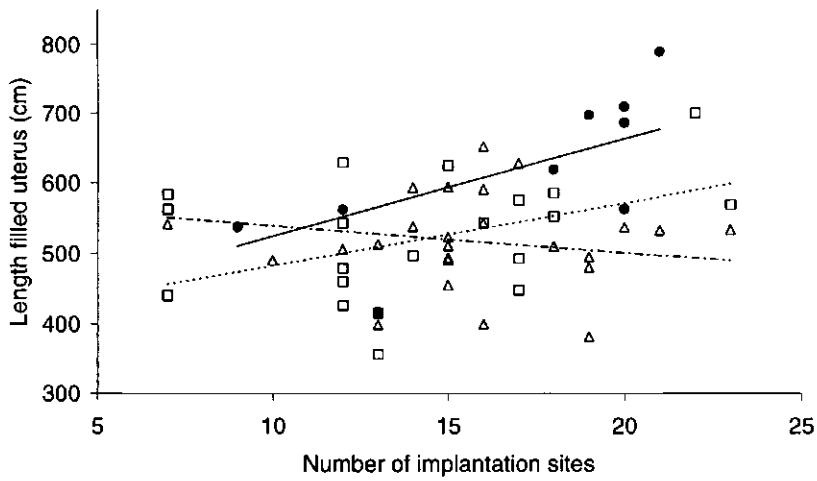


Figure 2. *Relation length empty uterus (a) or filled uterus (b) to number of implantation sites (● —— = AA ; Δ -·-·- = AB ; □ ····· = BB)*

intermediate. The lack of significance of these differences (which still ran up to a difference of three vital embryos between AA and BB gilts) is probably a result of the increased between-gilt variation, as the average percentages of implantation sites, embryos and vital embryos were similar. Thus, provided that uterine capacity is not limiting for the three genotypes, we would expect AA gilts to have the highest litter size. Litter size results of a Landrace synthetic line, a line with similar genetic backgrounds as our Meishan/Landrace synthetic line, are in agreement with this expectation (Vincent et al., 1998). Total number born of a Large White/Meishan Synthetic line however, was highest for AB sows (Vincent et al., 1998). Results of Vincent *et al.* (1998) are based on litter size results of sows with different parities, while our results are based on gilts only. Litter size results of Large White X Meishan F2 crossbred gilts, bred under the same management system as the gilts in this study, were also in agreement with our expectation. For this line, the total number of piglets born was 11.4, 10.8, and 8.8 for AA, AB and BB gilts, respectively (Van Rens and Van der Lende, 2000).

Uterine weight and length were higher for AA gilts, compared to AB and BB gilts. As uterine length adapts to litter size (Wu et al. 1987), the differences in uterine length found, might very well be a consequence of the difference in number of vital embryos found at Day 35/36. The fact that empty uterine length still tended to be higher for AA gilts after including the number of implantation sites as a covariable in the model, indicates that the embryos of AA gilts had potentially more uterine space available than embryos of BB gilts. AB gilts on the other hand, appeared to be unable to adapt uterine length to litter size, which might lead to capacity problems later in pregnancy.

The extra potential uterine space in AA gilts actually seemed to be utilized by the embryos for implantation, as implantation surfaces for embryos from AA gilts were larger compared to AB and BB gilts and their placentae were heavier and tended to be longer. This larger surface area of contact of endometrium with placental membranes might lead to a potentially better exchange of nutrients and waste products between uterus and embryo in AA gilts compared to AB and BB gilts. This advantage has not led to an increase in embryonic weight or length at Day 35/36, as gilts with different PRLR genotypes still had embryos with similar weights and lengths.

AA, AB and BB gilts had a similar degree of endometrial folding of the uterine surface area, as the ratio between placental length and length of implantation site was the same for the three genotypes.

The total weight of ovaries was larger for AA and AB gilts, compared to BB gilts. This difference was not a direct result of the difference in number of corpora lutea, as the total weight of corpora lutea was similar for the three genotypes. Still the difference in number of corpora lutea found between the genotypes seemed to be involved in ovarian weight, as correction for ovulation rate reduced the genotype effect. Thus the difference in ovarian weight might be caused by a difference in connective tissue and blood supply, or by a difference in number or size of follicles present. Clark et al. (1973) reported a positive relationship between number of corpora lutea and the development of antral follicle pool and thus support the second suggestion. The high ovulation rate of AA gilts might have been accompanied by a larger antral follicle pool than in AB and BB gilts, which in turn might lead to a higher ovulation rate in the next estrus.

Unlike AA and AB gilts, for BB gilts both implantation length and placental length were dependent on bodyweight. The reason for this phenomenon is not known. However, it is not surprising that both variables react in the same way, as for all three genotypes there is a positive relation between placental length and implantation length.

The mutation that has caused the polymorphism at the PRLR locus was found in the last exon of the gene (M.F. Rothschild, A.L. Vincent and G. Evans, personal communication), suggesting a difference in structure of prolactin receptor rather than a difference in expression of the PRLR gene leading to different numbers of prolactin receptors in the tissues. The last exon of the PRLR gene encodes for the intracellular domain of the membrane anchored PRLR (Ormandy et al., 1998, Bole-Feysot et al., 1998). A difference in structure at the intracellular domain probably will not affect the capacity of the receptor to bind its ligand and to dimerize with another PRLR. However, it might very well lead to differences in receptor mediated signal transduction following ligand binding and dimerization, and thus might lead to a change in action of prolactin.

Vincent et al. (1998) studied the effect of PRLR genotype on litter size of different breeds. Because the favorable alleles were different in the different populations, they suggested that either PRLR gene is just a linked marker or that background

genes play a large role in determining the effect of PRLR. Although the possibility of the PRLR polymorphism being a marker for a closely linked major gene for litter size can probably never be excluded, results of this experiment do not exclude the possibility that the PRLR gene is the major gene itself either. The gene seems to affect the number of ovulations on one hand and the capability to increase uterine length and subsequently potency to enlarge placentae and implantation surfaces on the other hand. Both porcine ovaries and porcine endometrium contain prolactin receptors distributed in a pregnancy dependent way and with quantitative differences throughout pregnancy (Rolland et al., 1976; Jammes et al., 1985; Young et al., 1989; Young *et al.*, 1990), indicating a substantial role of PRLR on these organs during pregnancy. As a matter of fact, various actions in which of PRLR is involved, both on ovarian and uterine level, have previously been described in various species: Female mice carrying a homozygous null mutation of the PRLR gene ($PRLR^{-/-}$) presented multiple reproductive abnormalities, including a reduced number of primary follicles in their ovaries and a reduced ovulation rate (Ormandy et al., 1997; Bole-Feysot, et al., 1998). Thus, polymorphism in the porcine PRLR might have led to a reduction or a stimulation of activities leading to a difference in ovulation rate and number of primary follicles between gilts carrying one or two B alleles and those carrying two A alleles.
Experiments with $PRLR^{-/-}$ mice furthermore showed that the uterus of these animals is refractory to implantation (Ormandy et al., 1997; Bole-Feysot, et al., 1998). Although attachment of embryos does not seem to be influenced in our experiment (as no differences in percentage of implantation sites were observed between the three genotypes), it indicates a role of PRLR in preparing or maintaining a suitable uterine environment for pregnancy.

Most research concerning the effect of PRLR on uterine properties have focused on involvement in endometrial secretory activity (Daniel *et al.*, 1984, Chilton and Daniel, 1987, Young *et al.*, 1989, Young et al., 1990). Apart from these secretory effects, prolactin has also been shown to affect uterine structure. In rats, prolactin promoted a thickening of the uterine endometrium and an increase in glandular differentiation (Chilton and Daniel, 1987). Hence, if these effects of prolactin were mediated through PRLR, porcine uterine structure (length, weight through different thickness) might also be affected by a difference in PRLR genotype.

It is remarkable that AB gilts are intermediate for ovulation rate, comparable to AA gilts for their ovarian weight and comparable to BB gilts for their placental and uterine properties (except for the lack of ability to adjust uterine length to number of

embryos). If indeed PRLR gene itself is the major gene for litter size, it will be a great challenge to unravel in which way the different alleles have their specific actions on the different reproductive organs.

Conclusion

The present study shows that prolactin receptor polymorphism affects porcine ovaries, uterus and placenta in a way that does not exclude the possibility of prolactin receptor gene being the major gene rather than a marker for a closely linked major gene for litter size. It is worthwhile to extend research with a larger number to confirm the results of this study. To increase knowledge about the physiological role of prolactin receptor gene on prolificacy in pigs, follow-up studies should focus on antral follicle development and ovulation on the one hand and on uterine properties (like thickness, glandular development, capability for enlargement) on the other hand. Despite the unequal sizes of experimental groups the data set proved to be very useful and might also be useful for the first physiological screening of other major genes for litter size.

REFERENCES

Bole-Feysot C, Goffin V, Edery M, Binart N, and Kelly PA (1998). Prolactin (PRL) and its receptor: Actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocrin Rev 19:225-268.

Chilton BS and Daniel JC (1987). Differences in the rabbit uterine response to progesterone as influenced by growth hormone or prolactin. J Reprod Fert 79:581-587.

Clark JR, Edey TN, First NL, Chapman AB, and Casida LE (1973). Effects of four genetic groups and two levels of feeding on ovulation rate and follicular development in puberal gilts. J Anim Sci 36:1164-1169.

Daniel JC, Jetton AE, and Chilton BS (1984). Prolactin as a factor in the uterine response to progesterone in rabbits. J Reprod Fert 72:443-452.

Jammes H, Schirar A, and Djiane J (1985). Differential patterns in luteal prolactin and LH receptors during pregnancy in sows and ewes. J Reprod Fert 73:27-35.

Ormandy CJ, Binart N, Helloco C, and Kelly PA (1998). Mouse prolactin receptor gene: Genomic organization reveals alternative promoter usage and generation of isoforms via alternative 3'-exon splicing. DNA Cell Biol 17:761-770.

Ormandy CJ, Camus A, Barra J, Damotte D, Lucas B, Buteau H, Edery M, Brousse N, Babinet C, Binart N, and Kelly PA (1997). Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. Genes Dev 11:167-178.

Rolland R, Gunsalus GL, and Hammond JM (1976). Demonstration of specific binding of prolactin by porcine corpora lutea. Endocrinology 98:1083-1091.

Rothschild MF, Vincent AL, Tuggle CK, Evans G, Short TH, Southwood OI, Wales R, and Plastow GS (1998). A mutation in the prolactin receptor gene is associated with increased litter size in pigs. 26th International Conference on Animal Genetics, ISAG (International Society for Animal Genetics), August 9-14, 1998, Auckland, NZ; pp 105 (Abstr.).

SAS/STAT (1990). User's guide SAS Institute, Cary, NC

SAS/STAT (1996). Changes and enhancements through release 6.11 SAS Institute, Cary, NC.

Van der Lende T, Hazeleger W, and De Jager D (1990). Weight distribution within litters at the early foetal stage and at birth in relation to embryonic mortality in the pig. Livest Prod Sci 26:53-65.

Van Rens BTTM, Hazeleger W, and Van der Lende T (2000). Periovulatory hormone profiles and components of litter size in gilts with different estrogen receptor (ESR) genotypes. Theriogenology 53:1375-1387.

Van Rens BTTM, and Van der Lende T (2000). Effect of prolactin receptor (PRLR) gene polymorphism on litter size and placental traits in gilts. J Reprod Fertil Abstracts series 26, 12.

Vincent AL, Evans G, Short TH, Southwood OI, Plastow GS, Tuggle CK, and Rothschild MF (1998). The prolactin receptor gene is associated with increased litter size in pigs. 6th WCGALP 27, 15-18.

Vincent AL, Wang L, Tuggle CK, Robic A, Rothschild MF (1997). Prolactin receptor maps to pig chromosome 16. Mamm Genome 8:793-794.

Wu MC, Hentzel MD, and Dziuk PJ (1987). Relationships between uterine length and number of fetuses and prenatal mortality in pigs. J Anim Sci 65:762-770.

Young KH, Kraeling RR, and Bazer FW (1989). Effects of prolactin on conceptus survival and uterine secretory activity in pigs. J Reprod Fert 86:713-722.

Young KH, Kraeling RR, and Bazer FW (1990). Effect of pregnancy and exogenous ovarian steroids on endometrial prolactin receptor ontogeny and uterine secretory response in pigs. Biol Reprod 43:592-599.

Chapter 7

Litter size and piglet traits of gilts with different prolactin receptor genotypes

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Accepted for publication in Theriogenology

ABSTRACT

77 Large White x Meishan F2 crossbred gilts with prolactin receptor (PRLR) genotype AA (n=26), AB (n=36) and BB (n=15) were compared for teat number (FTm), age at first estrus, gestation length (GL), litter size, and litter means of functional teat number (FTp), birthweight (BW), and pre-weaning growth rate (GR). For 88% of 620 liveborn piglets (62 gilts) own placental information was available, since placentae were labeled during farrowing. The effect of PRLR genotype of the mother on average placenta weight (PLW) and placenta efficiency (EFF = BW/PLW) was therefore also analyzed. PRLR genotype significantly ($P<0.05$) affected age at first estrus, and as a result (since the gilts were inseminated at a fixed estrus number) age and bodyweight at insemination. Furthermore PRLR genotype affected total number of piglets born (TNB; $p=0.056$) and number of piglets born alive (NBA; $p=0.072$), but did not affect ($p>0.3$) GL, BW or GR, neither before nor after correction for litter size. BB gilts were significantly younger at first estrus and younger and lighter at insemination than AA gilts ($p<0.05$). AA gilts had larger TNB ($p=0.047$) and tended to have a larger NBA ($p=0.062$) than BB gilts. TNB was 11.4±0.7, 10.8±0.6, and 8.8±0.9, NBA 11.1±0.6, 10.5±0.6, and 8.7±0.9, BW 1309±40, 1277±34, and 1290±53 g and GL 113.6±0.3, 113.8±0.3, and 113.5±0.4 d for AA, AB and BB gilts, respectively. The effects on litter size and age at first estrus are independent effects. PRLR affected PLW ($p=0.050$) and EFF ($p=0.066$), resulting in a difference between AA and BB gilts. PLW was 160±9, 181±7 and 196±11 g and EFF was 7.6±0.2, 7.3±0.2 and 6.7±0.3, for AA (n=19), AB (n=29) and BB (n=14) gilts, respectively. After correction for TNB, the differences disappeared. Functional teat number of the AA, AB and BB gilts was 15.35±0.22, 15.53±0.18, and 15.60±0.29, respectively, and was not affected by PRLR genotype ($P=0.7$). Functional teat number of piglets from AA, AB and BB mothers was 14.20±0.10, 14.37±0.08, and 14.63±0.13, respectively. Piglets from BB mothers had on average larger numbers of functional teats compared to piglets from AA mothers ($P=0.028$). In conclusion, PRLR gene is a major gene or a marker for age at first estrus, litter size, and litter average of number of functional teats in the Large White x Meishan F2 crossbred gilts studied. The favorable allele for litter size (A allele) is the unfavorable allele for age at first estrus and litter mean of functional teat number.

Keywords: pigs, prolactin receptor gene, litter size, teat number, puberty, placenta

INTRODUCTION

In the pig, the prolactin receptor (PRLR) gene has been mapped to chromosome 16 (17). Since the discovery of a polymorphism at the PRLR locus (two alleles, A and B), PRLR gene has become a candidate gene for litter size in pigs (12, 16). The polymorphism was associated with total number of piglets born and(or) number born alive in three genetic lines tested (12, 16). The direction and magnitude of the effects, however, varied between lines. The mechanism through which this gene affects litter size is not yet known. Furthermore, it is not known whether the PRLR polymorphism itself causes differences in litter size or whether this polymorphism provides a marker for a closely linked major gene for litter size.

The present study was conducted to examine effects of PRLR gene polymorphism on reproductive traits in Large White x Meishan F2 crossbred gilts. Reproductive traits investigated were: number of functional teats, age at first estrus, litter size, gestation length and litter means of functional teat number, birthweight, placental weight at term, and placental efficiency.

MATERIALS AND METHODS

Animals

The Large White x Meishan F2 crossbred gilts used in this experiment were bred and raised at the experimental farm of Wageningen University. At birth, the number of functional teats (i.e. the morphologically normal teats) of the gilts were counted. At an average age of 8 weeks, the gilts were housed in groups of 4. By preference, groups consisted of littermates with similar bodyweights. Once housed in groups, the animals were checked once daily (1600) for estrus by the back pressure test in presence of a vasectomized adult boar. When the gilts showed their fourth estrus, they were artificially inseminated twice with an interval of 24 hours with semen of a Great York-S boar ($3*10^9$ sperm cells per dose). Semen from a different boar was used for first and second insemination. Animals that returned to estrus, were inseminated again, following the same protocol. Animals that returned to estrus for a second time were excluded from the experiment. Animals that did not return to estrus, remained in the original group until not later than day 107 after insemination. They were then transferred to individual farrowing pens (Danish

Farrowing Pen 220*380 cm^2, farrowing crate 265x66 cm^2, concrete floor covered with saw dust).

Within 16 h after farrowing, live born, stillborn and non-fresh stillborn piglets were distinguished, and sex, birthweight and teat number of the piglets was determined. All expelled placentae were meticulously investigated to recover all mummified fetuses.

Pregnant gilts were fed 1.25 running up to 1.5 kg of a commercial sow ration (12.56 MJ metabolizable energy/kg, crude protein 144 g/kg, ileal digestible lysine 7.5 g/kg) twice daily at 0830 and 1630. Water was always available ad libitum.

The gilts were weighed at 17 days after showing their third estrus. Gilts which had returned to estrus and thus had been inseminated again, were weighed just preceding insemination. Furthermore, bodyweight of the sows was determined one day after farrowing.

Of 62 of the 77 F2 females that had farrowed, placentae at term were examined as described below.

Placentae at term

In order to match individual piglets with their placentae, the placentae of a random sample of the F2 females (i.e. 62 of 77 gilts) were labeled during farrowing as described by Wilson et al. (19), with slight modifications. The gilts were observed every 1h beginning at 18:00 h on Day 112 of gestation for signs of impending parturition. Once milk let-down or vulvar swelling and mucus secretion was observed, the gilts were monitored continuously until farrowing and placental expulsion was completed. As a piglet was expelled, it was caught, and the umbilical cord was ligated with surgical silk (35165, Linnen Braun EP-5 USP 2, Instruvet, Amerongen, The Netherlands) containing a specific code. The umbilical cord was then cut between piglet and tag, allowing the placental end of the cord with its tag to retract into the vagina. Subsequently the piglet was earmarked with a number corresponding to the specific code on the tag. Immediately after expulsion, the placentae were identified and stored at 4 °C.

Within 24 hours after expulsion, the placentae were carefully isolated from each other. The allantochorion was opened over the whole length at the anti-

mesometrial side and the umbilical cord and amniotic membranes were removed. Subsequently, placental weight was determined.

DNA Isolation and Genotyping

Only the gilts that had farrowed successfully were genotyped for PRLR. DNA was isolated using a PurGene Genomic DNA isolation kit for human and mammalian whole blood and bone marrow cells (D-5000, Gentra Systems Inc.,Minneapolis, USA). DNA was then genotyped for the PRLR marker at PIC's Research Center (Cambridge, UK).

The region of the PRLR gene containing the polymorphic *Alu* I site was amplified using primers GTGTCTGCAGTGGCCCG and CTCGAAACGTGGCTCCG in a 10 µl PCR containing 1x PCR Buffer II (Perkin Elmer), 0.2 mM dATP, dTTP, dGTP and dCTP, 1.5 mM $MgCl_2$ and 0.5 units AmpliTaq Gold (Perkin Elmer). Primers were used at a concentration of 0.25 µM with a thermal cycling regime of 94 °C for 12 minutes followed by 35 cycles of 94 °C for 1 minute, 60 °C for 1 minute and 72 °C for 1 minute and ending with a final step of 72 °C for 4 minutes. To each reaction 1.5 µl of 10x Alu I Buffer (Stratagene), 2.5 units of *Alu* I and H_2O to make up to 15 µl was added. Reactions were incubated at 37 °C for 2 hours. Following digestion, 5 µl of loading buffer was added and the products subject to electrophoresis on a 4% agarose gel composed of 3% NuSieve GTG agarose (FMC BioProducts) and 1% Seakem ME agarose (FMC BioProducts). The region of the gene amplified contained one *Alu* I site that was present in both of the PRLR alleles and acted as a positive control for digestion giving fragments of 60 and 50 base pairs in length. In the presence of the polymorphic *Alu* I site the 50 bp fragment is digested to fragments of 31 and 19 bp. The 50 bp fragment was designated the B allele and the 31 bp fragment was designated the A allele.

Statistical Analysis

All data were analyzed with SAS/STAT (13). The procedure GLM was used for analyzing linear models.

Traits of the gilts. Results are expressed as least squares means and standard errors of least squares means, estimated after stepwize elimination of non-

significant effects (except for 'PRLR'), i.e. effects with P > 0.05, using one of the following models:

$$y_{ijk} = m + PRLR_i + fam_j + PRLR*fam_{ij} + e_{ijk}$$

[model 1]

$$y_{ijklmn} = m + PRLR_i + fam_j + season_k + enr_l + kgins + ageins + PRLR*kgins + PRLR*ageins + e_{ijklm}$$

[model 2]

in which in which 'fam' (j=1-18) is the family the F2 gilt belonged to (i.e., the combination F1boar x F1 sow the F2 gilt descended from), 'season' (l=1-3) is the season in which the gilt was inseminated (autumn, winter and spring), 'enr' (m=1,2) is the estrus number at insemination (4, or >4), 'kgins' is the weight at insemination, and 'ageins' is the age at insemination.

When PRLR genotype had or tended to have a significant effect (i.e. P<0.1), multiple comparisons were performed with adjustments according to Tukey-Kramer (14). In addition, for some of the traits tested, TNB and its interaction with PRLR has been included in the model as a covariable.

Traits that were examined using model 1 were: Number of functional teats, age at first estrus, and age and bodyweight at insemination. Traits that were examined using model 2 were: Gestation length, total number of piglets born including mummies (TNBm), total number of piglets born (TNB), and number of piglets born alive (NBA).

For TNB, several additional aspects were examined. Firstly, age at first estrus or age or bodyweight at insemination and its interaction with PRLR genotype have been included in the final model, to examine the possible effect of the mentioned traits on TNB along with PRLR genotype. If the interaction was not significant (i.e. P>0.05), it was eleminated from the model, to examine the effect of PRLR genotype on TNB after correction for one of the three traits. Secondly, age at first estrus and age and bodyweight at insemination were examined for their effect on TNB in the absence of PRLR.

Piglet traits and placentae at term. As far as piglet or placental traits were concerned, all statistical analyses were performed on average values per gilt. These values represent only the liveborn piglets. Results are expressed as least squares means and standard errors of least squares means, estimated after stepwize elimination of non-significant effects (except for 'PRLR'), i.e. effects with $P > 0.05$, using model 2, or following model:

$$y_{ijklmn} = m+PRLR_i+fam_j+season_k+enr_l+kgfar+agefar+ PRLR*kgfar+PRLR*agefar+e_{ijklm}$$

[model 3]

in which 'fam' (j=1-18) and 'enr' (l=1,2) are the same as before, 'season' (k=1,2) is the season in which the gilt was inseminated (autumn and winter), 'kgfar' is the weight after farrowing, and 'agefar' is the age at farrowing.

When PRLR genotype had or tended to have a significant effect (i.e. $P<0.1$), multiple comparisons were performed with adjustments according to Tukey-Kramer (14). In addition, for some of the traits tested, TNB or NBA and its interaction with PRLR had been included in the model as a covariable.

Traits that were examined using model 2 were: Placental weight, placental efficiency calculated by dividing birthweight by placental weight (only for liveborn piglets of which own placental information was known since its placenta was labeled succesfully), and number of functional teats. Traits that were examined using model 3 were: Piglet weight at day 21 and growth of the piglets during the first three weeks.

RESULTS

In total 77 gilts (26 AA, 36 AB, and 15 BB) had farrowed, of which 62 (19 AA, 29 AB, and 14 BB) were examined for individual placenta weights of their piglets. Labeling success was 88 %, i.e. placentae from 543 of 620 liveborn piglets were identified succesfully.

Table 1. Effects of PRLR genotype on reproductive traits of Large White x Meishan F2 crossbred gilts (least squares means±SEM)

		PRLR genotype			
	n	AA	AB	BB	P
	n_{max}	n=26	n=36	n=15	
gilt traits					
functional teats	77	15.35±0.22	15.53±0.18	15.60±0.29	0.733
age first estrus (d)	77	228±9^{a}	213±8^{ab}	187±12^{b}	0.032
age at insemination (d)	77	306±10^{a}	280±8^{ab}	257±13^{b}	0.009
weight at insemination (kg)	77	117±4^{a}	110±3^{ab}	100±5^{b}	0.030
gestation length (d)	77	113.58±0.30	113.78±0.25	113.53±0.39	0.819
gestation length (d)^{TNB}	77	113.59±0.30	113.78±0.25	113.50±0.41	0.806
TNBm[1]	75	11.69±0.71	11.29±0.62	9.33±0.94	0.122
TNB[1]	77	11.42±0.66^{a}	10.78±0.56^{ab}	8.80±0.87^{b}	0.056
NBA[1]	76	11.12±0.64^{c}	10.51±0.55^{cd}	8.67±0.85^{d}	0.072
average piglet traits					
placenta weight (g)	62	160±9^{c}	181±7^{cd}	196±11^{d}	0.0498
placenta efficiency	62	7.56±0.20^{c}	7.26±0.15^{cd}	6.73±0.25^{d}	0.0658
birthweight (g)	77	1309±40	1277±34	1290±53	0.837
weight day 21 (g)	77	4862±227	4983±193	5345±299	0.430
growth rate d 0-21 (g/d)	77	168±10	175±8	191±13	0.366
placenta weight (g)^{TNB}	62	167±8	184±6	184±11	0.2319
placenta efficiency ^{TNB}	62	7.57±0.21^{c}	7.26±0.15^{cd}	6.72±0.27^{d}	0.0944
birthweight (g)^{TNB}	77	1288±35	1290±29	1214±52	0.472
weight day 21 (g)^{NBA}	76	5065±145	5081±124	4839±194	0.705
growth rate d 0-21 (g/d)^{NBA}	76	177±7	179±6	172±9	0.803
functional teats	76	14.20±0.10^{a}	14.37±0.08^{ab}	14.63±0.13^{b}	0.037
functional teats^{TNB}	76	14.18±0.10^{a}	14.36±0.08^{ab}	14.67±0.13^{b}	0.015

[1] TNBm = total number of piglets born including mummies; TNB= total number of piglets born; NBA = number of piglets born alive

^{a,b} Means in the same row with different superscripts differ significantly (pdiff<0.05)

^{c,d} Means in the same row with different superscripts differ significantly (pdiff<0.08)

^{TNB} TNB was included as a covariable in the model

^{NBA} NBA was included as a covariable in the model

Gilts

The number of functional teats of the gilts was not affected by their PRLR genotype ($P>0.5$, Table 1, Figure 1). Age at first estrus, and age and bodyweight at insemination, however, were significantly affected by PRLR genotype ($P<0.05$, Table 1). For all three traits, BB gilts had a lower value than AA gilts, and AB gilts were in between. Gestation length was not affected by PRLR genotype (Table 1).

Since the gilts were inseminated at a fixed estrus number, the differences in age and bodyweight at insemination were probably the result of the difference in age at first estrus. Age and bodyweight at insemination were highly correlated with age at first estrus ($r=0.94$ and $r=0.70$, respectively). Furthermore, including age at first estrus and estrus number in the model as a covariable, reduced the effect of PRLR on age at insemination ($P=0.58$, 292±2, 290±2, and 289±3 d, for AA, AB and BB gilts respectively), and bodyweight at insemination ($P=0.69$, 113±3, 112±3, and 109±4 kg for AA, AB and BB gilts, respectively).

PRLR genotype did not affect TNBm, but tended to affect TNB and NBA (Table 1, Figure 2), leading to a difference of 2.45 live born piglets between AA and BB gilts. AA gilts had largest and BB gilts had smallest litters, while litter size of AB gilts was in between. When age at first estrus, or age or bodyweight at insemination and its interaction with PRLR was included in the final model for TNB, none of the three covariables had a significant interaction with PRLR ($P>0.5$). After elimination of the interaction from the model, the regression also appeared to be nonsignificant ($P=0.52$, $P=0.20$, and $P=0.17$, for age at first estrus, age and body weight at insemination, respectively). The relation of TNB to age at first estrus for the three PRLR genotypes is shown in Figure 3. This relation was not affected by including estrus number in the model.

Piglet traits and Placentae at term

Average birthweight, weight at d 21 and pre-weaning growth rate were not affected by PRLR genotype (Table1). Placental weight, however, was affected by PRLR genotype ($P<0.05$), and as a result, placental efficiency tended to be affected as well. Piglets of BB gilts tended to have larger ($P=0.055$), less efficient ($P=0.052$) placentae compared to piglets of AA gilts (Table 1).

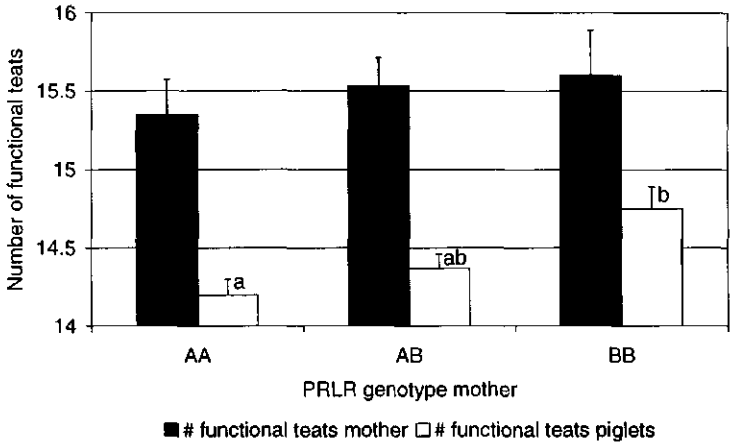


Figure 1. *Effect of prolactin receptor (PRLR) genotype of the mother on number of functional teats (least squares means ± SEM).*
Teats piglets: a, b $P < 0.05$

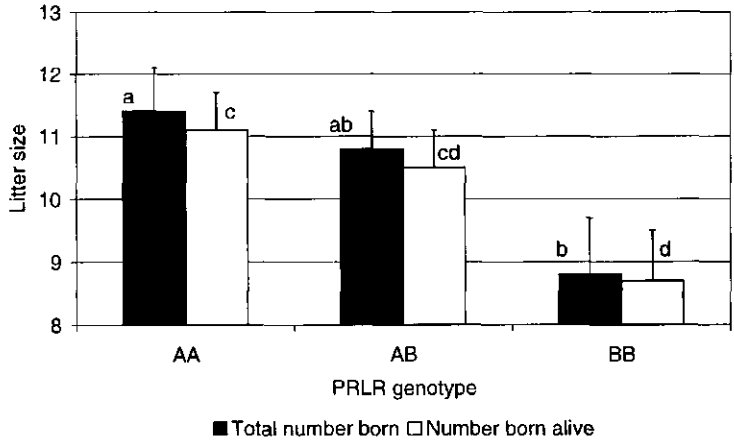


Figure 2. *Average litter size results of Large White x Meishan F2 crossbred gilts with different prolactin receptor (PRLR) genotypes (ls means ± SEM).*
TNB: a,b $P < 0.05$ NBA: c,d $P < 0.065$

Including TNB or NBA in the model did not change the effect of PRLR genotype on birthweight, weight at d 21 or pre-weaning growth rate (Table 1). For all three genotypes, an additional piglet resulted in a significant (P=0.0001) decrease in average birthweight, weight at d 21 and preweaning growth rate of 36 g, 268 g and 11.4 g/d, respectively. Including TNB in the model, reduced the effect of PRLR genotype on placental weight and placental efficiency (Table 1).

Average number of fuctional teats of the piglets was significantly affected by PRLR genotype (P<0.05). Piglets from BB gilts had on average more functional teats than piglets from AA gilts (P=0.028; Table 1, Fig. 1). Including number of functional teats of the mother, percentage males in the litter, or both did not change these results.

DISCUSSION

In the present study, three independent effects of maternal PRLR on reproductive traits of Large White x Meishan F2 crossbred gilts were found, i.e. an effect on age at first estrus, litter size, and litter mean of funtional teat number.

PRLR genotype significantly affected age at first estrus. BB gilts showed their first estrus 41 days earlier than AA gilts, and AB gilts were in between. This implies that the PRLR gene is a marker or a major gene for age at puberty in these gilts. For all three genotypes age at puberty (average 213 d) was closer to that of crossbred (217±3 d) than of Meishan (118±3 d) gilts as reported by Christenson (3). Since the gilts were inseminated at a fixed estrus number, the differences in age and bodyweight at insemination were the result of the difference in age at first estrus.

Several quantitative trait loci (QTL) have been reported for age at first estrus. None of the detected QTL however, was located on chromosome 16, the chromosome PRLR gene is located on. Rohrer et al. (11) reported two regions that possessed suggestive evidence for quantitative trait loci (QTL) affecting age at first estrus on chromosomes 1 and 10, while Cassady et al. (2) reported QTL affecting age at first estrus on chromosomes 7, 8 (P<0.05), and 12 (P<0.1).

PRLR genotype tended to affect litter size of the gilts in the present study, i.e. AA gilts had the largest litters, BB the smallest and AB were in between. Thus we hypothesize that PRLR gene is a marker or a major gene for litter size in Large White x Meishan F2 crossbred gilts, with the A allele as the favorable allele.

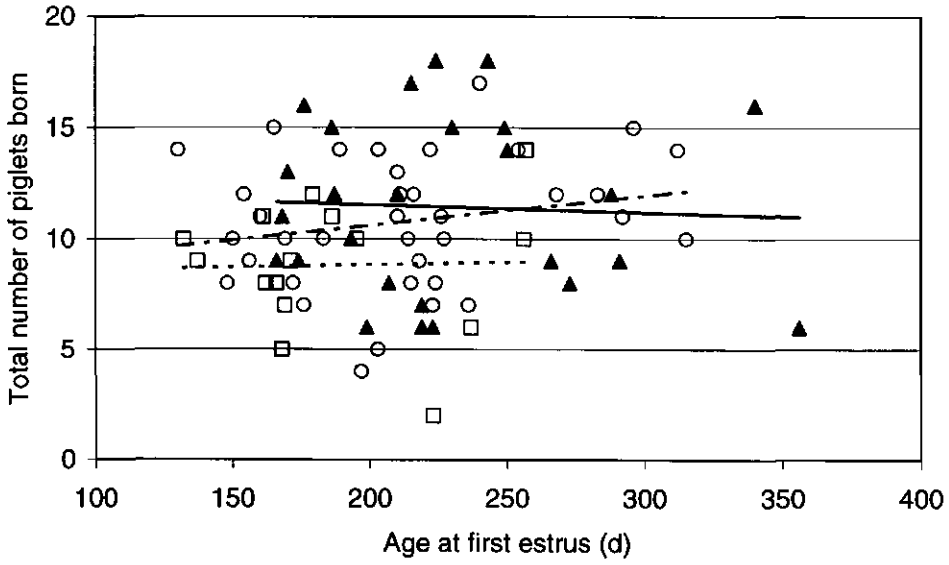


Figure 3. *Relation of TNB to age at first estrus in Large White x Meishan F2 crossbred gilts with different PRLR genotypes.*
▲ —— = *AA* ○ — = *AB* □ ····· = *BB*

Vincent et al. (16) reported comparable results in a Large White synthetic line and a Landrace synthetic line. In the Large White synthetic line, AA sows had 0.66 live born piglets more than AB and BB animals ($P<0.05$), while in the Landrace synthetic line, TNB and NBA was more than one piglet larger for AA animals compared to BB animals ($P<0.1$). TNB and NBA of a Meishan synthetic line however, was highest for AB sows ($P<0.05$; 16). Results of Vincent et al. (16) are based on litter size results of sows with different parities, while our results are based on gilts only. In L93 Meishan Synthetic gilts that were kept under the same management system as the gilts in this study, Van der Lende and Van Rens (15) reported effects of PRLR genotype on ovulation rate and number of Day 35 embryos that were comparable to the current litter size results, i.e. a difference of 2.8 corpora lutea and 2.9 embryos between AA and BB gilts in favour of AA.

The results of the present study show that the difference in age at first estrus (and as a result age and bodyweight at insemination) and the difference in litter size are two independent effects of PRLR genotype polymorphism. In other words, when gilts are inseminated at the same estrus number (i.e. physiological age), their chronological age and bodyweight do not affect litter size. Several authors have published relations between age at first estrus, age or weight at insemination and ovulation rate or litter size (3, 4,8). The results of these studies however, can not be compared with the present results, since in each study, one of the three traits age at first estrus, estrus number and age or weight at insemination was not known. In a recent publication, Grigoriadis et al. (6) concluded that gilts that were bred at the same age but either at second or third estrus had a similar litter size. Since Grigoriadis et al. (6) had induced puberty, however, their results can not be not compared with the present results either.

The difference in litter size between the PRLR genotypes was not accompanied with differences in birthweight or growth rate, although for all three genotypes the traits decreased with an increase in litter size. The difference in litter size, however was accompanied with differences in placental weight, i.e. piglets of BB gilts had on average heavier placentae compared to piglets of AA gilts. Since birthweights were not different between the genotypes, the larger placentae have had no additional advantage in terms of fetal growth. After correction for TNB, the differences in placental size disappeared.

The average number of functional teats of the piglets was significantly affected by the PRLR genotype of their mother. The number of functional teats of the mother

however, was not affected by its own PRLR genotype. Apparently, a maternal factor influences the number of functional teats of the piglets. According to Patten (9) the nippels can already be recognized in embryos of 2 cm (approximately 28 d), which implies a maternal effect of PRLR genotype in the embryonic stage of pregnancy. The biological background behind this phenomenum is not known, but is worthwhile to investigate. Several suggestive and significant QTL for teat number have been reported on different porcine chromosomes, e.g on chromosome 1 (2,10, 18), 3 (10), 6 (2), 7 (1, 2,18,), 8 (2), 10 (10), 11 (2), and also 16 (1), the chromosome the PRLR gene has been mapped to (17). All these QTL however, refer to own (maternal) teat number, and not to the teat number of the piglets.

Irrespective of PRLR genotype, functional teat number of the mothers was approximately one nipple higher than of the litter mean. This might be due to the fact that the mothers were 50% Meishan, while their piglets were only 25% Meishan. Teat number has been reported to increase as the proportion of Meishan genes in the sow increase (7).

Drickamer et al. (5) suggested that the average teat number of female piglets is related to the proportion of males in the litter on the one hand and the number of teats of the dam on the other hand. In the current study, however, average teat number of the female piglets was not affected by the proportion of males (P=0.9) or functional teat number of the dam (P=0.3). The effect of PRLR genotype on the average number of functional teats of the female piglets was comparable to its effect on the average number of functional teats of all piglets, i.e PRLR significantly affected number of functional teats of female piglets (P=0.0031; 14.17±0.11, 14.40±0.10, and 14.82±0.15 teats on female piglets of AA, AB and BB gilts, respectively). Females from BB mothers had significantly more functional teats than females from AA (P=0.002) and AB (P=0.0492) mothers.

In conclusion, PRLR gene appears to be a major gene or a marker for age at first estrus and litter size in Large White x Meishan F2 crossbred gilts. If an early puberty is preferred, the favorable allele for litter size (the A allele) is the unfavorable allele for age at first estrus. Litter size is affected independenly from age at first estrus. Furthermore, the gene is a major gene or a marker for litter means of number of functional teats, with the B allele being the favorable allele.

REFERENCES

1. Bidanel JP, Milan D, Iannuccelli N, Amigues Y, Boscher MY, Bourgeois F, Caritez JC, Gruand J, Le Roy P, Lagant H, Bonneau M, Lefaucheur L, Mourot J, Prunier A, Désautés C, Mormède P, Renard C, Vaiman M, Robic A, Gellin J, Ollivier L, Chevalet C (2000). Détection de locus à effets quantitatifs dans le croisement entre les races porcines Large White et Meishan: Résultats et perspectives. Journées Rech Porcine en France 32:369-383.
2. Cassady JP, Johnson RK, Pomp D, Rohrer GA, Van Vleck LD, Spiegel EK, and Gilson KM (2001). Identification of quantitative trait loci affecting reproduction in pigs. J Anim Sci 79:623-633.
3. Christenson RK (1993). Ovulation rate and embryonic survival in Chinese Meishan and White crossbred pigs. J Anim Sci 71:3060-3066.
4. Dourmad J-Y, Noblet J, Père MC, and Étienne M (1999). Mating, pregnancy and prenatal growth. In: A quantitative biology of the pig. Ed. Kyriazakis A. CABI Publishing, Oxon, UK. 1999;129-153.
5. Drickamer LC, Rosenthal TL, and Arthur RD (1999). Factors affecting the number of teats in pigs. J Reprod Fert 115:97-100.
6. Grigoriadis DF, Edwards SA, English PR, and Davidson F (2001). The effect of oestrous cycle number, at constant age, on gilt reproduction in a dynamic service system. Anim Sci 72:11-17.
7. Haley CS, Lee GJ and Ritchie M (1995). Comparative reproductive performance in Meishan and Large White pigs and their crosses. Anim Sci 60:259-267.
8. Mösch VW (1991). Untersuchungen zum Einfluß der Lebendmasse auf die Entwicklung der Uterus- und Ovarmassen und auf die Ovulationsrate bei Jungsauen. Mh Vet Med 46:835-838.
9. Patten BM (1948). Embryology of the pig. 3rd edition. McGraw-Hill book company inc. New York, Toronto, London.
10. Rohrer GA (2000). Identification of quantitative trait loci affecting birth characters and accumulation of backfat and weight in a Meishan-White composite resource population. J Anim Sci 78:2547-2553.
11. Rohrer GA, Ford JJ, Wise TH, Vallet JL, and Christenson RK (1999). Identification of quantitative trait loci affecting female reproductive traits in a multigeneration Meishan-White composite swine population. J Anim Sci 77:1385-1391.
12. Rothschild MF, Vincent AL, Tuggle CK, Evans G, Short TH, Southwood OI, Wales R, and Plastow GS (1988). A mutation in the prolactin receptor gene is associated with increased litter size in pigs. 26th International Conference on Animal Genetics, ISAG (International Society for Animal Genetics), August 9-14, 1998, Auckland, NZ; pp 105 abstr.
13. SAS/STAT (1990). User's guide. Cary NC, SAS Institute.
14. SAS/STAT (1996). Changes and enhancements through release 6.11. Cary NC, SAS Institute.
15. Van der Lende T, and Van Rens BTTM (2000). The effect of the prolactin receptor gene on ovulation rate and embryonic survival in gilts. 14th ICAR Abstracts 2000;1:142.
16. Vincent AL, Evans G, Short TH, Southwood OI, Plastow GS, Tuggle CK, and Rothschild MF (1998). The prolactin receptor gene is associated with increased litter size in pigs. 6th WCGALP 27:15-18.
17. Vincent AL, Wang L, Tuggle CK, Robic A, Rothschild MF (1997). Prolactin receptor maps to pig chromosome 16. Mamm. Genome 8:793-794.
18. Wada Y, Akita T, Awata T, Furukawa T, Sugai N, Inage Y, Ishii K, Ito Y, Kobayashi E, Kusumoto H, Matsumoto T, Mikawa S, Miyake M, Murase A, Shimanuki S, Sugiyama T, Uchida Y, Yanai S, Yasue H (2000). Quantitative trait loci (QTL) analysis in a Meishan x Göttingen cross population. Animal Genetics 31:376-384.

19. Wilson ME, Biensen NJ, Youngs CR, and Ford SP (1998). Development of Meishan and Yorkshire littermate conceptuses in either a Meishan or Yorkshire uterine environment to day 90 of gestation and to term. Biol Reprod 58:905-910.

Chapter 8

General discussion

CONTENTS

In the present study the first steps to a physiological explanation of the effects of two candidate genes for litter size on reproductive performance in pigs have been taken. Until now, only little research into the physiology behind such candidate genes has been published (Isler et al., 1999[a]), which makes comparison with relevant literature difficult. The major conclusions of the previous chapters will be combined and discussed in the present chapter. Furthermore, the knowledge provided by these chapters will be used to speculate on why litter size differences are more pronounced in gilts than in sows with different ESR genotypes, whether it is wise to select for candidate genes like ESR and PRLR gene, and whether the two genes are major genes or markers. To make the discussion more accessible for the reader, several aspects of the biology of porcine reproduction are briefly reviewed where necessary. Furthermore, since this thesis concerns "genomics", i.e. the study of genotypes and their physiological function, the structure and function of the two genes and their products are briefly reviewed as well. The design of the present chapter is such that the separate paragraphs can be read independently from each other.

COMPONENTS OF LITTER SIZE

A litter is the result of a chain of sequential events, beginning with ovulation. To get one liveborn piglet, the whole chain of events consisting of ovulation, fertilization, embryonic development and implantation, fetal development and parturition, has to be passed successfully. A major gene for litter size will affect at least one of the components of litter size (ovulation rate, fertilization rate, embryonic and fetal survival), resulting in differences in litter size. To identify which component (or components) of litter size was (were) affected by ESR or PRLR genotype, in the first experiment, L93 Meishan Synthetic gilts were slaughtered at Day 35 of pregnancy, and number of corpora lutea, number of implantation sites, and number of embryos were determined (Chapters 2 & 6).

ESR genotype

ESR genotype does not affect ovulation rate or embryonic survival, but appears to affect fetal survival (Chapter 2). Irrespective of whether the ESR gene is a major gene or a marker for litter size, this implies that the polymorphism is a valuable tool

to improve litter size, especially when it is used in combination with selection for ovulation rate. The only other reported procedure to select for fetal survival is to select for uterine capacity, i.e. the maximum number of fetuses that can be maintained to term when the number of potential viable embryos is not a constraint (Christenson et al., 1987, 1993). To measure uterine capacity, uterine horns have to be challenged by more Day 35 embryos than can be nurtured to parturition, which can be obtained by means of superovulation (e.g. Dziuk, 1968; Webel and Dziuk, 1974), embryo transfer (e.g. Dziuk, 1968; Pope et al., 1972) or unilateral hysterectomy-ovariectomy (UHOX, e.g. Dziuk, 1968; Knight et al., 1977; Christenson et al., 1987). Due to failure of some gilts to respond to exogenous hormone treatment and to greater labor and donor gilt requirements for embryo transfer, the UHOX procedure is preferred (Christenson et al., 1987). This invasive method, however, is still a time consuming, expensive procedure, resulting in offspring from one uterine horn per sow only. Furthermore the trait uterine capacity is expressed in females only, and can not be measured until after sexual maturity.

Marker assisted selection for fetal survival (using the ESR gene), on the other hand, can be applied on both sexes at a very early age. One has to keep in mind, however, that the selection for fetal survival by means of selection for the favorable ESR allele will result in an improvement of potential fetal survival once-only. As a matter of fact, this counts for every selection for a major gene or marker. The question whether it is advisable to select on a major gene will be discussed elsewhere in this chapter.

PRLR genotype

PRLR genotype appears to affect ovulation rate (Chapter 6). The (non significant) difference in the number of implantation sites and number of D35 embryos between L93 Meishan synthetic gilts with differerent PRLR genotypes is suggested to be a result of this effect. Uterine length and placental size at Day 35 of pregnancy confirmed this suggestion. Therefore, differences in litter size caused by the difference in ovulation rate were predicted for this breed, presumed that the PRLR genotype does not affect fetal survival (Chapter 6). Further studies on large data sets will have to be performed to verify this prediction. In another breed, however, the found differences in litter size between the three PRLR genotypes pointed in the predicted direction (Chapter 7).

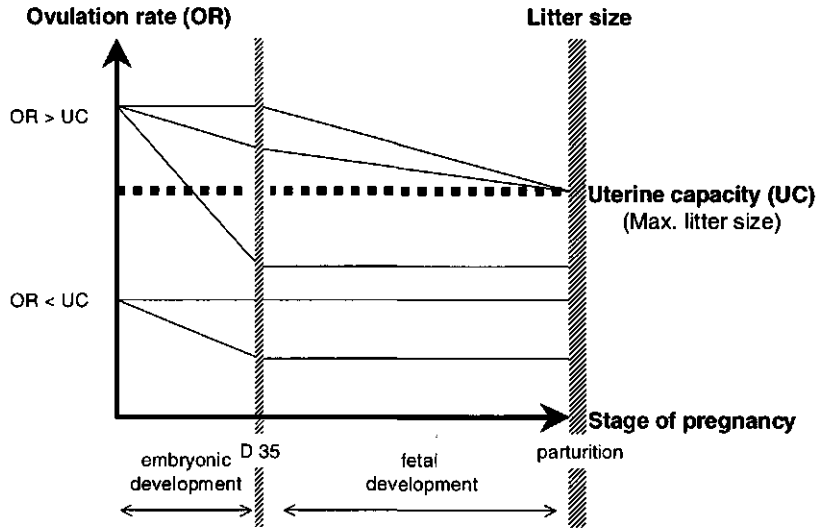


Figure 1. *Ovulation rate and uterine capacity as limiting traits for litter size: some examples of possible interactions.*
Ovulation rate sets the first limit on the potential number of piglets born. Subsequent reductions in this number may occur during fertilization, embryonic development (until Day 35) and implantation (around Days 13-16), fetal development (Day 35 until term), and parturition. Dependent on ovulation rate, uterine capacity may reduce the initial potential number of piglets born (OR > UC) or not (OR < UC).
(Design: Van der Lende)

Table 1. *Combined effects of PRLR and ESR genotype on components of litter size in L93 Meishan synthetic gilts*

	ESR AA		ESR BB	
PRLR AA	ovulation rate	+	ovulation rate	+
	fetal survival	-	fetal survival	+
PRLR BB	ovulation rate	-	ovulation rate	-
	fetal survival	-	fetal survival	+

Combination of ESR and PRLR genotypes

PRLR and ESR genotype appear to affect litter size at different links of the previously mentioned chain of events, namely ovulation (Chapter 6) and fetal survival (Chapter 2), respectively. Ovulation rate sets the first limit on the size of a litter. All other subsequent events may further reduce this number. Thus, dependent on the initial ovulation rate, a high potential fetal survival will actually affect litter size or will have no effect at all (Figure 1). This might explain the lack of effect of ESR genotype on litter size in some of the published studies (Table 4 in Chapter 4). Pigs homozygous for the favorable ESR allele for litter size (i.e. fetal survival), might have had a limiting number of Day 35 fetuses, e.g. because they were homozygous for the unfavorable PRLR allele for litter size (i.e. had a limiting ovulation rate) as well, or because they possessed an unfavorable genotype for another not yet discovered major gene for litter size which affects an event before Day 35 of pregnancy.

The fact that the two genotypes affect different components of litter size that are expressed at different stages of pregnancy, indicates that they can oppose each others effects. This implies that there are favorable PRLR/ESR combinations and unfavorable ones. The largest potential changes in litter size are expected after selection for both genes at the same time.

For the L93 Meishan synthetic gilts, the ESR B allele was the favorable allele for litter size (Southwood et al., 1995), while the PRLR A allele was predicted to be the favorable allele for litter size (Chapter 6). Thus, PRLR/ESR genotype combination AA/BB should be the ideal combination for an optimum increase in litter size in gilts belonging to this line (Table 1). Unfortunately, in the present study numbers were too small to test the effect of an interaction of ESR and PRLR genotype on the studied Day 35 components of litter size. (Theoretically, however, PRLR AA gilts are expected to have the highest numbers of ovulations and Day 35 embryos, independent on their ESR genotype).

For the Large White x Meishan F2 crossbreds studied in the present thesis, the PRLR A allele (Chapter 7), and the ESR A allele (Chapter 4) appear to be the favorable alleles for litter size. Thus PRLR/ESR genotype combination AA/AA is expected to be the best combination for an increase in litter size, while the combination BB/BB is expected to be the worst (Table 2).

Table 2. Effects of PRLR and ESR genotype on litter size of Large White x Meishan F2 crossbred gilts

		ESR			PRLR
		AA	AB	BB	
PRLR	AA	+ / +	+ / +	+ / -	+
	AB	± / +	± / +	± / -	±
	BB	- / +	- / +	- / -	-
ESR		+	+	-	

Table 3. Total numbers of piglets born in Large White x Meishan F2 crossbred gilts with different ESR and PRLR genotypes

		ESR [1]			PRLR [2]
		AA	AB	BB	
PRLR [1]	AA	11.80 ± 1.06 (10)	11.11 ± 1.11 (9)	11.29 ± 1.26 (7)	11.35 ± 0.66 [a] (26)
	AB	11.36 ± 0.89 (14)	10.27 ± 1.01 (11)	10.55 ± 1.01 (11)	10.74 ± 0.56 [ab] (36)
	BB	9.00 ± 1.93 (3)	11.00 ± 1.36 (6)	6.50 ± 1.36 (6)	8.90 ± 0.87 [b] (15)
ESR [3]		10.83 ± 0.68 (27)	10.53 ± 0.67 (26)	9.62±0.69 (24)	

[1] *Lsmean estimates and standard errors of means ((..) = number of gilts) of the interaction between ESR and PRLR genotype, using model*
$TNB_{ijk} = \mu + ESRm_i + PRLRm_j + ESRm*PRLRm + e_{ijk}$
*(ESR*PRLR, P=0.335)*

[2,3] *Lsmean estimates and standard errors of means ((..) = number of gilts), for gilts with different PRLR genotypes[2] and different ESR genotypes[3], using model*
$TNB_{ijk} = \mu + ESRm_i + PRLRm_j + e_{ijk}$ *(ESR, P=0.426; PRLR, P=0.086)*

[a,b] *Different superscripts in the same column differ (P=0.07)*

For 77 gilts with litter size data, both PRLR and ESR genotype were known. For this population the effect of the interaction of PRLR and ESR genotype on total number of piglets born was tested, using a model without any covariables (Table 3). Although the interaction of PRLR and ESR genotype did not significantly affect TNB (P=0.33), gilts with PRLR/ESR genotype combination AA/AA indeed had the largest litters, while gilts with PRLR/ESR genotype combination BB/BB had the smallest litters (Table 3). Grouping the PRLR/ESR genotype combinations according to expected litters, i.e. "Large" (AA/AA, AA/AB, AB/AA, and AB/AB), "Medium" (BB/AA, BB/AB, AA/BB and AB/BB), and "Small" (BB/BB), resulted in lsmeans of 11.14±0.49, 10.67±0.63, and 6.50±1.33 for TNB of Large, Medium, and Small, respectively. TNB of Small differed significantly from Large (P=0.005) and Medium (P=0.016).

MATERNAL GENOTYPE

Besides the identification of components of litter size that are affected by ESR and PRLR gene, the purpose of the two experiments was to obtain as much information as possible about underlying reproductive physiology. In the following paragraphs the main results and conclusions will be summarized. Most of the issues already have been discussed in the separate chapters of the present thesis.

ESR genotype

Estrogen receptor genotype did not affect estrus length or estrus cycle length of the L93 Meishan Synthetic gilts studied in the present thesis. Furthermore, no differences in periovulatory plasma LH, estrogen or progesterone profiles between AA and BB gilts were detected, and the temporal aspects of these profiles were not different for both genotypes.

Numbers of corpora lutea, implantation sites and vital embryos at Day 35 of pregnancy were not affected by ESR genotype. The vital embryos from AA and BB gilts seemed to be at the same developmental stage, since their length and weight did not differ. From these results it was concluded that the difference in litter size between AA and BB gilts, as shown by Southwood et al. (1995), probably was due to a difference in uterine capacity between the two genotypes (see Chapter 2).

Mechanisms that might lead to a difference in uterine capacity are differences in placental efficiency and differences in the use of available uterine space (Chapter 2). Placentae of Day 35 embryos from AA gilts were significantly shorter than placentae of Day 35 embryos from BB gilts. Being shorter, the placentae of AA gilts might be less efficient in supporting further development of all embryos, thus leading to the expected higher fetal mortality. As discussed in Chapter 2, the difference in placental length might have been related to a difference in the timing of embryonic mortality.

Uterine size (length and weight) was similar for AA and BB gilts. Therefore the potentially available uterine space for the vital embryos was the same for AA and BB gilts. Due to the suggested difference in timing of embryonic mortality (in BB gilts probably more embryos died before implantation, while in AA gilts more

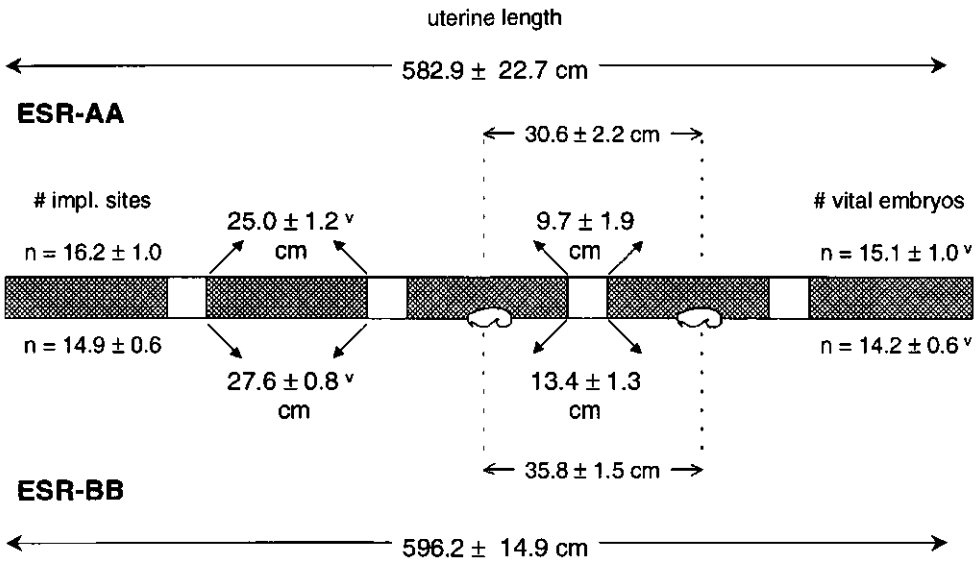


Figure 2. Measurements on D35 uteri of L93 Meishan Synthetic gilts differing in ESR genotype (Results from Chapter 2). v = For vital embryos only.
Uterine length was the same for both genotypes (P=0.63), interval between adjacent implantation sites was the same for both genotypes (P=0.12), but length of implantation sites tended to be longer for vital embryos of BB gilts (P=0.08), and the distance between adjacent embryos tended to be longer for BB gilts (P=0.05). Since percentage of implantation sites but not percentage of vital embryos tended to be higher for AA gilts, a difference in timing of embryonic mortality was suggested.

embryos died after implantation), however, vital embryos of AA gilts probably had less space available than vital embryos of BB gilts. All measurements on uterine traits confirm this suggestion (summarized in Figure 2).

Lamberson and Eckardt (1996) showed that surviving fetuses do not make use of space vacated by nonsurviving littermates after Day 28 of pregnancy. This implies that all the non-occupied Day 35 implantation sites, observed in the L93 Meishan synthetics, will not be available for the developing fetuses.

In the F2 crossbreds studied in the present thesis, ESR genotype significantly affected litter size, i.e. AB gilts had larger litters than BB gilts, while AA gilts appeared to have similar litter sizes as AB gilts (Chapter 4). The average birthweight of the piglets and average placental length, surface area and weight at term were not affected by ESR genotype (Chapter 4). This might imply that the growth retarded fetuses with the smaller placentae (found at Day 35 of pregnancy in the L93 Meishan Synthetics) have been sifted out during fetal development, leaving a population of viable piglets at term with on average equal placental sizes and birthweights for the different ESR genotypes. One has to be careful in comparing the results of the two experiments however, since the ESR gene is probably a marker rather than the major gene itself (discussed in Chapter 4, and below). This means that the A allele in the L93 Meishan synthetics not per se has to be coupled to the same allele of the actual major gene for litter size, as the B allele in the F2 crossbreds. Other unknown alleles might be involved.

An intriguing effect of ESR genotype, detected in the F2 crossbreds, was the effect on the areola density on placentae at term (Chapter 4). The number of areolae represents the number of uterine glands to which the placenta had contact (Abromavich, 1926, Brambel, 1933, Chen et al., 1975, Friess et al., 1981, Dantzer 1984, Dantzer and Leiser, 1993). Despite the fact that AA and AB gilts had comparable litter sizes, and also comparable placental sizes for their piglets, the areola density differed. This might be related to a difference in density of endometrial glands, a difference in length of implantation sites, and/or a difference in uterine length, uterine folding, possibly combined with a difference in size of unoccupied parts of the uterus (Chapter 4). Therefore further research on uterine length, distribution and size of implantation sites, and number and distribution of endometrial glands in relation to the different ESR genotypes are recommended (Chapter 4).

Average placental efficiencies, as calculated by dividing birthweight by placental length, weight or surface area were not affected by ESR genotype. As a result of the differences in number of areolae, birthweight divided by number of areolae was affected (Chapter 4).

A small series of reports on the physiology behind the ESR gene polymorphism has been published by another research group (Isler et al., 1998, 1999[a-e]). The reports were based on one study of a population of Yorkshire, Large White, and crossbred (Yorkshire x Large White and Large White x Yorkshire) females with varying parities. Litter data of 212 dams and reproductive traits of 146 pregnant females at approximately Day 75 of pregnancy, were studied in relation to their ESR genotype. ESR genotype did not affect TNB or NBA ($P>0.05$) of the studied population (Isler, personal communication). Furthermore, number of stillborn piglets, number of piglets alive at weaning, and total litter weights at birth (all piglets or liveborn piglets) and at weaning were not affected (Isler et al., 1999[a]) by ESR genotype. No effects of ESR genotype on reproductive traits at Day 75 of pregnancy (i.e. uterine length, total fetal weight per uterus, average fetal weight, and number of mummies) were detected in the mentioned population ($P>0.2$, Isler et al., 1999[a]). The reported significant ESR effect on the number of Day 75 fetuses per horn ($P=0.04$; Isler et al., 1999[a]) is difficult to interpret, since it was accompanied with a significant ESR genotype x breed interaction ($P=0.02$), and the two horns of the sows had been used as separate units in the statistical analysis (Isler et al., 1999[a]). Furthermore, when the data were analyzed for total number of fetuses, ESR was not significant (Isler, personal communication). Since the results of Isler et al. (1999[a-e]) were based on different breeds and parities, and a different stage of pregnancy, they are not comparable to the results of the present thesis.

PRLR genotype

For the L93 Meishan synthetic gilts studied (Chapter 6), PRLR genotype did not affect estrus length, but tended to affect estrus cycle length, i.e. AA gilts tended to have longer estrus cycles compared to AB and BB gilts. Periovulatory LH, progesterone and estradiol profiles were hardly affected by PRLR genotype. Ovulation rate was significantly affected by PRLR genotype, and, though not significant, number of implantation sites, total number of embryos and vital embryos at Day 35 of pregnancy showed a similar trend, i.e. AA gilts had the highest numbers, BB gilts the lowest, and AB gilts were intermediate. Since the

difference still ran up to a difference of three vital embryos, the prediction that the AA gilts would get larger litters than the BB gilts was postulated under the assumption that the animals have comparable uterine capacities (Chapter 6). Uterine length and weight on Day 35 of pregnancy probably had adapted to the difference in number of embryos, since they were higher for AA gilts compared to AB and BB gilts. After correction for number of implantation sites, AA gilts still tended to have longer empty uteri compared to BB gilts, indicating that the embryos of AA gilts had potentially more space available. The extra uterine space in AA gilts actually seemed to be utilized by the embryos for implantation, since implantation surface areas and placentae were larger for embryos from AA gilts compared to embryos from AB and BB gilts. The AB gilts appeared to be unable to adapt uterine length to litter size, which might lead to capacity problems later in pregnancy. The weight of the ovaries was significantly affected by PRLR genotype as well. The difference in weight (AA and AB gilts tended to have heavier ovaries than BB gilts) was not a direct result of the difference in number of corpora lutea (AA gilts had heavier ovaries after subtraction of weight of corpora lutea than BB gilts), but probably due to a difference in connective tissue and blood supply, or a difference in number or size of follicles present. As discussed in Chapter 6, the high ovulation rate of the AA gilts might have been accompanied by a larger antral follicle pool than in AB and BB gilts, which in turn might lead to a higher ovulation rate in the next estrus.

In the F2 crossbreds studied in the present thesis (Chapter 7), PRLR genotype significantly affected age at first estrus, i.e. BB gilts showed their first estrus 41 days earlier than AA gilts, and AB gilts were in between. Furthermore PRLR genotype tended to affect litter size, i.e. AA gilts had the largest litters, BB gilts had the smallest litters and AB gilts were in between. The difference in litter size ran up to a difference of 2.45 liveborn piglets between AA and BB gilts, and therefore PRLR gene was hypothesized to be a major gene or a marker for litter size, with the A allele as favorable allele (Chapter 7). The effect of PRLR genotype on age at first estrus and the difference in litter size were two independent effects of PRLR polymorphism. The effects of PRLR genotype on the average placental weight and placental efficiency, were a result of the differences in litter size. Piglets of BB gilts, i.e. of the smaller litters, had heavier placentae. The difference, however, disappeared after correction for TNB. A remarkable effect of PRLR gene polymorphism was the effect on the average number of functional teats of the piglets. Although PRLR genotype did not affect the number of functional teats of the gilt itself, the genotype did affect litter means of number of functional teats of

her piglets, i.e. piglets of BB gilts had on average more functional teats than piglets of AA gilts.

THE GENOTYPE OF THE CONCEPTUS ^{ESR}

All published research concerning ESR genotype in pigs in relation to litter size has been restricted to the maternal ESR genotype. In the present thesis the ESR genotype of the conceptus (i.e. Day 35 fetus or liveborn piglet) also has been taken into consideration (Chapter 3, Chapter 5). The purpose was twofold: The first purpose was to examine whether ESR genotype of the conceptus nested within maternal ESR genotype would affect fetal, piglet, and placental traits. The second purpose was to evaluate relations of fetal bodyweight and heart weight to various placental traits and relations of piglet birthweight and number of areolae to various placental traits, all relative to ESR genotype.

Interactions

Fetal ESR genotype nested within maternal ESR genotype did not affect fetal weight, fetal length, placental length, placental weight, or implantation surface area at Day 35 of pregnancy in the L93 Meishan synthetic gilts (Chapter 3). This implies that the difference in average placental length between embryos from AA and BB gilts found in the same experiment (Chapter 2) is entirely due to the genotype of the gilt.

At term, piglet ESR genotype nested within maternal ESR genotype neither affected birthweight, placental length, weight or surface area, nor number of areolae per placenta in the Large White x Meishan F2 crossbreds (Chapter 5). This implies that the difference in average number of areolae per placenta between AB and BB gilts found in the same experiment (Chapter 4) is entirely due to the ESR genotype of the mother.

Traits that actually were affected by an interaction between maternal and conceptus genotype were D35 fetal heart weight, amnion weight at term, and placental efficiency calculated as piglet birthweight divided by placental weight at term. Hearts of Day 35 AB fetuses from AA mothers were significantly heavier than

those of fetuses from BB mothers, and tended to be heavier than those of their AA littermates. The reason for this hypertrophy is unclear, but might be related to a difference in placental vascularity (Chapter 3). At term, amnions of AA piglets from AA mothers were heavier than amnions from three other ESR genotype combinations, suggesting that the amions were larger or thicker, which might be associated with a better protection to mechanical injury and with a better materno-fetal barrier, respectively (Chapter 5).

Relations

At Day 35 of pregnancy, several significant effects of fetal ESR genotype nested within maternal ESR genotype were found in L93 Meishan synthetic gilts (Chapter 3). Except for the relation of heart weight to fetal weight, the differences in relationships between different ESR genotype combinations appeared to be mainly due to outlyers (Figures 1 to 3 in Chapter 3). Heart weight of fetuses from BB gilts, however, increased with fetal weight, while heart weights of fetuses from AA gilts did not.

Day 35 fetal weight was related similarly to placental length, placental weight, and implantation surface area: up to a certain threshold value, an increase in the trait was associated with an increase of fetal weight. Thereafter, fetal weight did not change anymore. Thus, at Day 35 of pregnancy porcine fetuses seem to have a maximum growth potential. The percentage of AA fetuses from AA mothers that had not reached this maximum growth potential was larger than for the other three genotype combinations studied, and therefore a higher subsequent fetal mortality was expected in this group (Chapter 3). Currently, PIC International Group is examining several porcine breeds for the existence of ESR genotype ratio distortion. Highly significant effects already have been found for segregation distortion at ESR in at least two lines (Bastiaansen, personal communication).

At term, piglet ESR genotype nested within maternal ESR genotype significantly affected two of the relations studied in the Large White x Meishan F2 crossbred gilts, i.e. the relation of birthweight to placental weight and the relation of birthweight to number of areolae (Chapter 5).

The reason that all published research concerning ESR genotype in pigs in relation to litter size has been restricted to the maternal ESR genotype, is the fact that the

only significant genetic contribution to the advantage in the number born alive in Meishans (the breed in which the B allele was initially discovered, Rothschild et al., 1991) was maternal (i.e. due to the dam of the litter), with no evidence of a contribution of the genes of the litter itself (Haley et al., 1995). Results of the present thesis, however, show that there actually are traits and relations between traits that are dependent on both maternal and fetal ESR genotype, which might lead to a difference in litter size. Therefore, the ESR genotype of the boar might be important for litter size as well.

GILT VERSUS SOW ^{ESR}

Since litter size differences, if found, between ESR AA and BB were more pronounced in gilts than in sows (Table 4 in Chapter 4), both experiments of the present thesis were performed with gilts. One might wonder, however, what would have happened when the experiments had been performed with sows. In other words: why are the litter size differences less pronounced in higher parities?

ESR genotype appears to affect fetal survival, and placental size and uterine capacity appear to play an important role in these expected differences in fetal survival (Chapter 2-4).

In general, multiparous sows carry more embryos to term than gilts (e.g. Legault 1985; Dourmad et al., 1999; Tummaruk et al., 2000), which partly might be due to an increase in ovulation rate (referred by Legault, 1985; Christenson, 1993). However, also a change in uterine capacity (as a result of the developmental history of the uterus) has been suggested to be a causative factor for the increase in litter size with increasing parity, especially for the difference between parity 1 and >1 (Bartol et al., 1993; Gama and Johnson, 1993). Thus, Legault (1983) suggested that uterine capacity may be a more limiting factor in first than in later parities. Culberson et al. (1997) found a large effect of age of the sow at breeding within parity 1 and parity 2 on number of piglets born alive, i.e. younger sows differed from older sows within parity 1 and 2.

No actual physiological explanations were given for the suggested change in uterine capacity with increasing parity (Bartol et al., 1993; Gama and Johnson,

1993). The changes in, and development of the uterus, however, might very well play an important role: The uterus of a pregnant pig is changing continuously to accommodate the conceptuses. At a macroscopical level, the uterus increases in weight, length and circumference. The growth in weight is due to hypertrophy of the myometrium and endometrium, while the growth in length and circumference is due to stretching of the uterine wall (Pomeroy, 1960). Besides these macroscopical changes, considerable microscopical changes are observed in the porcine uterus throughout pregnancy. Examples of these microscopical changes are the temporal local proliferations of the uterine epithelium at the mesometrial side (probably to anchor the conceptus to the uterus) just before implantation (Dantzer 1985; Van Rens, 1988; Van Rens and Stroband, 1989; Stroband and Van der Lende, 1990), appearance of primary and secondary ridges on the macroscopic endometrial folds (Dantzer, 1984), the change of orientation and height of these folds and ridges (Dantzer, 1984; Dantzer et al., 1988; Leiser and Dantzer, 1988), the change of size and position of blood vessels and capillary network (Dantzer et al., 1988; Leiser and Dantzer, 1988), the continuous gradual transition of the glands from a resting condition to their fullest activity in late pregnancy (Perry and Crombie, 1982), the appearance of interlocking microvilli (Perry, 1981; Dantzer 1985), and the local differentiation of uterine epithelial cells (on the top and bottom of the endometrial ridges; Goldstein, 1926; Wislocki and Dempsey, 1946; Leiser and Dantzer 1988; Friess et al., 1980).

After parturition, uterine length and weight decrease again until 21 to 28 days after farrowing (Palmer et al., 1965[a], 1965[b]; Graves et al., 1967; Smidt et al., 1969; Svajgr et al., 1974; Kirakofe, 1980). The highest weight loss appears to occur during the first five days after farrowing, as a result of all tissue components involuting at a high rate (Graves et al., 1967). The uterine epithelium, which is degenerated during the first days post partum, starts regeneration 7 days post partum and appears to be complete (low columnar or pseudostratified in appearance) at the 21st day (Palmer et al., 1965[b]). Involution after Day 6 is primarily at the expense of the myometrium (Palmer et al., 1965[b]; Graves et al., 1967; Svajgr et al., 1974), and is a result of a decline in number of muscle cells, as well as a decrease in size of muscle fibers and a reduction in amount of interdispersed connective tissue (Palmer et al., 1965[b]; Graves et al., 1967).

None of the publications concerning uterine involution contain data of the initial uterine status before pregnancy. It is very unlikely however, that all tissues will return to their original state after farrowing. Of 119 Large White x Meishan F2

crossbred gilts that have farrowed, all uteri had curled blood vessels after weaning (personal observation), which implies that at least the blood vessels have grown permanently during the first pregnancy, resulting in a more developed uterus. At a next pregnancy, certain uterine traits thus will already be present to accommodate the piglets, and fewer investments might have to be made for the subsequent litters.

Uteri of gilts with the favorable ESR genotype (for litter size) may be more mature than uteri of gilts with the unfavorable allele, in terms of capacity to sufficiently accommodate the fetuses during first pregnancy. Subsequent uterine development during first pregnancy might (partly) abolish this difference in uterine capacity, and as a result, the difference in litter size.

TO SELECT OR NOT TO SELECT

Both ESR and PRLR gene have been suggested to be a candidate gene for litter size because of the important role their products play in reproductive processes. Both ligands, and thus both receptors, however, play important roles in many other different processes as well. Prolactin for example, has been reported to have over 300 separate biological activities (Bole-Feysot et al., 1998). Aside from its actions on reproductive processes, prolactin plays a role in maintaining the constancy of the internal environment by regulation of the immune system, osmotic balance, and angiogenesis (Bole-Feysot et al., 1998, Freeman, et al., 2000). Estrogens regulate the growth, differentiation, and functioning of diverse target tissues, both within and outside of the reproductive system (Katzenellenbogen, 1996).

The present thesis deals with eventual associations of both polymorphisms with aspects of reproductive physiology only, with the emphasis on periovulatory hormone profiles, Day 35 components of litter size, placental traits at Day 35 and at term, gestation length, birthweight and growth of piglets until weaning, and number of functional teats.

Until now, all published data concerning the effect of ESR locus on reproduction and production traits are restricted to litter size (Table 4 in Chapter 4), gestation length and birthweight of the piglets (Korwin-Kossakowska et al., 1999), average

daily gain over a certain test period, teat number and ultrasonic back fat (Rothschild et al., 1994, 1995, 1996, Short et al., 1997). Published data concerning effects of PRLR polymorphism also are restricted to reproduction associated traits like litter size and birthweight of the piglets (Rothschild et al., 1998, Vincent et al., 1998).

Selection for the favorable allele for litter size implies that the unfavorable allele will be excluded from the population. These unfavorable alleles, however, might very well play an important and even major role in other (non-reproductive) processes. Two phenomena described in the present thesis confirm this statement. In the Large White x F2 crossbred gilts studied, the ESR A allele appears to be the favorable allele for litter size, while the B allele appears to be the favorable allele for growth until weaning (Chapter 5). For the same crossbred gilts studied, the PRLR A allele appears to be the favorable allele for litter size, while the B allele of the mother appears to be the favorable allele for number of functional teats of the piglets (Chapter 7). Both growth and teat number are important culling criteria after traditional selection for litter size. Piglets that are selected because of their potency to give large litters, may be culled if they have a teat number or growth which lies below a certain threshold value. Phenomena like this might explain why the unfavorable alleles for litter size are still present in lines that have undergone selection on prolificacy. The French INRA hyperprolific line, for instance, has been selected on prolificacy, but has an ESR B allele frequency of 0.52 (Legault et al., 1996).

Thus, if breeders want to select for litter size without losing the animals robustness, it is questionable whether they should select directly for genes that have such a broad biological function. The presence of different alleles, especially in already long existing selection lines might indicate the importance of these alleles. Specific experiments designed to compare different genotypes in a constant, controlled, environment (i.e. experiments like the ones of the present thesis) are needed to give more insight in this difficult matter.

An alternative option to select for the advantage of the favorable allele is to include the genotype as a factor in the selection index. Thus, effects as high uterine capacity, high ovulation rate, early age of puberty, will not be lost.

MAJOR GENE OR MARKER?

Both ESR and PRLR gene have been suggested to be a candidate gene for litter size because of the important role their products play in reproductive processes. The fact that for both genes an association of their polymorphism with litter size has been described in various genetic lines tested, does not incontestably imply that the genes are actually major genes for litter size. The possibility of the polymorphism being a marker for another mutation in the same gene or a mutation in a closely linked gene affecting litter size can not be excluded.

There are different approaches to search for an answer to the question whether the polymorphism found is the actual major gene for litter size or a marker. One approach is to look at the *site of the mutation*, in combination with the *structure and mechanism of action of the gene product*. Another approach is to compare *the biological role of the gene product*, with eventual differences in physiology found between animals with different genotypes (this thesis). A third approach is the association with *litter size in various porcine breeds.*

None of the three mentioned approaches can actually give a decisive answer to the question, in other words, the possibility of the polymorphism being a marker rather than a major gene probably can not be excluded at present. Combining the results of the different approaches, however, will give more insight, and thus can give a better indication about the probability that the polymorphism actually is the major gene.

In the following paragraphs the three mentioned approaches will be discussed and combined for the ESR gene and PRLR gene separately. Especially for the first two approaches, relevant biological information will be reviewed. Both ESR gene and PRLR gene encode for hormone receptors that will be activated by a ligand. Therefore there are different levels on which the biological role of the gene product can be studied. The presence of the ligand and the receptor, and their (temporal) changes in concentrations and activities in certain organs, might give more insight in the biological role of the receptor. Furthermore, much about the biological role of the receptors has been confirmed and learned by studying animals in which the gene has been disrupted (knocked-out) by the use of gene targeting technology. Results of physiological studies on ligand, receptor, eventual target organs, and the animal as a whole, will be compared with results found in the present study.

ESR gene

Site of the mutation, and structure and functioning of the gene product [ESR]

The ESR gene examined in the present thesis, is located on the p region of porcine chromosome 1 (Rothschild et al, 1996). Its polymorphism was identified using a cDNA insert of a human ESR gene probe (Rothschild et al., 1991). This human ESR gene, located on chromosome 6 (Green et al., 1986), encodes for human ESRα, and not for the later discovered human ESRβ, whose gene is located on chromosome 14 (Enmark et al., 1997). ESRα and ESRβ are reported to form heterodimers though (Pettersson and Gustafsson 2001), indicating an interaction between the functioning of the two different receptors.

The estrogen receptor is a nuclear receptor which functions as a ligand activated transcription factor (e.g. Katzenellenbogen, 1996; Parker, 1995). After binding of the ligand estrogen, the ligand-receptor complex will bind to DNA hormone response elements, thereby modulating the expression of target genes, either by repressing or by enhancing transcription (Figure 3).

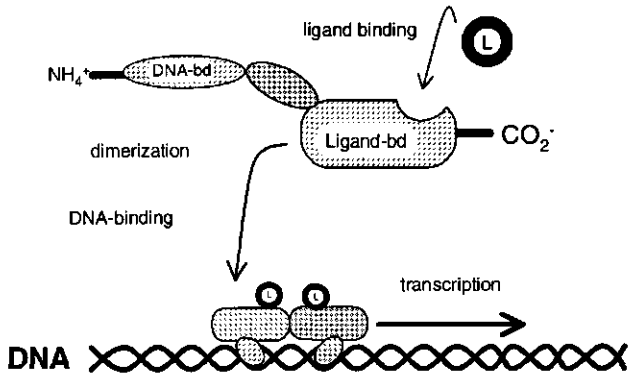


Figure 3. Schematic representation of the functioning of ESR (for explanation, see text).

Prior to estrogen binding, ESR forms an inactive oligomeric complex with other proteins (including heat shock protein 90, hsp90) which maintain the receptor in its inactivated state. It is assumed that hsp90 plays a role in the folding of the receptor

Biological role of the gene product ^{ESR}

The two reasons why a gene is proclaimed a candidate gene for litter size are the existence of a polymorphism and the important role its product plays in reproduction. In the quest for an answer to the question "is ESR a major gene or a marker for litter size?", comparing the biolocal role of the gene product and its ligand as described in literature with physiological differences found in gilts with different ESR genotypes therefore seems to be a logical action.

The name "estrogen receptor" implies an inseparable functional connection of the receptor with its ligand estrogen and vice versa. For both estrogen and ESR, however, independent actions have been reported. Nongenomic actions of estrogen in reproductive tissues have been described (granulosa cells, endometrial cells, oocytes and spermatozoa), of which most involved Ca^{2+} as a second messenger (reviewed by Revelli et al. 1998). On the other hand, estrogen independent regulation of ESR by pathway "cross-talk" from membrane receptors for growth factors like epidermal growth factor (EGF; Curtis et al, 1996), transforming growth factor α (TGFα), and insulin-like growth factor-1 (IGF-1) have been described (reviewed by Smith, 1998). Growth factors are able to activate ESR independently from estrogen, but are also able to synergize with estrogen. Furthermore, estrogens are able to increase the expression of some growth factors and their receptors (Smith, 1998).

The main differences in reproductive biology found in gilts with different ESR genotypes are described in the first paragraphs of this chapter. In summary they are related to placental size, uterine length, distribution and size of implantation sites, endometrial folding, and number and distribution of uterine glands. The differences in placental size, may be related to a difference in the timing of embryonic mortality (Chapter 2). Besides the mentioned differences in reproductive biology, a difference in Day 35 fetal heart size has been detected (Chapter 2).

Hormone profiles.

Despite the fact that estrogen is not always related to its receptor and vice versa, the changes of estrogen concentration during pregnancy will be reviewed briefly:
The changes of estrogen concentration in maternal plasma, uterine flushings, and allantoic and amniotic fluids during pregnancy of pigs have been reviewed by Knight (1994). The concentration of estrone sulphate in the blood plasma of a

pregnant sow indicates a triphasic production, with peaks on Day 10 to12 (Stoner et al., 1981), Day 16 to 30 (Robertson and King, 1974; Knight et al., 1977; Stoner et al., 1981), and Day 60 to term (Robertson and King, 1974; Knight et al., 1977). Estrone and estradiol 17β first appear in the plasma around Day 70 to 80, rise and then reach a peak just before parturition (Robertson and King, 1974). The changes of estrogen and estronsulphate in maternal plasma of a pregnant pig appear to be mainly due to the changes of estrogen production by the conceptus (Perry et al., 1973; Robertson and King 1974; Perry et al.,1976; Knight et al., 1977; Knight 1994). Conceptus estrogen production, measured in vitro as well as in uterine flushings is also multiphasic (Knight 1994). The first peak estrogen levels (conjugated and free) measured in uterine flushings are associated with the period of conceptus elongation. Production increases during conceptus elongation on Day 11-12 (Perry et al., 1973, 1976; Geisert et al., 1982[a], 1990), declines on Day 13 and 14, followed by a second increase after Day 14 (Geisert et al., 1982[a], 1990). In vitro placenta release of estrone show a peak between Day 14-18 and around Day 30 and an increase from Day 70 until parturition (Knight 1994). A similar biphasic pattern of change after Day 20 is reported for estrogen concentrations in the allantoic and amniotic fluid (Knight et al., 1977). Compared to the relatively low plasma concentrations of estradiol and estrone, allantois and amnion contain extremely high concentrations. In both fluids, estrone and estradiol concentrations are highly correlated, and estrone is the predominant estrogen (Knight et al., 1977). Following parturition, the plasma estrogen concentrations decline rapidly (Robertson and King, 1974). An example of the published changes in estrone concentration in the different tissues is shown in Figure 5.

Both placenta and endometrium are capable of changing the form (conjugated or free) and ratios of estrogens produced to mediate intrauterine events without adversely affecting systemic events (Knight 1994).

Distribution of the receptor.

If the ESR gene is directly involved in the differences found in the present thesis, estrogen receptors should be present in the mentioned tissues, i.e. endometrium, placenta and fetal heart.

Published research concerning the distribution of estrogen receptors in the adult porcine endometrium is restricted to the pre-implantation period (Pack, et al., 1979; Deaver and Guthrie, 1980; Rexroad and Guthrie, 1984; Geisert et al., 1993; Van

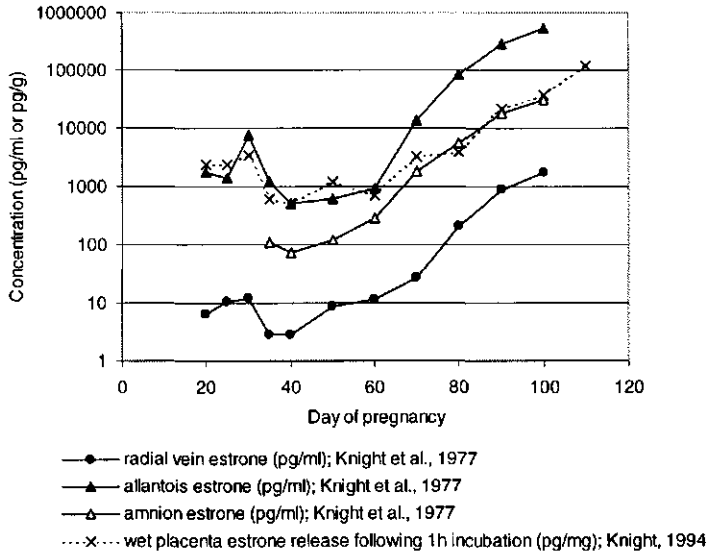


Figure 5. *Intrauterine and systemic changes in estrone concentration during pregnancy of the pig (Knight et al., 1977; Knight 1994).*

der Meulen et al., 1994), the period in which estrogen secretion by the conceptus is elevated for the first time (Perry et al., 1973; Geisert et al., 1982[a], 1990; Knight 1994). This elevation of estrogen secretion seems to be related to many phenomena (Roberts et al., 1993) like the maternal recognition of pregnancy, i.e. prolonging of the functional lifespan of the corpora lutea (Geisert et al., 1990), myometrial activity and migration of the expanding blastocyst (Pope et al., 1982, 1986), increase in uterine blood flow (Ford and Christenson, 1979; Ford et al., 1982), and endometrial secretory response (Geisert et al., 1982[b], 1982[c], 1990; Stroband and Van der Lende, 1990). The results of the reported distribution of endometrial ESR around implantation are not consistent, which might at least partly be due to a difference in methods used. Cell sampling methods, estrus detection, type of ESR measured (nuclear versus cytoplasmic), and ESR detection levels were not always comparable (Pack, et al., 1979; Deaver and Guthrie, 1980; Rexroad and Guthrie, 1984; Geisert et al., 1993; Van der Meulen et al., 1994). The developmental stage of the blastocyst significantly affected the endometrial cytoplasmic ESR concentration at Day 12 of pregnancy (Van der Meulen et al., 1994).

For the L93 Meishan synthetic gilts studied, a difference in timing of embryonic mortality between AA and BB gilts was suggested (Chapter 2). Whether this difference is associated to a difference in distribution of endometrial ESR in the preimplantation period can not be deduced from the published inconsistent results. Furthermore, the suggestion was based upon a non-significant tendency, which has to be studied in more detail.

Uterine capacity.

Differences in the capacity of the adult uterus to accommodate the conceptuses sufficiently, might already be the result of differences in neonatal uterine development. A brief description of neonatal uterine development in the pig is necessary for a discussion of the biological role of ESR in the neonatal uterine development: During the first 70 days of life, porcine uterine weight and length increase slowly but linearly and are related to age and bodyweight (Dyck and Swierstra, 1983). During this period, the uterine wall undergoes dramatic remodelling events, including appearance and proliferation of endometrial glands, formation of endometrial folds, and growth and development of the myometrium (Bal and Getty, 1970; Spencer et al., 1993[a]). Hereafter, uterine weight as well as growth rate increase abruptly and continue to increase until near the time of puberty (Dyck and Swierstra, 1983; Evans et al., 1988; Wu and Dziuk, 1988; Bartol et al., 1993; Tarleton et al., 1998).

Endometrial and myometrial thickness start to increase between Days 14 and 49 after birth (Spencer et al., 1993[b]). Endometrial thickness continues to increase until at least Day 120 (Tarleton et al., 1998).

The endometrial glands, which are absent at birth, grow from the luminal surface into the endometrial stroma (Spencer et al., 1993[a]). They are present in the shallow stroma (stratum compactum) and have begun to coil on Days 7 and 14, and extend to the myometrium from Day 28 through Day 56 (Spencer et al., 1993[a], 1993[b]; Tarleton et al., 1998). Their postnatal growth is completed between 1 and 3 months (Bal and Getty, 1970). Endometrial folds have developed then, and the glands have proliferated such that deep stratum spongiosum has become intensely glandular (Tartleton et al., 1998). The appearance of the uterine glands is associated with an increase in glandular epithelial DNA synthesis which is maximal on Days 7 and 14 and declines thereafter (Spencer et al., 1993[a]). Furthermore, adenogenesis is associated temporally with development of ESR positive (ESR

protein as well as mRNA) endometrial glandular epithelium and stroma (Tarleton et al., 1998). At birth the porcine uterus is ESR negative.

ESR is required for normal uterine wall development and in particular for proliferation of endometrial glands (Tarleton et al., 1998). Antiuterotropic effects of the antiestrogen ICI 182,780 (ICI; inhibition of adenogenesis and endometrial thickness, and increase of stromal compaction) suggest that normal postnatal maturation of the uterine wall requires both development and activation of an endometrial ESR system in the pig (Tarleton et al., 1998, 1999). This does not imply that the critical uterine organizational events are estrogen dependent, since ovariectomy (OVX) at birth did not affect uterine weight or endometrial thickness until after Day 60 (Wu and Dziuk, 1988; Tarleton et al., 1998). Furthermore OVX did not affect adenogenesis or ESR expression patterns between Day 0 and 120 (Bartol et al., 1993; Tarleton et al., 1998). Still, the mentioned events were sensitive for estradiol, since treatment with estradiol 17β valerate (EV) increased uterine wet weight, endometrial thickness, reflecting stromal hypertrophy and disorganization and edema, and myometrial thickness. Furthermore patterns of endometrial gland and fold development were altered, and fewer endometrial gland openings were observed, at least at Day 14 and 49 (Spencer et al., 1993[b]). Between birth and Day 6, plasma estradiol-17β declines to a nearly undetectable level, remaining low until near the time of puberty (Elsaesser et al., 1982). Early postnatal development of the uterine wall thus seems to occur in a steroid independent, but ESR dependent manner (Tarleton et al., 1998, 1999). Factors of local origin might be essential for the support of uterine wall development, or withdrawal from inhibiting prenatal endocrine conditions during birth could initiate the events (Spencer et al., 1993[a]). Furthermore, the presence of a functional ESR system may enhance the ability of the target cells to respond to uterotrophic growth factors.

Summarized, endometrial maturation and adenogenesis in the neonatal pig require expression and activation of a functional ESR system. Differences in this system, caused e.g. by differences in ESR genotype, might therefore lead to differences in potency of the adult uterus to respond competently to conceptuses. The difference in areola density found between the different ESR genotypes at term (Chapter 4) might be a result of such a difference in neonatal adenogenesis due to a difference in ESR genotype. The detected difference in areola distribution however was not related to a difference in litter size, since ESR AA and AB gilts with comparable litter sizes, differed in areola density (Chapter 4).

Placental size.

Until now, no studies concerning the ESR distribution in porcine placenta have been published. The differences in placental size found in the present thesis (D35 fetuses), were due to the differences in genotype of the mother, and not of the placenta itself (Chapters 2 and 3). Therefore it seems that the maternal receptors for estrogen are important for placental size. The relation of fetal weight to placental size, however, indicates an effect of fetal ESR genotype on placental size as well (Chapter 3; the percentage of AA fetuses with relatively small placentae is smaller than the percentage of BB or AB fetuses with relatively small placentae). I.m. estradiol 17β administration to Meishan gilts at the time of conceptus elongation increased placental size at term, leading to reduction of the placental efficiency (Wilson and Ford, 2000). Furthermore, exogenous progesterone (P4) and estrone supplement increased number of areolae, probably due to an increase in placental size, since the number of areolae per surface area of the placentae was not affected (Dalton and Knight 1983). It is not known, however, through what physiological pathway the ESR ligand might increase placental size. Estradiol or estrone might have activated maternal ESR or fetal ESR, or even both, to induce the reported change of placental size.

Knock-out.

Much about the biological role of ESR has been confirmed and learned by studying animals in which the ESR gene has been disrupted by the use of gene targeting technology. This methodology has allowed for the generation of transgenic mice that lack the functional gene for the classical (in the present study examined) ESRα, the ESRα knockout mice (Lubahn et al., 1993), and the more recently discovered ESRβ (Kuiper et al., 1996; Mosselman et al., 1996; Tremblay et al., 1997), the ESRβ knockout mice (Krege et al., 1998), as well as germline passage of these mutations. Furthermore, recently, even mice lacking both estrogen receptors α and β have been generated and studied (Couse et al., 1999).

Since the ESR gene studied in the present thesis is comparable to the gene that encodes for ESRα, the major part of this paragraph focuses on the biological role of ESRα. Until now, no studies about the existence of a porcine ESRβ gene have been published. Since certain results indicate that ESRα and ESRβ have different or even opposite biological actions (Gustafsson 1999), however, its possible

existence should not be neglected (especially when the biological role of ESR is deduced from the presence of its ligand estrogen). The biological role of ESRβ is reviewed by Couse and Korach (1999), Gustafsson (1999) and Pettersson and Gustafsson (2001).

ESRα knockout (αERKO) mice are viable, but infertile (Lubahn et al., 1993), and exhibit normal expression of the ESRβ gene (Couse and Korach, 1999). The female reproductive tract undergoes normal pre- and neonatal development, i.e. possesses all major uterine cell types, but is hypoplastic, resulting in weights that are half the weight of uteri of wild type litter-mates. Furthermore the uterus is insensitive to estradiol, DES and hydroxy tamoxifen during adulthood (Lubahn et al., 1993), and lacks mitogenic response to EGF (Curtis et al., 1996). The ovaries also undergo normal, though hyperemic, pre- and neonatal development, but are anovulatory during adulthood, exhibit multiple hemorrhagic cysts and no corpora lutea. Mammary gland undergoes normal, though underdeveloped prenatal development, but is insensitive to estrogen-induced development during puberty and adulthood (Couse and Korach, 1999). The females do not display estradiol and progesterone induced sexual behavior (Rissman et al., 1997), show increased aggression and infanticide, and greatly reduced levels of parental behavior towards newborn pups placed in their home cage (Ogawa et al., 1998).

Besides the mentioned reproduction related traits, αERKO mice have been studied for cardiovascular traits, neuroendocrine system, growth of bones, adipose tissue regulation and several immunological characteristics (reviewed by Couse and Korach, 1999). Functional ESRα appears to be essential for the increase of basic fibroblast growth factor induced angiogenesis by exogenous estradiol in female mice (Johns et al., 1996). Furthermore ESRα absence results in marked increases in white adipose tissue, i.e. adipocyte hyperplasia and hypertrophy, insulin resistance, and glucose intolerance (Heine et al., 2000). αERKO mice have reduced post natal thymic weights, and large decreases in overall absolute numbers of CD4+ and CD4+CD8+ T cells (Yellayi et al., 2000).

Summarized, many reproductive but also non-reproductive traits are affected in ESRα knockout mice, indicating the important physiological role of ESR in general. Among these traits is uterine development. The suggested differences in uterine capacity between ESR AA and BB gilts therefore might be the result of a difference in functioning of the ESR itself.

The disruption of the ESR gene and as a result the absence of ESR, of course is not the same as the existence of ESR gene polymorphism possibly resulting in the existing of two different functioning receptors. Therefore it does not make sense to produce ESRα knockout swine to verify the possible differences between AA and BB pigs. Still, in future, gene modification might be used to examine which of the differences described in the present study are actually related to the ESR gene rather than to a linked gene. Hereto, animals homozygous for one of the ESR alleles will have to be compared with animals that used to be homozygous for the other ESR allele but that have been modified solely for their ESR genotype into the opposite genotype. For the time being, this is merely a theoretical option, since gene targeting in the pig is not yet available.

Litter size results [ESR polymorphism]

Litter size results of different porcine breeds differing in their ESR genotype have been reviewed in Chapter 4. Briefly, if in gilts differences in litter size were found, they were always in favor of the B allele (Table 4 in Chapter 4). In the Large White x Meishan F2 crossbred gilts of the present study, however, the A allele was favorable. These results imply that the ESR polymorphism studied is a marker rather than a major gene for litter size (for discussion, see Chapter 4).

Combination of the three approaches [ESR]

Both the knowledge about the site of mutation and the litter size results imply that ESR gene is a marker rather than a major gene for litter size. The differences in physiology found, however, imply at least partly potential differences in functioning of the ESR itself. The question remains, whether these differences in functioning of the ESR itself are actually causing differences in litter size. The differences in areola density detected on placentae at term, for instance, were not related to the differences in litter size.

The eventual marker might be a marker for another mutation in the same gene, or for a mutation in another closely linked gene. Screening of the whole ESR gene will give a definite answer to the existence of more mutations in the ESR gene. Screening of the DNA closely linked to the ESR gene, might give an answer to the existence of mutations in a closely linked gene.

If the ESR gene actually is a marker for another closely linked gene, the differences in physiology found in the present study, can at least partly still be related to the ESR polymorphism itself.

Despite the great effort that has been made to perform the two described experiments, only two pregnancy stages (Day 35 and term) have been investigated for the effects of ESR genotype on reproductive traits. Comparable experiments at a later stage of pregnancy are recommended.

PRLR gene

Site of the mutation and structure and functioning of the gene product ^{PRLR}

The PRLR gene examined in the present thesis, is located on the q region of porcine chromosome 16 (Vincent et al, 1997). The locus is closely linked to the growth hormone receptor gene 1 (Vincent et al., 1997).

The structure and functioning of PRLR after activation by PRL is reviewed by Bole-Feysot et al. (1998) and Freeman et al. (2000): The prolactin receptor is a single membrane bound protein. It contains an extracellular, transmembrane and intracellular domain. Different PRLR isoforms, varying in the length and composition of their cytoplasmic domain (Figure 6), have been described in different tissues. These isoforms are results of transcription starting at alternative initiation sites of the different PRLR promotors as well as alternative splicing of noncoding and coding exon transcripts (Ormandy et al., 1998). In addition to the membrane bound isoforms, also soluble prolactin binding products of the same PRLR gene have been described. It is not certain whether latter proteins are results of alternative splicing of the primary transcripts or products of proteolytic cleavage of the mature receptor.

The extracellular domain of PRLR can be divided into two fibronectine like subdomains, referred to as D1 and D2. Subdomain D1 contains two disulphide bonds. The amino acids bordering the first disulfide bridge are involved in ligand binding. Subdomain D2 contains a pentapeptide, termed "WS motif" in the membrane proximal region that is probably required for correct folding and cellular trafficking. The involvement of the 24 amino acids long transmembrane domain in

the functional activity of the receptor is not known. The intracellular domain contains two relatively conserved regions, called box1 and box2. The membrane proximal box1, due to its particular structure, is assumed to have a function in the folding specifically recognized by transducing molecules. The membrane proximal region of the intracellular domain is associated with a tyrosine kinase termed Janus Kinase 2 (Jak2).

The activation of the PRLR involves ligand-induced sequential receptor dimerization driven by the PRL molecule containing two binding sites (Figure 7). The interaction of prolactin binding site 1 with the NH_2-terminal subdomain D1 of PRLR, induces the interaction of binding site 2 on the same prolactin molecule with a second PRLR. After receptor dimerization, within 1 minute the Jak2 kinases transphosphorylate each other and phosphorylate tyrosine residues from the receptor itself and a family of transducing proteins termed stat (signal transducer and activator of transcription). Both the presence of box 1 and a homodimeric stoichiometry of PRLR dimers are necessary for the activation of JAK2. Although Jak/stat are the most important pathways initiated by activation of PRLR, other signaling pathways are described (Bole-Feysot et al., 1998; Hu et al., 1998; Freeman et al., 2000).

Although PRLR is mainly a cell surface receptor, endocytosis of PRLR has been shown in several cell types, and nuclear translocation of PRLR accompanied by nuclear actions has been described (Freeman et al., 2000).

The mutation that has caused the polymorphism at the porcine PRLR locus was found in the last exon of the gene (M.F. Rothschild, personal communication), suggesting a difference in structure of prolactin receptor rather than a difference in expression of the PRLR gene leading to different numbers of prolactin receptors in the tissues. The last exon of the PRLR gene encodes for the intracellular domain of the membrane anchored PRLR. A difference in structure at the intracellular domain probably will not affect the capacity of the receptor to bind its ligand and to dimerize with another PRLR. However, it might very well lead to differences in receptor mediated signal transduction following ligand binding and dimerization, and thus might lead to a change in action of prolactin. It is not known whether there also are different porcine PRLR isoforms, as described above.

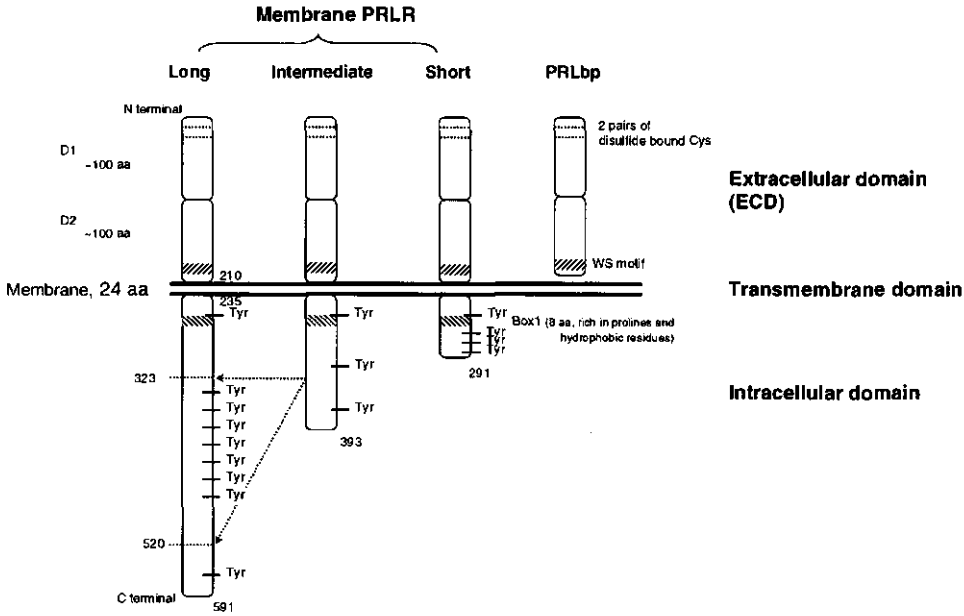


Figure 6. Structure of PRLR. (Modified after Bole-Feysot et al., 1998).

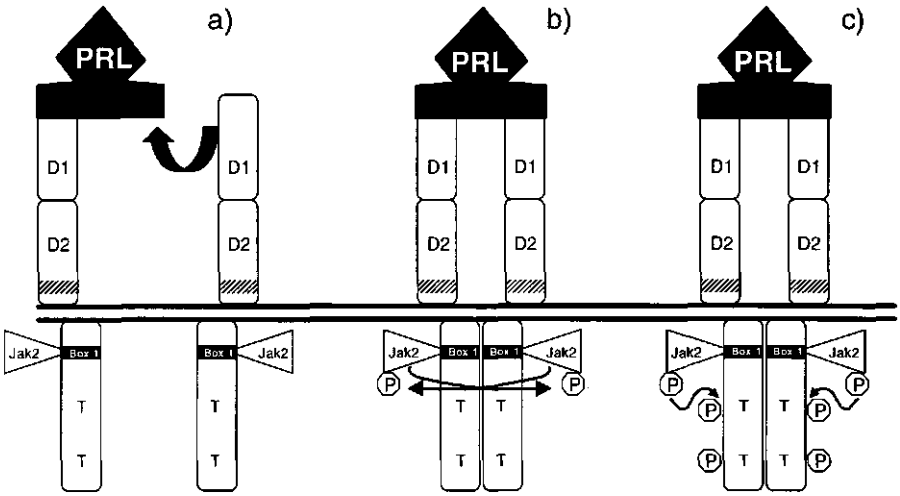


Figure 7. Schematic representation of the ligand-binding and activation of the PRLR a) receptor dimerization b) activation Jak2; transphosphorylation c) phosphorylation thyr residues of PRLR itself. (Modified after Freeman et al., 2000).

Biological role of the gene product ^{PRLR}

Like for ESR gene, comparison of the biological role of the gene product and its ligand (as described in literature) with physiological differences found in gilts with different PRLR genotypes might result in more insight in the question "is PRLR gene a marker or a major gene?".

The main differences in reproductive biology found in gilts with different PRLR genotypes are described in the first paragraphs of this chapter. In summary they are related to ovulation rate, age at first estrus, number of funtional teats of the piglets, and the capability to increase uterine length and subsequent potency to enlarge placentae and implantation surfaces (Chapters 6 and 7). Since the number of functional teats of the piglets is already determined at Day 28 of pregnancy (Patten, 1948), most of the differences found in the present thesis are related to the first days of pregnancy.

The eventual association of PRLR gene polymorphism with difference in ovulation rate and adjustment of uterine and placental environment to litter size, are already discussed in Chapter 6. Briefly, both porcine ovaries and endometrium contain prolactin receptors distributed in a pregnancy dependent way and with quantitative differences throughout pregnancy (Rolland et al., 1976; Jammes et al., 1985; Young et al., 1989, 1990), indicating a substantial role of PRLR on these organs during pregnancy.

Knock-out

Female mice carrying a homozygous null mutation of the PRLR gene ($PRLR^{-/-}$) were sterile due to a complete failure of embryonic implantation, and presented multiple reproductive abnormalities, including a reduced number of primary follicles in their ovaries, a failure of a significant proportion of eggs to undergo germinal vesicle break down, a reduced ovulation rate, irregular cycles, reduced fertilization rates, and defective preimplantation embryonic development (Ormandy et al., 1997; Bole-Feysot et al., 1998). In $PRLR^{-/-}$ mice, mammary development is normal up to puberty (Ormandy et al., 1997; Bole-Feysot et al., 1998). Two functional PRLR alleles, however, are required for efficient lactation (Bole-Feysot et al., 1998; Brisken et al., 1999). Embryonic mammary epithelium develops independent of ovarian and pituitary influence but is already responsive to hormonal stimuli (referred by Brisken et al., 1999).

The results of PRLR knockout mice demonstrate the importance of the presence of PRLR during oocyte maturation and ovulation. The difference in ovulation rate and weight of the ovaries described in the present study therefore could be due to a difference in functioning of the PRLR itself, as a result of the PRLR gene polymorphism.

PRL hormone profiles.

Peri-estrus patterns of circulating PRL in the pig have been described by Van de Wiel et al. (1981), and Prunier et al. (1987). During the cycle, plasma PRL showed two main peaks (Van de Wiel et al., 1981), one at the beginning of pro-estrus rise of E2 (4-5 days before estrus, Dusza and Tilton, 1990) and one during estrus (beginning at 2 h after the time of maximum E2 concentration). The mean duration of the PRL surge during estrus lasted ~ 50 h, and practically coincided with estrus behaviour (Van de Wiel et al., 1981). Furthermore, during the period of estrus, every exposure to the boar induced a PRL peak, the amplitude of which decreased towards the end of estrus (Prunier et al., 1987). During the luteal phase, pulsatile secretion patterns were observed for PRL (though to a lesser degree than for LH), in a diurnal rhythm, with minimal concentrations during the late night (Van der Wiel et al., 1981). Prunier et al. (1987) could not confirm this diurnal rhythm. The peripheral PRL plasma level during pregnancy was similar to the basal values in cyclic sows, i.e. 4-13 ng/ml (Dusza and Krzymowska, 1981). The second day before parturition, the level increased to 20 ng/ml, and at one day before and during farrowing the average PRL level was very high (with a maximum at the start of farrowing, 147 ng/ml). After farrowing plasma PRL level decreased gradually to 43 ng/ml on the fifth day of lactation (Dusza and Krzymowska, 1981).

It is not known whether PRL and PRLR (like estrogen and ESR) have actions independent from each other. If not, the suggested relation between estrus behavior and PRL level (Van der Wiel et al., 1981; Prunier et al., 1987) might be related to PRLR genotype as well, and might be involved in the found differences in age at first estrus for the Large White x Meishan F2 crossbred gilts between the different PRLR genotypes (Chapter 7).

Summarized

PRLR tissue distribution, as well as peripheral prolactin distribution indicate a possible role of PRLR in the found differences between the different PRLR genotypes.

Litter size results [PRLR polymorphism]

Compared to the estrogen receptor gene polymorphism, relatively few porcine litter size results in relation to PRLR gene are available (Vincent et al., 1998, Chapter 7 present thesis). Vincent et al. (1998) examined five lines, in three of which TNB and/or NBA was affected by PRLR genotype (Chapter 7). The direction and magnitude of the effects varied between these lines, suggesting that PRLR gene is a marker, or that background genes play a role. For three lines, including the crossbred gilts from the present study, AA animals had the largest litters, while for one line, the AB animals had the largest litters, followed by the BB animals.

Combination of the three approaches [PRLR]

The site of the mutation as well as at least part of the physiological effects, do not exclude the possibility that PRLR itself is the major gene for litter size instead of a marker. The few reported litter size results, however, do not confirm this statement. Assumed that PRLR gene affects ovulation rate, the direction and magnitude of the effect of PRLR polymorphism on litter size can very well differ, dependent on what will happen after ovulation (Figure 1, see also the first paragraph of this chapter). Therefore, the possibility of PRLR gene being a major gene for litter size, by affecting ovulation rate, can not be excluded. Further litter size studies and also physiological studies with larger numbers should be performed to confirm this statement.

Since other genes (e.g. ESR gene) and also environmental factors might change the effect caused by the PRLR polymorphism within the 112 days to parturition, it is preferable to state that PRLR gene is a major gene for ovulation rate rather than for litter size.

THE OTHER WAY AROUND

Using physiological data for screening candidate genes for litter size

The results of the present study give an impression of the usability of existing physiological data for a first screening of another candidate gene for litter size, the PRLR gene. For this gene it was not yet clear whether its polymorphism would affect litter size. The first experiment of the present study was designed in order to determine the first three components of litter size (ovulation rate, number of implantation sites, and number of embryos) on the one hand, and to obtain physiological data that are related to the measured components of litter size on the other hand. Based on the number of embryos, a prediction of litter size could be made for the different PRLR genotypes, under the assumption that fetal survival was similarly affected by these genotypes. To confirm latter assumption, the genotypes ultimately will have to be tested for their effect on litter size, by preference on the same line that has been used for the first experiment. Part of the physiological data obtained, however, already pointed in the predicted direction. Because of their relation to litter size, the size of uterus, placenta and implantation site, for example, confirmed the suggestion that the difference in ovulation rate associated with PRLR polymorphism might hold for the number of vital embryos (and litter size) as well.

From the PRLR results it can be concluded that, because of its design, the first experiment can be used for screening any candidate gene for litter size for its effect on number of vital Day 35 embryos (and its underlying components ovulation rate and number of implantation sites). Part of the obtained physiological data are related to the measured components of litter size, and therefore may play an important role in the interpretation and confirmation of differences or tendencies found. The only restriction will be the number of alleles involved, and the allele frequency in the population of experimental animals (i.e. the number of observations per genotype should be adequate).

Under the same restrictions as for the first experiment, the data from the second experiment of the present study can be used for screening a candidate gene for litter size for its effect on first parity litter size. Litter size data, and also early neonatal growth curves and estrus data of 275 Large White x Meishan F2 crossbred gilts that were kept under a controlled management system, are

available. Furthermore, detailed information of placental traits is available for the individual piglets of 62 of these gilts.

Unfortunately, the two experiments of the present study were performed with different lines of gilts, and therefore, one has to be careful in combining the conclusions based on the results of both experiments.

To evaluate in general the possibility to use physiological studies to confirm the role as major gene once a candidate gene has been identified or (in combination with comparative genome mapping) to identify major genes once markers have been described, will be a full study on itself. The results of the present study, however, can be used as a starting point for the first mentioned evaluation.

The results clearly demonstrate that there are several conditions which have to be fulfilled in order to use a physiological study to confirm the role as major gene once a candidate gene has been identified. If the study already has been performed, DNA has to be available. The study has to be designed in a way that the trait of interest (i.e. the trait that is presumed to be affected by the candidate gene), or a component of this trait can be measured. Furthermore, as many as possible physiological traits that can be related to the traits of interest should be included in the study. To be able to relate the physiological results to the gene of interest, knowledge about the physiological function of the gene product should be available. Furthermore, any knowledge about the site of mutation in combination with the make-up of the gene (e.g. the DNA sequence and its function; intron-exon structure, promotor region), will be of help. In summary, knowledge from different disciplines will have to be combined (without loosing the initial objective) in order to get an answer. The broader the physiological function(s) of the product of the candidate gene, however, the more difficult the interpretation of the physiological results may be. Candidate genes that act upon a single determining physiological process, will be more difficult to find, but will be easier to examine for actually being a major gene.

When a gene has been identified as a candidate gene through comparative mapping of genome regions with existing flanking marker genes, the structure and function of such a gene might be unknown in the species of interest. In such case, knock-out studies might be helpful. These studies, however, are very difficult and time consuming, since knock-outs are not yet available for many species.

CONCLUSIONS

Without knowing anything about the physiological background of the effect of the studied genes on litter size, breeders can (and are willing to) use both polymorphisms for selection on litter size. The results of the present thesis, however, demonstrate the value and advantages of additional knowledge of differences in the reproductive physiology between gilts with different ESR or PRLR genotypes for breeding companies: The results clearly indicate that both polymorphisms have their effects on different components of litter size that are expressed at different stages of pregnancy, and therefore can have synergistic but also antagonistic effects. This implies there are favorable PRLR/ESR genotype combinations and unfavorable ones. The fact that synergistic/antagonistic genes with major effects on litter size actually exist, can be extended to the hypothesis, or even warning, that more of such (not yet discovered) genes will exist. Although selection on the favorable genotype combination will have the largest impact, it will lead to an enlargement of litter size once-only. Furthermore, keeping in mind the highly variable biological functions both gene products have besides their effects on reproduction, it is questionable whether total elimination of the unfavorable alleles for litter size is a wise action. Still, both genes at the moment are unique, being the only current major genes or marker genes for porcine uterine capacity (ESR gene), ovulation rate (PRLR gene), age at first estrus (PRLR gene) and teat number of the piglets (PRLR gene), and therefore, they should get the recognition they deserve.

A problem that makes it difficult to use the polymorphisms directly for selection, is the fact that for both genes studied the favorable allele for litter size has negative effects on other traits. This problem on the other hand, also clearly demonstrates the importance of physiological research parallel to and coherent with the search for QTLs and markers for any trait. Regarding the complexity of pregnancy, it is advisable to make a distinction between gilts and sows and even different parities, when physiological aspects of major genes for litter size are studied.

Combining the limited knowledge about the site of the mutation, structure and mechanism of action of the gene product, the biological role of the gene product in relation to differences in physiology found between different genotypes, and the litter sizes in relation to the different genotypes, has resulted in the hypothesis that the ESR gene is a marker for litter size. Using the same strategy for the PRLR

gene does not exclude the possibility of PRLR gene being the major gene rather than a marker for a closely linked major gene for litter size, although further investigation on larger data sets is recommended. Since other genes (e.g. ESR gene) and also environmental factors might change the effect caused by the PRLR polymorphism within the 112 days to parturition, it is preferable to state that PRLR gene is a major gene for ovulation rate rather than for litter size.

In contrast with the general opinion, there actually seem to be traits and relations between traits that are dependent on both maternal and fetal ESR genotype, which might lead to a difference in litter size. Therefore, the ESR genotype of the boar might be important for litter size as well.

The results of the present thesis are the first published, concerning the unraveling of specific actions of the different ESR and PRLR alleles, and therefore have a pioneering character. Besides the intruiging results, the thesis clearly delimitates diverse areas for follow-up research (see Chapters 2-7). Furthermore, the datasets generated in the course of this study might be very useful for the first physiological screening of other major genes or marker genes for litter size.

REFERENCES

Abromavich CE (1926). The morphology and distribution of the rosettes on the foetal placenta of the pig. Anat Rec 39:69-72.

Bal HS and Getty R (1970). Postnatal growth of the swine uterus from birth to six months. Growth 34:15-30.

Bartol FF, Wiley AA, Spencer TE, Vallet JL, and Christenson RK (1993). Early uterine development in pigs. J Reprod Fertil Suppl 48:99-116.

Bennett GL, and Leymaster KA (1989). Integration of ovulation rate, potential embryonic viability and uterine capacity into a model of litter size in swine. J Anim Sci 67:1230-1241.

Bökenkamp D, Jungblut PW, and Thole HH (1994). The C-terminal half of the porcine estradiol receptor contains no post-translational modification: determination of the primary structure. Mol Cell Endocrinol 104:163-172.

Bole-Feysot C, Goffin V, Edery M, Binart N, and Kelly PA (1998). Prolactin (PRL) and its receptor: Actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocrin Rev 19:225-268.

Brambel CE (1933). Allantochorionic differentiations of the pig studied morphologically and histochemically. Am J Anat 52:397-459.

Brisken C, Kaur S, Chavarria TE, Binart N, Sutherland RL, Weinberg RA, Kelly PA, and Ormandy CJ (1999). Prolactin controls mammary gland development via direct and indirect mechanisms. Dev Biol 210:96-106.

Chen TT, Bazer FW, Gebhardt BM, and Roberts RM (1975). Uterine secretion in mammals: synthesis and placental transport of a purple acid phosphatase in pigs. Biol Reprod 13:304-313.

Christenson RK, Leymaster KA, and Young LD (1987). Justification of unilateral hysterectomy-ovariectomy as a model to eveluate uterine capacity in swine. J Anim Sci 65:738-744.

Christenson RK, Vallet JL, Leymaster KA, and Young LD (1993). Uterine function in Meishan pigs. J Reprod Fertil Suppl 48:279-289.

Couse JF, Curtis Hewitt S, Bunch DO, Sar M, Walker VR, Davis BJ, and Korach KS (1999). Postnatal sex reversal of the ovaries in mice lacking estrogen receptors α and β. Science 286:2328-2331.

Couse JF, and Korach KS (1999). Estrogen receptor null mice: what have we learned and where will they lead us? Endocrine reviews 20:358-417.

Culbertson MS, Mabry JW, Bertrand JK, and Nelson AH (1997). Breed-specific adjustment factors for reproductive traits in Duroc, Hampshire, Landrace, and Yorkshire swine. J Anim Sci 75:2362-2367.

Curtis SW, Washburn T, Sewall C, Di Augustine R, Lindzey J, Couse JF, and Korach KS (1996). Physiological coupling of growth factor and steroid receptor signaling pathways: Estrogen receptor knockout mice lack estrogen-like response to epidermal growth factor. Proc Natl Acad Sci USA 93:12626-12630.

Dauvois S, White R, and Parker MG (1993). The antiestrogen ICI 182780 disrupts estrogen receptor nucleocytoplasmic shuttling. J Cell Sci 106:1377-1388.

Dantzer V (1984). Scanning electron microscopy of exposed surfaces of the porcine placenta. Acta Anat 118:96-106.

Dantzer V (1985). Electron microscopy of the initial stages of placentation in the pig. Anat Embryol 172:281-293.

Dantzer V, and Leiser R (1993). Microvasculature of regular and irregular areolae of the areola-gland subunit of the porcine placenta: structural and functional aspects. Anat Embryol 188:257-267.

Dantzer V, Leiser R, Kaufmann P, and Luckhardt M (1988). Comparative morphological aspects of placental vascularization. Trophoblast Research 3:235-260.

Deaver DR, and Guthrie HD (1980). Cytoplasmic estrogen receptor, estradiol and progesterone concentrations in endometrium of nonpregnant and pregnant pigs. Biol Reprod 23:72-77.

Dourmad J-Y, Noblet J, Père MC, and Étienne M (1999). Mating, pregnancy and prenatal growth. In: A Quantitative biology of the pig. Ed. I Kyriazakis. CABI publishing Oxon/New York. 1999:129-153.

Drögemüller C, Hamann H, Thieven U, Krieter J, Distl O, and Harlizius B (1999). Influence of the genome region surrounding the estrogen receptor (ESR) gene on litter size in a German Landrace population. Archiv für Tierzucht 42:special issue, 1-3.

Drögemüller C, Thieven U, and Harlizius B (1997). An Ava I and a MspA1 I polymorphism at the porcine oestrogen receptor (ESR) gene. Animal Genetics 28:59.

Dusza L and Krzymowska H (1981). Plasma prolactin levels in sows during pregnancy, parturition and early lactation. J Reprod Fert 61:131-134.

Dusza L and Tilton JE (1990). Role of prolactin in the regulation of ovarian function in pigs. J Reprod Fert Suppl 40:33-45.

Dyck GW, and Swierstra EE (1983). Growth of the reproductive tract of the gilt from birth to puberty. Can J Anim Sci 63:81-87.

Dziuk PJ (1968). Effect of number of embryos and uterine space on embryo survival in the pig. J Anim Sci 27:673-676.

Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjöld M, and Gustafsson J-Å (1997). Human estrogen receptor β-gene structure, chromosomal localization, and expression pattern. J Clin Endocr Metab 82:4258-4265.

Evans FD, Christopherson RJ, and Aherne FX (1988). The comparative physiological development of the reproductive and adrenal axis of the gilt from weaning to puberty. Can J Anim Sci 68:1113-1119.

Ford SP, and Christenson RK (1979). Blood flow to uteri of sows during the estrous cycle and early pregnancy: local effect of the conceptus on the uterine blood supply. Biology of Reproducion 21:617-624.

Ford SP, Christenson RK and Ford JJ (1982). Uterine blood flow and uterine arterial, venous and luminal concentrations of oestrogens on Days 11, 13 and 15 after oestrus in pregnant and non-pregnant sows. J Reprod Fertil 64:185-190.

Freeman ME, Kanyicska B, Lerant A, and Nagy G (2000). Prolactin: Structure, function, and regulation of secretion. Physiological reviews 80:1523-1631.

Friess AE, Sinowatz F, Skolek-Winnisch R, and Träutner W (1980). The placenta of the pig. I Finestructural changes of the placental barrier during pregnancy. Anat Embryol 158:179-191.

Friess AE, Sinowatz F, Skolek-Winnisch R, and Träutner W (1981). The placenta of the pig. II The ultrastructure of the areolae. Anat Embryol 163:43-53.

Gama LLT and Johnson RK (1993). Changes in ovulation rate, uterine capacity, uterine dimensions, and parity effects with selection for litter size in swine. J Anim Sci 71:608-617.

Geisert RD, Brenner RM, Moffatt RJ, Harney JP, Yellin T, and Bazer FW (1993). Changes in oestrogen receptor protein, mRNA expression and localization in the endometrium of cyclic and pregnant gilts. Reprod Fertil Dev 5:247-260.

Geisert RD, Brookbank JW, Roberts RM, and Bazer FW (1982[b]). Establishment of pregnancy in the pig: II Cellular remodelling of the porcine blastocyst during elongation on day 12 of pregnancy. Biol Reprod 27:941-955.

Geisert RD, Renegar RH, Thatcher WE, Roberts RM, and Bazer FW (1982[a]). Establishment of pregnancy in the pig: I Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol Reprod 27:925-939.

Geisert RD, Thatcher WW, Roberts RM, and Bazer FW (1982[c]). Establishment of pregnancy in the pig: III Endometrial secretory response to estradiol valerate administered on day 11 of the estrous cycle. Biol Reprod 27:957-965.

Geisert RD, Zavy MT, Moffatt RJ, Blair RM, and Yellin T (1990). Embryonic steroids and the establishment of pregnancy in pigs. J Reprod Fertil Suppl 40:293-305.

Goldstein SR (1926). A note on the vascular relations and areolae in the placenta of the pig. Anat Rec 34:25-35.

Graves WE, Lauderdale JW, Kirkpatrick RL, First NL, and Casida LE (1967). Tissue changes in the involuting uterus of the postpartum sow. J Anim Sci 26:365-371.

Green S, Walter P, Kumar V, Krust A, Bornert B-M, Argos P, and Chambon P (1986). Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature 320:134-139.

Gustafsson J-Å (1999). Estrogen receptor β - a new dimension in estrogen mechanism of action. J Endocrinol 163:379-383.

Haley CS, Lee GJ, and Ritchie M (1995). Comparative reproductive performance in Meishan and Large White pigs and their crosses. Anim Sci 60:259-267.

Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, and Cooke PS (2000). Increased adipose tissue in male and female estrogen receptor-α knockout mice. Proc Natl Acad Sci USA 97:12729-12734.

Hu Z-Z, Zhuang L, and Dufau ML (1998). Prolactin receptor gene diversity: Structure and Regulation. TEM 9:94-102.

Isler BJ, Irvin KM, and Neal SM (1998). Investigation of the estrogen receptor gene and its association with reproductive tract traits in swine. Research and Reviews: Poultry and Swine: OARDC special circular 164:49-51.

Isler BJ, Irvin KM, Neal SM, Moeller SJ, Davis ME, and Meeker DL (1999[a]). Examination of the relationship between the estrogen receptor gene and repoductive traits in swine. Record of proceedings of the National Swine Improvement Federation conference and annual meeting 42:14-19.

Isler BJ, Irvin KM, Neal SM, Moeller SJ, Davis ME, and Meeker DL (1999[b]). The effect of the estrogen receptor gene on litter traits in swine. Research and Reviews: Poultry and Swine: OARDC special circular 171:50-53.

Isler BJ, Irvin KM, and Neal SM (1999[c]). Examination of the relationship between the estrogen receptor gene and reproductive tract components in swine. Research and Reviews: Poultry and Swine: OARDC special circular 171:54-59.

Isler BJ, Irvin KM, Neal SM, Moeller SJ, and Davis ME (1999[d]). Association between the estrogen receptor gene and reproductive components in swine. J Anim Sci 77 Suppl 1:32 (abstr).

Isler BJ, Irvin KM, Neal SM, Moeller SJ, Davis ME, and Meeker DL (1999[e]). The effect of estrogen receptor genotype, breed, and parity on litter traits and reproductive tract traits in swine. J Anim Sci 77 Suppl1:131 (abstr).

Jammes H, Schirar A, and Djiane J (1985). Differential patterns in luteal prolactin and LH receptors during pregnancy in sows and ewes. J Reprod Fert 73:27-35.

Johns A, Freay AD, Fraser W, Korach KS, and Rubanyi GM (1996). Disruption of estrogen receptor gene prevents 17β estradiol-induced angiogenesis in transgenic mice. Endocrinology 137:4511-4513.

Katzenellenbogen B (1996). Estrogen receptors: Bioactivities and interactions with cell signaling pathways. Biol Reprod 54:287-293.

Kiracofe GH (1980). *Uterine involution: its role in regulating postpartum intervals.* J Anim Sci Suppl 51 II:16-28.

Knight JW (1994). Aspects of placental estrogen synthesis in the pig. Exp Clin Endocrinol 102:175-184.

Knight JW, Bazer FW, Thatcher WW, Franke DE, and Wallace HD (1977). Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: Interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 44:620-637.

Korwin-Kossakowska A, Kuryl J, Pierzchala M (1999). An analysis of relations between the polymorphism of estrogene receptor gene and some reproduction traits in Zlotnicka Spotted x Polish Large White pigs. Animal Science Papers and Reports 17:155-161.

Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson J-Å, and Smithies O (1998). Generation and reproductive phenotypes of mice lacking estrogen receptor β. Proc Natl Acad Sci USA 95:15677-15682.

Krust A, Green S, Argos P, Kumar V, Walter P, Bornert J-M, and Chambon P (1986). The chicken oestrogen receptor sequence: homology with v-erbA and the human oestrogen and glucocorticoid receptors. EMBO J 5:891-897.

Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, and Gustaffson J-Å (1996). Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925-5930.

Lamberson WR, and Eckardt GR (1996). Local changes in uterine dimensions in the pregnant pig. Anim Reprod Sci 43:35-41.

Legault C (1985). Selection of breeds, strains and individual pigs for prolificacy. J Reprod Fertil 33 Suppl 2:151-166.

Legault C, Gruand J, Lebost J, Garreau H, Ollivier L, Messer LA, Rothschild MF (1996). Fréquence et effet sur la prolificité du gène ESR dans deux lignées Large White en France. Journées Rech Porcine en France 28:9-14.

Leiser R and Dantzer V (1988). Structural and functional aspects of porcine placental microvasculature. Anat Embryol 177:409-419.

Little M, Szendro PI, and Jungblut PW (1973). Hormone-mediated dimerization of microsomal estradiol receptor. Hoppe-Seyler's Z Physiol Chem 354:1599-1610.

Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, and Smithies O (1993). Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc Natl Acad Sci USA 90:11162-11166.

Mosselman S, Polman J, Dijkema R (1996). ERβ: identification and characterization of a novel human estrogen receptor. FEBS Lett 392:49-53.

Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, and Pfaff DW (1998). Roles of estrogen receptor-α gene expression in reproduction-related behaviors in female mice. Endocrinology 139:5070-5081.

Ormandy CJ, Binart N, Helloco C, and Kelly PA (1998). Mouse prolactin receptor gene: Genomic organization reveals alternative promoter usage and generation of isoforms via alternative 3'-exon splicing. DNA Cell Biol 17:761-770.

Ormandy CJ, Camus A, Barra J, Damotte D, Lucas B, Buteau H, Edery M, Brousse, N, Babinet C, Binart N, and Kelly PA (1997). Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. Genes Dev 11:167-178.

Pack BA, Brooks CL, Dukelow WR, and Brooks SC (1979). The metabolism and nuclear migration of estrogen in porcine uterus throughout the implantation process. Biol Reprod 20:545-551.

Palmer WM, Teague HS, and Venzke WG (1965[a]). Macroscopic observations on the reproductive tract of the sow during lactatino and early postweaning. J Anim Sci 24:541-545.

Palmer WM, Teague HS, and Venzke WG (1965[b]). Histological changes in the reproductive tract of the sow during lactation and early weaning. J Anim Sci 24:1117-1125.

Parker MG (1995). Structure and function of estrogen receptors. Vitamins and Hormones 51:267-287.

Patten BM (1948). Embryology of the pig. 3rd edition. McGraw-Hill book company inc. New York, Toronto, London.

Perry JS (1981). The mammalian fetal membranes. J Reprod Fertil 62:321-335.

Perry JS, and Crombie PR (1982). Ultrastructure of the uterine glands of the pig. J Anat 134:339-350.

Perry JS, Heap RB, and Amoroso EC (1973). Steriod hormone production by pig blastocysts. Nature Lond 245:45-47.

Perry JS, Heap RB, Burton RD, and Gadsby JE (1976). Endocrinology of the blastocyst and its role in the establishment of pregnancy. J Reprod Fertil Suppl 25:85-104.

Pettersson K and Gustafsson J (2001). Role of the estrogen receptor beta in estrogen action. Annu Rev Physiol 63:165-192.

Pomeroy RW (1960). Infertility and neonatal mortality in the sow. III Neonatal mortality and foetal development. J Agric Sci 54:31-56.

Pope CE, Christenson RK, Zimmerman-Pope VA, and Day BN (1972). Effect of number of embryos on embryonic survival in recipient gilts. J Anim Sci 35:805-808.

Pope WF, Lawyer MS, and First NL (1986). Intrauterine migration of the porcine embryo: coordination of bead migration with estradiol. J Anim Sci 63:848-853.

Pope WF, Maurer RR, and Stormshak F (1982). Intrauterine migration of the porcine embryo: influence of estradiol-17β and histamine. Biol Reprod 27:575-579.

Prunier A, Martinat-Botté, Ravault JP , and Camous S (1987). Perioestrous patterns of circulating LH, FSH, prolactin and oestradiol-17β in the gilt. Anim Reprod Sci 14:205-218.

Rexroad CE, and Guthrie HD (1984). Cytoplasmic and nuclear estrogen receptors and leucin incorporation in endometrium of cyclic and pregnant pigs to day 19 postestrus. J Anim Sci 59:1286-1294.

Revelli A, Massobrio M, and Tesarik J (1998). Nongenomic actions of steroid hormones in reproductive tissues. Endocrine Reviews 19:3-17.

Rissman EF, Early AH, Taylor JA, Korach KS, and Lubahn DB (1997). Estrogen receptors are essential for female sexual receptivity. Endocrinology 138:507-510.

Roberts RM, Xie S, and Trout WE (1993). Embryo-uterine interactions in pigs during week 2 of pregnancy. J Reprod Fertil Suppl 48:171-186.

Robertson HA, and King GJ (1974). Plasma concentrations of progesterone, oestrone, oestradiol-17β and of oestrone sulphate in the pig at implantation, during pregnancy and at parturition. J Reprod Fertil 40:133-141.

Rolland R, Gunsalus GL, and Hammond JM (1976). Demonstration of specific binding of prolactin by porcine corpora lutea. Endocrinology 98:1083-1091.

Rothschild MF, Jacobson C, Vaske DA, Tuggle CK, Short TH, Sasaki S, Eckardt GR, and McLaren DG (1994). A major gene for litter size in pigs. Proc World Congr Genet Appl Livest Prod 21:225-228.

Rothschild, M, Jacobson C, Vaske D, Tuggle C, Wang L, Short T, Eckardt G, Sasaki S, Vincent A, Mc Laren D, Southwood O, Van der Steen H, Mileham A, and Plastow G (1996). The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc Natl Acad Sci USA 93:201-205.

Rothschild MF, Larson RG, Jacobson C, and Pearson P (1991). Pvu II polymorphisms at the porcine oestrogen receptor locus (ESR). Anim Genet 22:448.

Rothschild MF, Vaske DA, Tuggle CK, Messer LA, Mc Laren DG, Short TH, Eckardt GR, Mileham AJ, and Plastow GS and in collaboration with Southwood OI and van der Steen HAM (1995). Estrogen receptor locus is a major gene for litter size in the pig. 46th Ann Mtg Eur Assoc Anim Prod. 5 pp.

Rothschild MF, Vincent AL, Tuggle CK, Evans G, Short TH, Southwood OI, Wales R, and Plastow GS (1998). A mutation in the prolactin receptor gene is associated with increased litter size in pigs. 26th International Conference on Animal Genetics, ISAG (International Society for Animal Genetics), August 9-14, 1998, Auckland, NZ, pp 105 (abstr).

Schwabe JWR, Chapman L, Finch JT, Rhodes D, and Neuhaus D (1993). DNA recognition by the oestrogen receptor: from solution to the crystal. Structure 1:187-204.

Short TH, Rothschild MF, Southwood OI, McLaren DG, De Vries A, Van der Steen H, Eckardt GR, Tuggle CK, Helm , Vaske DA, Mileham AJ, and Plastow GS (1997). Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. J Anim Sci 75:3138-3142.

Smidt VD, Thume O, und Jöchle W (1969). Untersuchungen zur geschlechtlichen Regeneration post partum by säugenden und nicht laktierenden Sauen. Zuchtungskunde 41:36-45.

Smith C (1998). Cross-talk between peptide growth factor and estrogen receptor signaling pathways. Biol Reprod 58:627-632.

Smith DF, and Toft DO (1993). Steroid receptors and their associated proteins. Molec Endocrinol 7: 4-11.

Southwood OI, Van der Steen HAM, Mileham AJ, Plastow GS, Cuthbert-Heavens D (1995). Evaluation of the oestrogen receptor (ESR) gene in Meishan synthetic pigs. 46th Ann Mtg Eur Assoc Anim Prod 1:53 (abstr).

Spencer TE, Bartol FF, Wiley AA, Coleman DA, and Wolfe DF (1993[a]). Neonatal porcine endometrial development involves coordinated changes in DNA synthesis, glycosaminoglycan distribution, and ^{3}H-glucosamine labeling. Biol Reprod 48:729-740.

Spencer TE, Wiley AA, and Bartol FF (1993[b]). Neonatal age and period of estrogen exposure affect porcine uterine growth, morphogenesis, and protein synthesis. Biol Reprod 48:741-751.

Stoner CS, Geisert RD, Bazer FW, and Thatcher WW (1981). Characterization of estrogen patterns in early pregnant and estrous gilts. J Anim Sci 53 Suppl 1:370 (abstr).

Stroband HWJ, and Van der Lende T (1990). Embryonic and uterine development during early pregnancy in pigs. J Reprod Fertil Suppl 40:261-277.

Svajgr AJ, Hays VW, Cromwell GL, and Dutt RH (1974). Effect of lactation duration on reproductive performance of sows. J Anim Sci 38:100-105.

Tarleton BJ, Wiley AA, and Bartol FF (1999). Endometrial development and adenogenesis in the neonatal pig: effects of estradiol valerate and the antiestrogen ICI 182,780. Biol Reprod 61:253-263.

Tarleton BJ, Wiley AA, Spencer TE, Moss AG, and Bartol FF (1998). Ovary-independent estrogen receptor expression in neonatal porcine endometrium. Biol Reprod 58:1009-1019.

Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F, Giguere V (1997). Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor-β. Mol Endocrinol 11:353-365.

Tummaruk P, Lundeheim N, Einarsson S, and Dalin, A-M (2000). Reproductive performance of purebred Swedish Landrace and Swedish Yorkshire sows: I Seasonal variation and parity influence. Acta Agric Scanc Sect A, Animal Sci 50:205-216.

Van de Wiel DFM, Erkens J, Koops W, Vos E, and Van Landeghem AAJ (1981). Periestrous and midluteal time courses of circulating LH, FSH, prolactin, estradiol-17β and progesterone in the domestic pig. Biol Reprod 24:223-233.

Van der Meulen J, Helmond FA, and Oudenaarden CPJ (1994). Oestrogen receptors in endometrial cytosol of gilts on days 10-13 of oestrus cycle and pregnancy. Netherlands J Agricultural Sci 42:217-223.

Van Rens BTTM (1988). Ultrastructuur van uterus an embryo van het varken gedurende de implantatie. Doctoraalverslag EDC, Landbouwuniversiteit Wageningen, 1988.

Van Rens BTTM and Stroband HWJ (1989). Ultrastructural study of porcine maternal endometrium and embryo during implantation. The Netherlands Journal of Zoology 39:107-108 (abstr).

Vincent AL, Evans G, Short TH, Southwood OI, Plastow GS, Tuggle CK, and Rothschild MF (1998). The prolactin receptor gene is associated with increased litter size in pigs. 6[th] WCGALP 27:15-18.

Vincent AL, Wang L, Tuggle CK, Robic A, Rothschild MF (1997). Prolactin receptor maps to pig chromosome 16. Mammalian Genome 8:793-794.

Webel SK, and Dziuk PJ (1974). Effect of stage of gestation and uterine space on prenatal survival in the pig. J Anim Sci 38:960-963.

Weigel NL, and Zhang Y (1998). Ligand-independent activation of steroid hormone receptors. J Mol Med 76:469-479.

Wilson ME and Ford SP (2000). Effect of estradiol 17β administration during the time of conceptus elongation on placental size at term in Meishan pigs. J Anim Sci 78:1047-1052.

Wislocki GB and Dempsey EW (1946). Histochemical reactions of the placenta of the pig. Am J Anat 78:181-225.

Wu MC and Dziuk PJ (1988). Ovarian influence on uterine growth in prepubertal gilts. J Anim Sci 66:2893-2898.

Yellayi S, Teuscher C, Woods JA, Welsh TH Jr, Tung KSK, Nakai M, Rosenfeld CS, Lubahn DB, Cooke PS (2000). Normal development of thymus in male and female mice requires estrogen/estrogen receptor-alpha signaling pathway. Endocrine 12:207-213.

Young KH, Kraeling RR, and Bazer FW (1989). Effects of prolactin on conceptus survival and uterine secretory activity in pigs. J Reprod Fert 86:713-722.

Young KH, Kraeling RR, and Bazer FW (1990). Effect of pregnancy and exogenous ovarian steroids on endometrial prolactin receptor ontogeny and uterine secretory response in pigs. Biol Reprod 43:592-599.

Summary

In the present thesis the effect of estrogen receptor (ESR) genotype (two alleles, A and B) and prolactin receptor (PRLR) genotype (two alleles, A and B) on various physiological traits has been examined in gilts of two different lines, in order to 1) increase knowledge about the physiological background of the effect of ESR polymorphism on litter size in gilts, 2) study the interaction between ESR genotype of mother and offspring and 3) get an impression about the usability of the existing physiological data for a first screening of another candidate gene for litter size, PRLR gene.

ESR gene

In L93 Meishan synthetics, a genetic line in which the ESR B allele is favorable for litter size, no differences in estrus length, estrus cycle length, or periovulatory plasma LH, estrogen or progesterone profiles were detected between AA and BB gilts. Furthermore, no differences in number of corpora lutea or number and percentage of vital Day 35 embryos were found between AA and BB gilts (Chapter 2). This indicates that the difference in litter size is likely caused by a difference in fetal survival. Thus, uterine capacity might be different for the two genotypes. The available uterine space per embryo, and the endometrial folding of uterine surface area seemed to be the same for both genotypes at Day 35 of pregnancy. A difference in placental size (i.e. embryos of BB gilts had longer placentae than embryos of AA gilts), however, was found, suggesting a higher chance for placental insufficiency in AA gilts, leading to the expected higher fetal mortality compared to the BB gilts (Chapter 2). The difference found was entirely due to the maternal ESR genotype, since fetal ESR genotype nested within maternal ESR genotype did not affect placental length, nor placental weight or implantation surface area (Chapter 3). Fetal weight was related similarly to placental length, placental weight and implantation surface area: up to a certain threshold value, an increase in the trait was associated with an increase of fetal weight. Thereafter, fetal weight did not change anymore. Thus, at Day 35 of pregnancy porcine fetuses seem to have a maximum growth potential. The percentage of AA fetuses (in AA mothers) that had not reached this maximum growth potential was larger than of the other three genotype combinations studied (AB fetuses in AB mothers, and BB and AB fetuses in BB mothers). Therefore a higher subsequent fetal mortality may be expected in this group (Chapter 3). Hearts of AB fetuses in AA mothers were significantly heavier than those of fetuses in BB mothers and tended to be heavier than those of AA fetuses in AA mothers. The reason for this hypertrophy is unclear,

but might be related to a difference in placental vascularity. Heart weight of fetuses from BB gilts increased with fetal weight, while heart weights of fetuses from AA gilts did not. Heart weight increased with an increase of placental length and implantation surface area up to 51 cm and 437 cm^2, respectively, and thereafter decreased again. For AB fetuses in BB mothers a similar relation was found between heart weight and placental weight, while heart weight of the other three genotype combinations remained unaffected as placental weight increased. The fetus and placenta are continuously changing during early pregnancy, therefore different mechanisms may change the demands for cardiac output. However, keeping in mind that placental size and blood volume are relatively large at Day 35 of pregnancy, placental vascularity and vascular development may play a major role (Chapter 3).

In Large White x Meishan F2 crossbred gilts, ESR genotype significantly affected litter size, i.e. AB gilts had larger litters than BB gilts, without affecting average birthweight (Chapter 4). The found effect differed from previous research, since in previous research the B allele was associated with largest litters, while in the present study the A allele was suggested to be the favorable allele. Therefore the hypothesis that ESR is a marker rather than the major gene itself was discussed (Chapter 4). In the same line, placental traits at term were examined in relation to the maternal ESR genotype (Chapter 4) as well as to the piglet ESR genotype nested within maternal ESR genotype (Chapter 5). At term, average placental length, surface area, and weight including and excluding amnion were not affected by maternal ESR genotype. The average number of areolae per placenta and the average number of areolae per cm^2 placenta, however was affected by maternal ESR genotype. The AB gilts had a lower number of areolae per placenta than BB gilts, and a lower number of areolae per cm^2 placenta than AA and BB gilts. Although the reason for the lower number of areolae on placentae in AB gilts is not yet known, the results suggest that the ESR linked major gene for litter size might be involved in the development and activity of the endometrial glands (Chapter 4). When the same placental traits at term were analyzed at a piglet level, piglet ESR genotype nested within maternal ESR genotype did not affect placental weight, length, surface area and number of areolae, indicating that the difference in litter means of number of areolae described in Chapter 4 was entirely due to the maternal ESR genotype (Chapter 5). Piglet ESR genotype nested within maternal ESR genotype, however, did affect amnion weight, placental weight after including placental surface area in the model, placental efficiency calculated as birthweight divided by placental weight, and the relations of birthweight to placental weight and

birthweight to number of areolae (Chapter 5). Although the effects did not always point in the same direction, the found differences imply an interaction of maternal and fetal ESR genotype on placental traits (especially placental weight and number of areolae) during fetal development. Furthermore, the found effects on placental and amnion weight might be the result of a difference in thickness and/or vascularisation. The favorable allele for litter size, i.e. the A allele, appears to be the unfavorable allele for pre-weaning piglet growth in the Large White x Meishan F2 crossbred gilts studied (Chapter 5).

PRLR gene

In L93 Meishan synthetic gilts, PRLR genotype did not affect estrus length, but estrus cycle length tended to be longer for AA gilts compared to AB and BB gilts (Chapter 6). AA gilts had a significantly higher ovulation rate than BB gilts, resulting in a (non significant) higher number of vital embryos at Day 35. The AB gilts were intermediate for these traits. The weight of the ovaries before and after subtraction of the weight of the corpora lutea was affected by PRLR genotype, i.e. the weight was highest for AA gilts, lowest for BB gilts and intermediate for AB gilts at Day 35 of pregnancy. Unlike AB gilts, AA and BB gilts were able to adapt uterine length to litter size, which led to longer uteri for AA gilts compared to BB at Day 35 of pregnancy. This was accompanied by heavier placentae and larger implantation surface areas for embryos of AA gilts compared to embryos of BB or AB gilts. The differences in uterine length and placental size confirm the suggestion that the difference in ovulation rate and vital embryos may lead to a subsequent difference in litter size, presumed that the PRLR genotype does not affect fetal survival (Chapter 6). This predicted difference in litter size actually was found in a different line studied. PRLR genotype affected total number born and number of piglets born alive of Large White x Meishan F2 crossbred gilts ($P<0.08$), without affecting litter mean of birthweight. PRLR AA gilts had the largest and BB gilts the smallest litters, while litter size of PRLR AB gilts was in between (Chapter 7). Furthermore, for the same line, PRLR genotype significantly affected age at first estrus, and as a result (since the gilts were inseminated at a fixed estrus number) age and body weight at insemination ($P<0.05$). BB gilts were significantly younger at first estrus and younger and lighter at insemination than AA gilts. The effects on age at first estrus and on litter size were two independent effects of PRLR polymorphism (Chapter 7). PRLR genotype affected placental weight and efficiency (calculated as birthweight divided by placental weight), resulting in a lighter, more efficient placenta for the

litter mean of AA gilts compared to the litter mean of BB gilts. The difference probably was related to the difference in litter size, since it disappeared after correction for total number of piglets born. Another effect of PRLR genotype in Large White x Meishan F2 crossbred gilts, was the significant effect on litter mean of functional teat number ($P<0.05$), despite the lack of effect of PRLR genotype on own teat number. Piglets from BB mothers had on average larger numbers of functional teats compared to piglets from AA mothers. In conclusion, PRLR gene is a major gene or a marker for age at first estrus, litter size and litter average of number of functional teats in the Large White x Meishan F2 crossbred gilts studied. The favorable allele for litter size (A allele) is the unfavorable allele for age at first estrus and litter mean of functional teat number (Chapter 7).

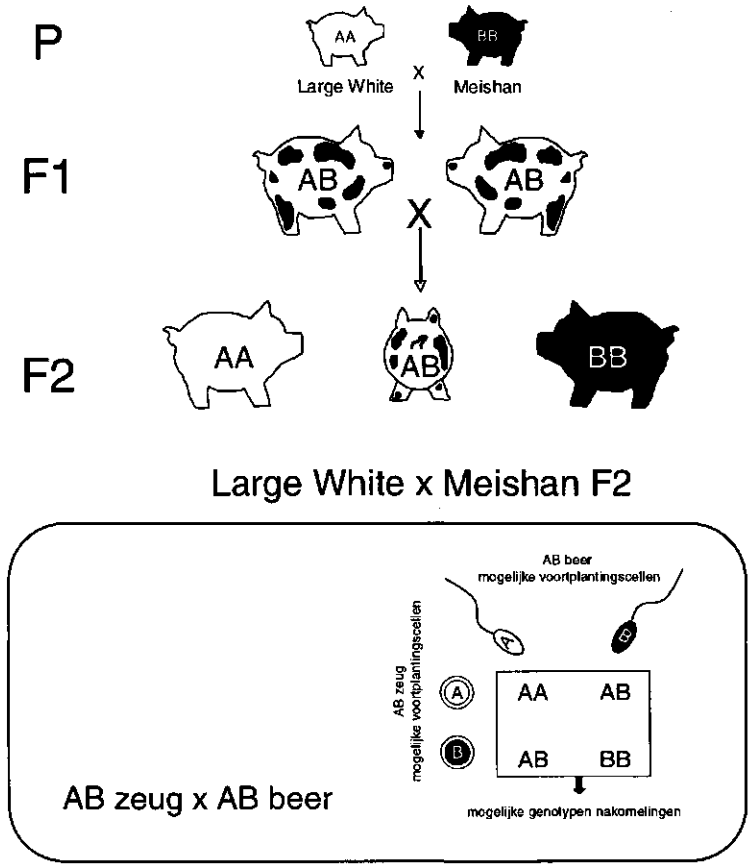
The results of the present thesis clearly indicate that the two polymorphisms affect different components of litter size that are expressed at different stages of pregnancy. PRLR genotype appears to affect ovulation rate, while ESR genotype appears to affect fetal survival. This implies that there are favorable and unfavorable PRLR/ESR genotype combinations for litter size. The favorable PRLR/ESR genotype combination for litter size of L93 Meishan Synthetic gilts and Large White x Meishan F2 crossbred gilts is AA/BB and AA/AB respectively (Chapter 8). Dependent on whether the genes are the actual major genes for litter size or merely markers for closely linked major genes for litter size, the favorable genotype combinations might be different in different lines. Combining the limited knowledge about the site of the mutation, structure and function of the gene product, the biological role of the gene product in relation to differences in physiology found between different genotypes, and the litter sizes in relation to the different genotypes, has resulted in the hypothesis that the ESR gene is a marker for litter size (Chapter 8). Using the same strategy for the PRLR gene does not exclude the possibility of PRLR gene being the major gene rather than a marker for a closely linked major gene for litter size, although further investigation on larger data sets is recommended (Chapter 8). Since other genes (e.g. ESR gene) and also environmental factors might change the effect caused by the PRLR polymorphism whithin the 112 days to parturition, at present it is preferable to state that PRLR gene is a major gene for ovulation rate rather than for litter size.

Like every marker assisted selection, selection on the favorable genotype combination will lead to an enlargement of litter size once-only. Furthermore,

results of the present thesis are an example of marker alleles having positive and negative effects at the same time, making it difficult to use the marker for selection. The favorable PRLR allele for litter size for example, appears to be the unfavorable allele for age at first estrus and litter average of teat number of the piglets. This problem seems to be a biological reality animal scientists will have to live with. It clearly demonstrates the importance of physiological research parallel to and coherent with the search for QTLs and markers for any trait.

Samenvatting

De Nederlandse samenvatting is uitgebreider dan de Engelse.
Bovendien bevat de samenvatting ter verduidelijking figuren en schema's.



Figuur 1. Uitleg basisinformatie. Tevens kruisingsschema van de productie van de proefdieren voor het tweede experiment.

Bij de bevruchting krijgt een embryo van elk gen twee allelen (die niet per se van elkaar hoeven te verschillen), één van de moeder en één van de vader. De allel-combinatie die het dan bezit voor een bepaald gen wordt genotype genoemd. Doordat er van het ESR gen twee verschillende allelen (A en B) bestaan, kan een dier dus drie verschillende ESR genotypen hebben: AA, AB, of BB.

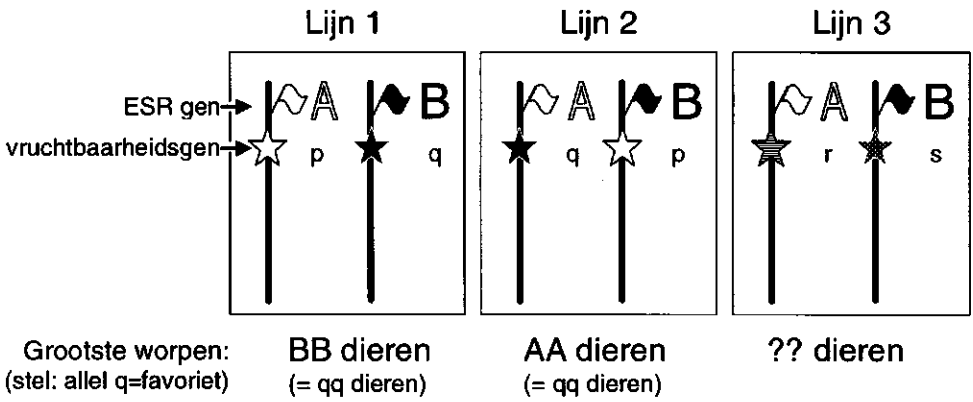
Het genotype van een dier hangt af van het genotype van zijn ouders. Een AA dier heeft van zowel vader als moeder een A allel gekregen, terwijl een AB dier van een van de twee ouders een A allel en van de andere ouder een B allel heeft gekregen. Een AA dier zal altijd een A allel aan zijn nakomelingen doorgeven. Als een AB dier zich echter voortplant, zal het voortplantingscellen (eicellen of spermacellen) hebben die een A allel bevatten, maar ook voortplantingscellen die een B allel bevatten. Welk allel doorgegeven wordt aan de nazaat is dus helemaal afhankelijk van welke voortplantingscel de gelukkige winnaar is.
Wanneer AA zeugen gedekt worden met AA beren, zullen al hun biggen dus AA zijn (immers, de zeug heeft alleen A voortplantingscellen en de beer ook). Wanneer AA zeugen gedekt worden met BB beren zullen al hun biggen AB zijn, omdat de zeug alleen A voortplantingscellen heeft en de beer alleen B voortplantingscellen. AA zeugen die gedekt worden met AB beren zullen echter gemiddeld voor de helft AA biggen en voor de helft AB biggen krijgen, omdat de helft van de voortplantingscellen van de beer een A allel bevatten en de helft een B allel. In het kader: Van de biggen van AB zeugen en AB beren zal de helft AB zijn, een kwart AA, en een kwart BB.

Inleiding

Voor de varkenshouderij is het economisch gezien belangrijk dat varkens grote worpen hebben. Men probeert dan ook op allerlei manieren de worpgrootte op peil te houden (goede voeding, goed stalklimaat, management). Bovendien proberen fokkerij-instanties door middel van selectie het aantal biggen per worp te verhogen. De oudste methode van selecteren op worpgrootte is het simpelweg alleen maar doorfokken met zeugjes die uit een grote worp komen. Tegenwoordig is het echter zelfs mogelijk te selecteren op gen-niveau. Het genpatroon van een organisme is al direct na de bevruchting bekend (de helft van het genetisch materiaal komt van de vader en de andere helft van de moeder). Men kan dus al in een zeer vroeg stadium nagaan of een big (of zelfs embryo) een gunstige genetische aanleg voor worpgrootte heeft. Om te selecteren hoeft men dan niet meer op de worpgegevens van een varken te wachten. Een voorwaarde hiervoor is wel, dat er genen bekend zijn met verschillende verschijningsvormen (*allelen*), waaronder tenminste één gunstigere, waarop men dan kan selecteren.

In Amerika heeft men inmiddels twee genen beschreven welke veelbelovend lijken te zijn wat betreft worpgrootte: Het *oestrogeen receptor (ESR)* gen en het *prolactine receptor (PRLR)* gen. Voor beide genen zijn twee verschillende verschijningsvormen (allelen) ontdekt (Voor uitleg "allelen" en "genotype", zie Figuur 1).

De allelen van het ESR gen worden A en B genoemd, en toevalligerwijs worden ook de allelen van het PRLR gen A en B genoemd. Voor verschillende varkenslijnen is aangetoond dat de ESR BB dieren grotere worpen hebben dan de ESR AA dieren. De verschillen zijn het duidelijkst bij de eerste worp (zie tabel 4 in hoofdstuk 4). Men noemt het ESR gen bij varkens dan ook wel een "vruchtbaarheidsgen", en er wordt ook al echt geselecteerd op het B allel in bepaalde lijnen. Het is echter helemaal niet zeker dat het ESR gen zélf voor het verschil in worpgrootte zorgt. Het is ook mogelijk dat de verschillende allelen van het ESR gen een soort van vlaggetjes zijn voor verschillende allelen van het eigenlijke oorzakelijke gen. Dat oorzakelijke gen moet dan zo dicht bij het ESR gen liggen, dat men als men op het ESR B allel selecteert, tegelijkertijd op het daaraan gekoppelde voor vruchtbaarheid gunstige allel van dat andere gen selecteert. In dit geval is het ESR gen niet het vruchtbaarheidsgen zelf, maar een *merker* voor een vruchtbaarheidsgen (Figuur 2).



Figuur 2. *Merkers.*
Het is niet bekend of het ESR gen het eigenlijke vruchtbaarheidsgen is; het kan ook een merker voor een ander gen (het echte vruchtbaarheidsgen) zijn dat er zeer dicht bij in de buurt ligt.
Stel dat het echte vruchtbaarheidsgen vier verschillende allelen, p t/m s kent, waarbij q het gunstige allel en p het ongunstige allel voor worpgrootte. De koppeling van het A en B allel met een allel van het vruchtbaarheidsgen hoeft dan niet bij iedere lijn per sé hetzelfde te zijn. Het ESR B allel kan in de ene lijn bijvoorbeeld gekoppeld zijn aan het gunstige allel van het eigenlijke vruchtbaarheidsgen (in dit voorbeeld "q"), terwijl het bij een andere lijn gekoppeld kan zijn aan het ongunstige allel ("p") van het echte vruchtbaarheidsgen, of aan een ander allel ("r", of "s"). Dit laatste is afhankelijk van het aantal allelen dat er van eigenlijke vruchtbaarheidsgen bestaan. Dieren van Lijn 1 met ESR genotype BB zullen de grootste worpen hebben (omdat ze dan tevens qq zijn). In Lijn 2 zullen de dieren met ESR genotype AA echter juist de grootste worpen hebben (omdat ze dan tevens "qq" zijn).

Over het PRLR gen is tot op heden slechts één publicatie verschenen in relatie tot worpgrootte. Hieruit blijkt nog niet duidelijk dat het PRLR gen de worpgrootte beïnvloedt. Toch wordt er geconcludeerd dat het gen zeer waarschijnlijk ook een vruchtbaarheidsgen is.

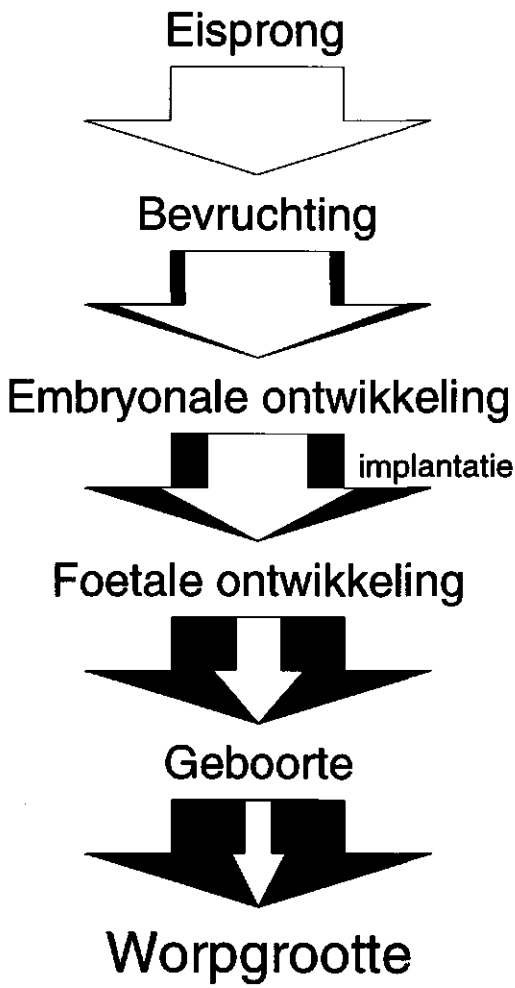
Het doel van het in dit proefschrift beschreven onderzoek was om een fysiologische verklaring te geven voor de relatie tussen het hebben van een bepaald genotype en het hebben van grotere of kleinere worpen. Met andere woorden: wat doet dat ene gen (wat gebeurt er in het varken), dat het zoveel invloed heeft op worpgrootte? Om deze vraag te kunnen beantwoorden zijn twee grote experimenten uitgevoerd. Het eerste experiment had als doel na te gaan op welk moment van de dracht de worpgrootteverschillen ontstonden en om zoveel mogelijk karakteristieken van de verschillende genotypen te registreren. Het tweede experiment had als doel de in het eerste experiment gevonden verschillen nader uit te diepen. Beide experimenten waren opgezet om het eerst beschreven kandidaat-gen voor vruchtbaarheid, het ESR gen, te toetsen. Pas later is van de proefdieren nagegaan wat voor PRLR genotype ze hadden. Voor beide experimenten zijn varkens die nog nooit eerder hebben geworpen (*gelten*) als proefdieren gebruikt. De worpgrootteverschillen tussen varkens met verschillende ESR genotypen bleken bij gelten namelijk groter dan bij meerdereworps zeugen.

Om verwarring te voorkomen zal het eerste deel van de samenvatting alleen betrekking hebben op het ESR gen en het tweede deel op het PRLR gen. Het laatste deel zal vervolgens het geheel combineren.

Oestrogeen receptor (ESR) gen

ESR gen, dag 35 van de dracht

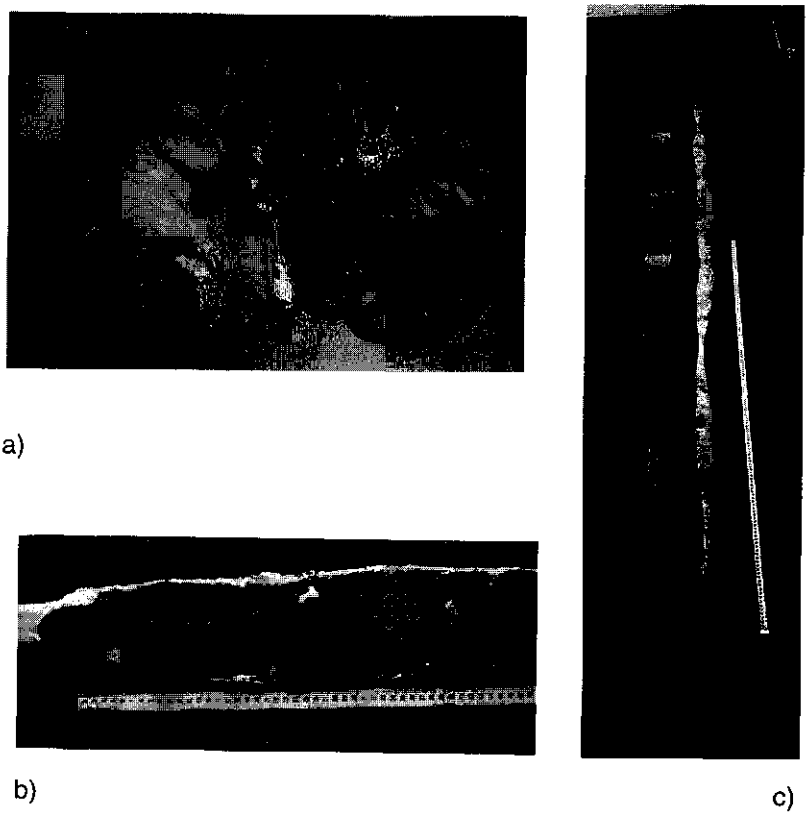
In het eerste experiment is onderzocht op welk moment van de dracht de verschillen in worpgrootte precies ontstonden. Het traject "dracht" is namelijk te verdelen in een aantal opeenvolgende gebeurtenissen die elk beperkend kunnen zijn voor de worpgrootte (Figuur 3). Zo is worpgrootte achtereenvolgens afhankelijk van het aantal eisprongen (*ovulaties*), het aantal eicellen dat bevrucht wordt, het aantal bevruchte eicellen dat zich tot embryo ontwikkelt, het aantal embryo's dat zich tegen de baarmoederwand "nestelt" (implantatie) en zich tot foetus ontwikkelt, het aantal foeten dat de baarmoeder kan dragen (baarmoedercapaciteit), en het



Figuur 3. *Het traject "dracht" is te verdelen in een aantal opeenvolgende gebeurtenissen die elk beperkend kunnen zijn voor de worpgrootte. Zo is worpgrootte achtereenvolgens afhankelijk van het aantal eisprongen (ovulaties), het aantal eicellen dat bevrucht wordt, het aantal bevruchte eicellen dat zich tot embryo ontwikkelt, het aantal embryos dat zich tegen de baarmoederwand "nestelt" (implantatie) en zich tot foetus ontwikkelt, het aantal foeten dat de baarmoeder kan dragen (baarmoedercapaciteit), en het aantal volgroeide foeten dat het geboorteproces (de partus) overleeft. Dag 35 van de dracht wordt meestal gezien als het moment waarop alle organen in aanleg aanwezig zijn, en men dus van een foetus gaat spreken.*

aantal volgroeide foeten dat het geboorteproces (de partus) overleeft. Dag 35 van de dracht wordt meestal gezien als het moment waarop alle organen in aanleg aanwezig zijn, en men dus een embryo een "foetus" gaat noemen. Nadat een eisprong heeft plaatsgevonden, wordt het blaasje waar die eicel uitkwam omgezet tot een duidelijk te onderscheiden bolletje weefsel op de eierstok, het *corpus luteum* genaamd. Dit corpus luteum blijft gedurende de hele dracht op die eierstok aanwezig, en produceert een hormoon dat de dracht in stand houdt. Op ieder moment van de dracht is dus nog te tellen hoeveel eisprongen er hebben plaatsgevonden.

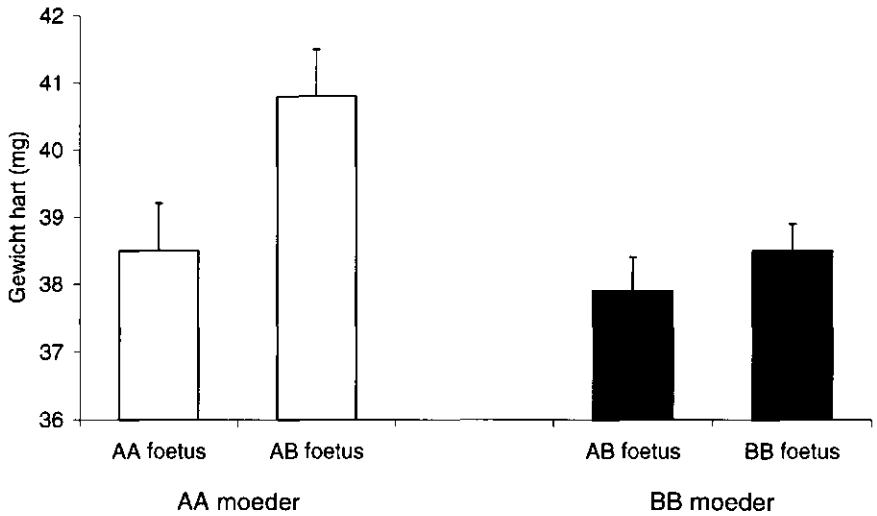
Voor het eerste experiment zijn L93 Meishan synthetic gelten gebruikt. Van deze lijn was aangetoond dat ESR BB dieren grotere worpen hadden dan ESR AA dieren. De gelten zijn geïnsemineerd en op dag 35 van de dracht geslacht. Vervolgens is het aantal corpora lutea op de eierstokken, het aantal embryo's en het aantal implantatieplaatsen (te onderscheiden als roodgekleurde vlakken in de baarmoeder; Figuur 4) bepaald. Bovendien zijn diverse gewichten en/of lengtes bepaald (eierstokken, corpora lutea, embryo's, nageboortes, baarmoederhoorns, implantatieplaatsen). De ESR AA en BB dieren bleken niet te verschillen qua aantal eisprongen en embryo's (hoofdstuk 2). Uit dit gegeven alleen kon al worden geconcludeerd (er van uitgaande dat de BB dieren grotere worpen zouden hebben gehad dan de AA dieren als ze niet op dag 35 waren geslacht) dat het verschil in worpgrootte ontstaat na dag 35 van de dracht. Kennelijk kan de baarmoeder van de AA dieren niet alle embryo's herbergen en grootbrengen tot voldragen biggen; kennelijk is de baarmoedercapaciteit van de AA dieren kleiner dan van de BB dieren. Het enige verschil dat gevonden werd tussen de AA en BB dieren was het verschil in grootte van de nageboorten: Gemiddeld hadden embryo's van BB moeders grotere (langere) nageboorten dan die van AA moeders. De grootte van de nageboorte en de mate van haar doorbloeding zijn zeer belangrijk voor de overlevingskans van een varkensfoetus. De nageboorte sluist namelijk voedingsstoffen van (baar)moeder door naar embryo en later foetus. Hoe groter de nageboorte, hoe groter het contactoppervlak met de baarmoeder, hoe beter de mogelijkheden om voedingsstoffen door te sluizen, en hoe hoger dus de kans op overleving van de foetus. Op dag 35 van de dracht is de nageboorte gemiddeld een factor 11 à 12 langer dan het embryo. Haar gemiddelde lengte zal dan over het algemeen nog niet beperkend zijn voor de groei van het embryo. De nageboorte stopt echter met groeien rond dag 60 van de dracht, het moment waarop de foetus juist aan een enorme groeispurt begint en dus hard bouwstoffen nodig heeft. De geringere gemiddelde lengte van de nageboorten van dag 35



Figuur 4. *a) Baarmoeder van een varken dat 35 dagen drachtig is.*
b) Geopende baarmoeder met een 35 dagen oud foetje in de vruchtvliezen; de vruchtvliezen worden samen "nageboorte" genoemd. De vruchtvliezen zijn normaalgesproken gevuld met vocht, het vruchtwater. Op de foto is het binnenste vruchtvlies, het amnion, nog intact. Het buitenste vruchtvlies (met de bloedvaatjes) is echter opengeknipt, en het vruchtwater verwijderd.
c) Losgeknipte baarmoederhoorns van een 35 dagen drachtig varken (meetlat = 1.5 m). De linker hoorn is opengeknipt; de vruchtvliezen en foeten zijn verwijderd. Er is echter nog precies te zien waar ze gelegen hebben: de implantatieplaatsen zijn als donkere vlakken te zien.

embryo's in AA moeders zal dus zeer goed een beperking op een later tijdstip van de dracht kunnen vormen, en er zodoende voor kunnen zorgen dat er foetussen vroegtijdig sterven.

Bij het varken is de nageboorte volledig ontstaan uit embryonaal weefsel. De grootte van deze nageboorte is afhankelijk van omgevingsfactoren (bijvoorbeeld de plooiing van de baarmoederwand en het aantal -voor ruimte concurrerende- foeten per baarmoederhoorn), maar ook van haar genetische aanleg, welke hetzelfde is als de genetische aanleg van de foetus. Het gevonden verschil in grootte van de nageboorten tussen AA en BB moeders zou dus ook best (mede) kunnen afhangen van het ESR genotype van de foetus zelf. Immers, een AA moeder kan, afhankelijk van het ESR genotype van de beer waardoor ze gedekt is, zowel AA als AB foeten in een worp hebben. Op dezelfde wijze kan een BB moeder zowel BB als AB foeten in een worp hebben (zie Figuur 1). Nageboorten van AB foeten zouden best wel eens anders binnen AA baarmoeders kunnen groeien dan binnen BB baarmoeders. Bovendien zouden de lengten van AA en AB nageboorten binnen een AA moeder ook best wel eens van elkaar kunnen verschillen. Om eventuele effecten van het ESR genotype van de foetus in samenhang met het ESR genotype van de moeder te onderzoeken, werden alle foeten getypeerd voor ESR. Geen van de kenmerken (implantatie-oppervlakte, lengte en gewicht van nageboorte of foetus) werd beïnvloed door het ESR genotype van de foetus in samenhang met het ESR genotype van de moeder (Hoofdstuk 3). Met andere woorden, er werden geen verschillen gevonden tussen de verschillende moeder-foetus ESR genotype combinaties. De verschillen tussen AA en BB moeders wat betreft de gemiddelde lengte van de nageboorten van hun embryo's was dus volledig toe te schrijven aan het genotype van de moeder. Het hartgewicht van de foetus daarentegen verschilde wel tussen de verschillende ESR genotype combinaties: AB foeten van AA moeders hadden op dag 35 van de dracht zwaardere hartjes vergeleken met de andere drie bestudeerde genotype combinaties (Figuur 5). Afhankelijk van het ESR genotype van de moeder, was het hartgewicht gerelateerd aan het gewicht van de foetus: hartjes van foeten van BB moeders waren zwaarder naarmate de foeten zwaarder waren, terwijl hartjes van foeten van AA moeders bij elk foetgewicht even zwaar waren (Figuur 1c in Hoofdstuk 3). Het is nog niet helemaal duidelijk wat dit betekent. Het hartje van een dag 35 foetus moet het bloed niet alleen door de foetus heenpompen, maar ook door alle bloedvaten van de op dat moment relatief kolossale nageboorte. Verschillen in grootte en doorbloeding van de nageboorte op dag 35 van de dracht zouden dus best een gevolg kunnen hebben voor de grootte (en dus het gewicht)



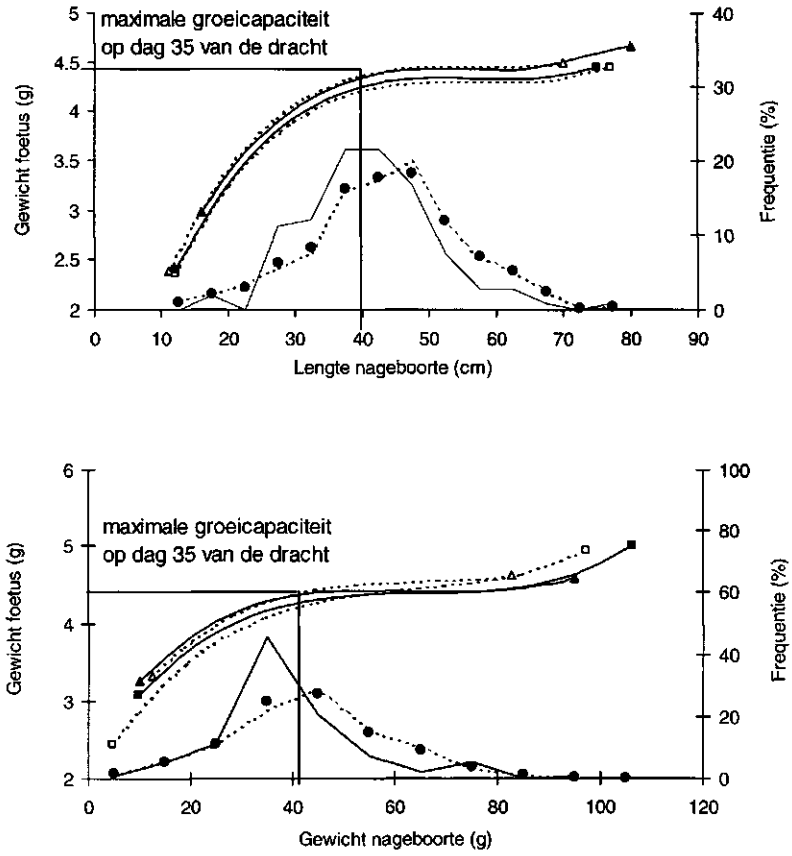
Figuur 5. *AB foeten van AA moeders hadden op dag 35 van de dracht zwaardere hartjes vergeleken met de andere bestudeerde genotype combinaties (AA foeten van AA moeders en AB en BB foeten van BB moeders).*

van het foetale hartje. Of andersom, ten gevolge van een verschil in hartgrootte kan de doorbloeding van de nageboorte zich wel eens verschillend ontwikkelen. Nader onderzoek in die richting wordt dan ook aanbevolen (Hoofdstuk 3).

Zoals reeds beschreven, wordt er aan de grootte en doorbloeding van de nageboorte een belangrijke functie wat betreft overlevingskans toegeschreven. Omdat een grotere nageboorte een groter oppervlakte heeft om voedingsstoffen door te sluizen, zou je verwachten dat foeten met een grotere nageboorte zelf ook groter zijn. Dit is op dag 35 van de dracht echter slechts ten dele het geval. Op dat ene moment in de dracht zijn de foeten die een heel erg kleine (lichte en korte) nageboorte hebben inderdaad het kleinst. Naarmate de nageboorte groter is, zijn de bijbehorende foeten ook zwaarder. Dit gaat echter alleen op voor de nageboorten die korter en lichter zijn dan 40 cm en 40 g. Foeten met langere en zwaardere nageboorten (ongeveer de helft van alle foeten die bekeken zijn), zijn op dag 35 van de dracht allemaal even zwaar (Figuur 6). Dit duidt erop dat de foeten op dag 35 een maximale groeicapaciteit hebben; op dat moment maakt het voor het gewicht van een foetus niet uit, of het een nageboorte van 40 cm heeft of eentje van 80 cm. De nageboorte van 80 cm heeft echter een grote lengte voorsprong op die van 40 cm, hetgeen op een later tijdstip in de dracht een voordeel op kan leveren (bijvoorbeeld als de beschikbare baarmoederruimte in het gedrang komt). De foeten welke op dag 35 een nageboorte hebben die kleiner en lichter is dan 40 cm en 40 g lopen qua gewicht op dat moment al achter op hun leeftijdgenootjes met een grotere nageboorte. Ze zullen daardoor een kleinere kans hebben om de dracht te overleven. Binnen de ESR AA moeders blijkt het percentage van de AA foeten dat de maximale groeicapaciteit heeft bereikt kleiner dan het percentage van de AB foeten dat de maximale groeicapaciteit heeft bereikt. Dientengevolge is het percentage dat qua gewicht achtergebleven is, groter. Binnen de BB moeders worden dit soort verschillen niet gevonden. Op grond van dit gegeven, wordt de voorspelling dat de kans op foetale sterfte in AA moeders groter zal zijn dan in BB moeders, uitgebreid tot de voorspelling dat binnen de AA moeders de kans of foetale sterfte van AA foeten groter zal zijn dan van AB foeten (Hoofdstuk 3).

ESR gen, rond ovulatie

Naast de hierboven vermelde bestudeerde kenmerken, is bij een gedeelte van de proefdieren bestudeerd hoe de zogenaamde profielen van drie verschillende



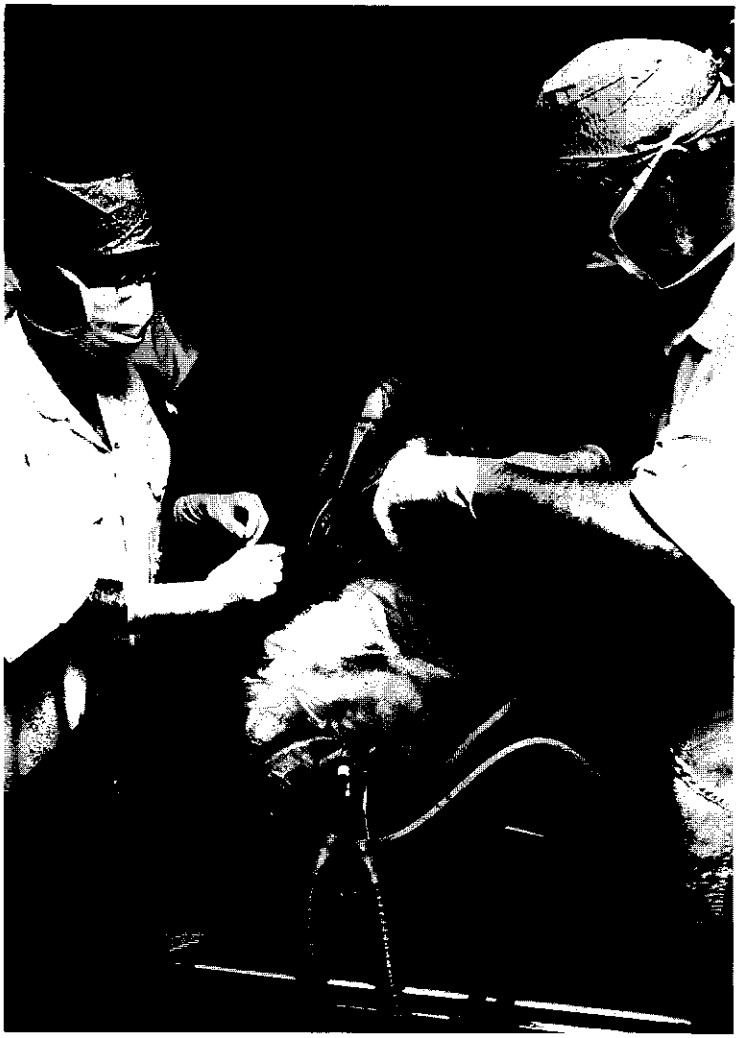
Figuur 6. *Relatie tussen de lengte (a) of het gewicht (b) van de nageboorte en het gewicht van de foetus op dag 35 van de dracht. Onafhankelijk van het genotype van de foetus en zeug (de vier lijnen in de grafiek met aan de uiteinden een vierkant of driehoek), zijn foetusen met de kleinste nageboorten lichter dan die met grotere nageboorten. Dit gaat op tot een gewicht of lengte van 40 g of 40 cm; foetusen met grotere nageboorten zijn op dag 35 van de dracht allemaal even zwaar. Onder op de assen staat de frequentieverdeling van de AA foeten (doorgetrokken lijn) en de gemiddelde frequentieverdeling van de overige foeten (gestippelde lijn). Er zijn relatief meer AA foeten met een kleinere nageboorte (de verdeling ligt iets meer naar links).*

hormonen er rond het moment van insemineren uitzagen. Hiertoe werden rond het voorspelde moment van berigheid (gemiddeld worden gelten en zeugen om de 21 dagen berig) om de vier uur bloedmonsters genomen. Bij de dieren was een canule aangebracht (Figuur 7), waardoor ze niet iedere keer geprikt hoefden te worden, maar het bloed stressloos "afgetapt" kon worden. De drie hormonen waarvan de concentraties in die bloedmonsters werden gemeten, zijn oestrogeen, LH en progesteron. Rond de eisprong en bevruchting veranderen de concentraties van deze hormonen enorm. De mate van deze veranderingen bleek niet te verschillen tussen ESR AA en BB gelten; de gemiddelde hormoonprofielen waren zo goed als identiek (zie Figuur 1 in hoofdstuk 2).

ESR gen, vlak na het werpen

Naar aanleiding van de resultaten van het eerste experiment, werd in het tweede experiment getoetst of kenmerken van de nageboorten direct na de geboorte ook nog beïnvloed werden door het ESR genotype van de moeder en/of van de big zelf. Tijdens het werpen komen bij het varken over het algemeen eerst alle biggen af, en pas later alle nageboorten. Er is dan niet meer te zien welke nageboorte bij welke big hoort. Een big die geboren wordt, zit vaak nog met de navelstreng vast aan de nageboorte (die nog in de baarmoeder zit; Figuur 8). Normaal gesproken zal deze navelstreng als de big begint te lopen knappen. Door de big bij de geboorte op te vangen, kan dit worden voorkomen. Door vervolgens een gelabeld touwtje om de navelstreng te binden, daarna de navelstreng tussen big en label door te knippen en de big direct te voorzien van een nummer dat correspondeert met het label, is later vast te stellen welke nageboorte bij welke big behoorde.

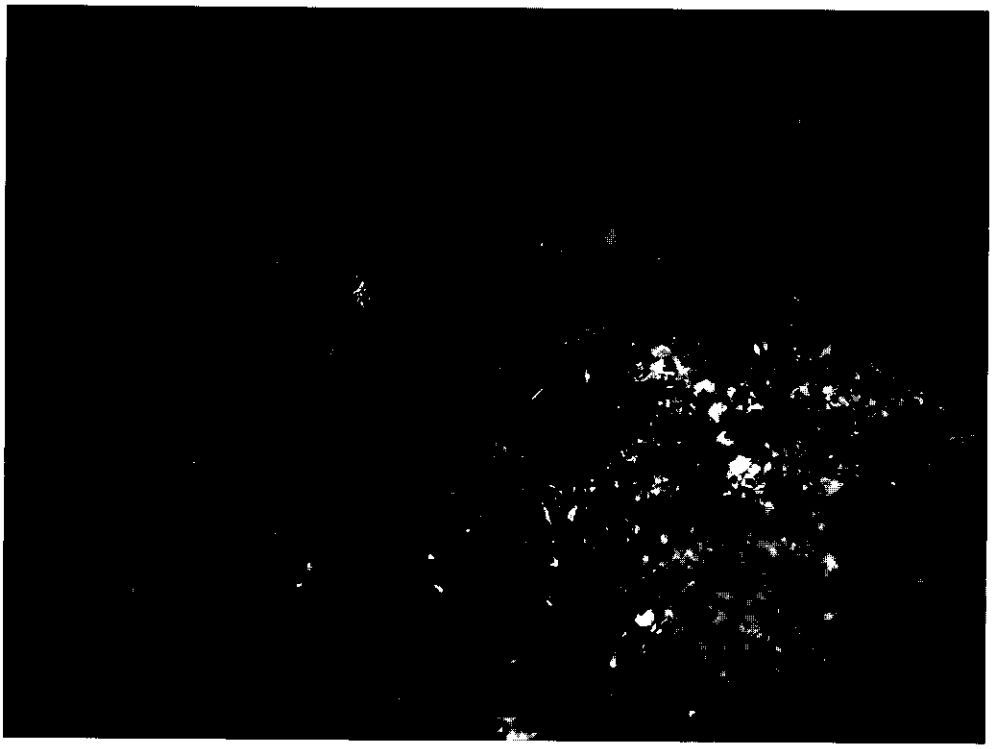
In het tweede experiment werden de nageboorten aldus tijdens het werpen van een label voorzien. Het experiment werd uitgevoerd met 62 op de proefaccommodatie De Haar Zodiac Wageningen gefokte Large White x Meishan F2 gelten (Figuur 9). Het kruisingsschema met ESR genotype staat weergegeven in Figuur 1. Van deze lijn was niet bekend of het ESR genotype effect had op worpgrootte. Daarom werd de worpgrootte voor een groot aantal (275) F2 gelten met een verschillend ESR genotype vergeleken. Het resultaat was verrassend: De AB dieren bleken de grootste worpen te hebben (Hoofdstuk 4). De worpen van de AB dieren waren significant groter dan die van de BB dieren, maar vergelijkbaar met die van de AA dieren. Verder waren er echter geen verschillen in worpgrootte. Op grond van deze resultaten werd geconcludeerd dat in de bestudeerde lijn, in



Figuur 7. *Bij de dieren werd een canule aangebracht, waardoor het bloed stressloos "afgetapt" kon worden.*

tegenstelling tot al het tot op heden gepubliceerd onderzoek, het ESR A allel het gunstige allel voor worpgrootte lijkt te zijn. Als dit zo is, is de kans ook groot dat het ESR gen niet het echte vruchtbaarheidsgen is, maar een merker, welke bij de "Large White x Meishan F2" dieren aan een ander allel gekoppeld zit, dan bijvoorbeeld bij de "L93 Meishan Synthetic" dieren (Figuur 2). In Hoofdstukken 4 en 8 wordt dit aspect bediscussiëerd.

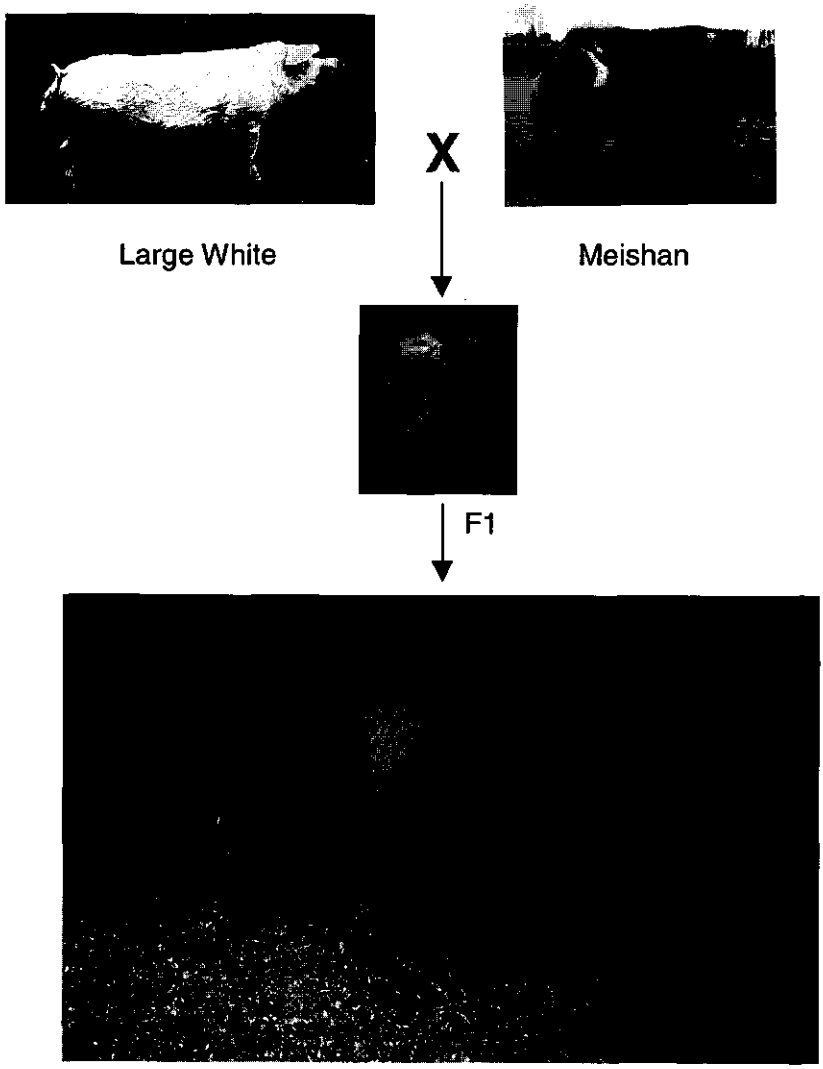
Het gemiddelde gewicht, gemiddelde oppervlak en de gemiddelde lengte van de nageboorten *a term* (dus precies na werpen) verschilde niet tussen ESR AA , AB en BB zeugen (Hoofdstuk 4). De biggen van AB zeugen hadden echter gemiddeld gezien een veel lager aantal areolae op de nageboorten dan de biggen van BB zeugen. Bovendien hadden de nageboorten van de biggen van de AB zeugen gemiddeld gezien een veel lagere areola dichtheid (aantal areolae per cm^2 nageboorte) dan de biggen van zowel AA als BB zeugen (Hoofdstuk 4). De *areolae* zijn als kleine witte rondjes op de nageboorte te onderscheiden. Het zijn gespecialiseerde deeltjes van de nageboorte welke elk precies over een klieropening van de baarmoeder liggen. Het aantal areolae op een nageboorte zegt dus iets over het aantal klieropeningen op de baarmoederwand waarmee die nageboorte in contact heeft gestaan. Het zegt echter nog niets over het totaal aantal klieropeningen dat de baarmoederwand heeft (ook niet als je alle areolae van alle nageboorten bij elkaar optelt). Dit heeft verschillende redenen. Mede afhankelijk van de worpgrootte, zullen stukjes baarmoeder welke wel klieropeningen bezitten niet bezet zijn. De baarmoederwand is tijdens de dracht zeer sterk geplooid. De nageboorte sluit zo goed als naadloos op die plooien aan. De baarmoederklieren en dus ook hun openingen komen in de eerste drie maanden van het varkensleven tot ontwikkeling, en blijven dan de rest van het leven op een vaste plaats in de baarmoederwand liggen. Afhankelijk van de plooiing van de baarmoederwand, welke pas tijdens de dracht echt goed op gang komt, komen de klieropeningen verder (sterke plooiing) of dichter (minder sterke plooiing) bij elkaar te liggen, alhoewel hun onderlinge afstand "hemelsbreed" misschien niet eens verschilt. Op het moment dat de nageboorte loslaat van de baarmoederwand, worden diens plooien (welke overeenkwamen met de plooiing van de baarmoederwand) rechtgestreken. De areola dichtheid op de nageboorte is dus mede afhankelijk van de mate van plooiing van de baarmoederwand. De "hemelsbrede" afstand tussen baarmoederklieropeningen is afhankelijk van de strekking van de baarmoeder. Tijdens de dracht neemt nl. de volledige baarmoeder in lengte toe (voor foto's van een varkensbaarmoeder, zie Figuur 4). De mate van toename in lengte is ten dele afhankelijk van het aantal embryo's dat zich in de



Figuur 8. *Een big die geboren wordt, zit vaak nog met de navelstreng vast aan de nageboorte (die nog in de baarmoeder zit).*

baarmoeder bevindt (onderzoek heeft aangetoond, dat voor ieder extra embryo, de baarmoeder ca 10 cm in lengte toeneemt), maar uiteraard ook van de genetische aanleg om in lengte toe te nemen. Al met al is er dus niet voldoende bekend over de baarmoederwand, om directe conclusies over de baarmoederklieren te trekken uit de gegevens van de areolae op de nageboorte. Toch blijft het gevonden gegeven dat biggen van AB dieren veel minder areolae op hun nageboorte hebben dan die van AA en BB dieren zeer frappant. De baarmoederklieren zijn gespecialiseerd om bepaalde voedingsstoffen te produceren. De areolae zijn erop gebouwd om deze stoffen op te nemen en door te sluizen naar de foetus. Als de biggen van AB dieren minder areolae op hun nageboorten hebben, hebben ze ook met relatief minder klieren in contact gestaan. Het zou dus kunnen zijn dat ze minder van die gespecialiseerde voedingsstoffen hebben ontvangen. Het zou echter ook kunnen zijn dat de klieren in een AB moeder veel actiever zijn geweest. Ondanks het verschil in areola dichtheid, verschilde het geboortegewicht van biggen van AB moeders namelijk niet van dat van de biggen van AA moeders (Hoofdstuk 4).

Evenals bij het eerste experiment zijn de kenmerken van de nageboorten ook geanalyseerd op bigniveau, om na te gaan of het ESR genotype van de big (binnen het genotype van de moeder) nog van belang was. Daar de moeders dit keer ESR genotype AA, AB of BB hadden, waren er in totaal 7 verschillende ESR moeder/big genotype combinaties (zie uitleg bij Figuur 1) Het gevonden verschil in gemiddeld aantal areoale tussen biggen van AB moeders en AA en BB moeders bleek volledig toe te schrijven aan het ESR genotype van de moeder. Ook het gewicht, lengte, en oppervlakte van de nageboorte bleek niet beïnvloed te worden door het genotype van de big (Hoofdstuk 5). Toch werden er diverse verschillen tussen de genotype combinaties gevonden. Kenmerken welke werden beïnvloed waren het amiongewicht (het amnion is het binnenste deel van de nageboorte, het eerste vruchtvlies dat het embryo en later de foetus omhult; het dient met name ter bescherming), het gewicht van de nageboorte wanneer gecorrigeerd wordt voor het oppervlak (wat dan overblijft is eigenlijk de dikte van de nageboorte), de efficientie van de nageboorte (berekend als het geboortegewicht gedeeld door het gewicht van de nageboorte), en de relatie van geboortegewicht tot het gewicht van de nageboorte en de relatie van geboortegewicht tot het aantal areolae (Hoofdstuk 5). Alhoewel het niet steeds dezelfde genotypecombinatie was die bij de genoemde kenmerken afweek, geven de resultaten aan dat tijdens de ontwikkeling van de foetus zowel ESR genotype van moeder als van big van belang zijn voor



Figuur 9. *Het tweede experiment werd uitgevoerd met zelf gefokte Large White x Meishan F2 gelten. (Voor kruisingsschema, zie Figuur 1).*

bepaalde kenmerken van de nageboorte, met name als het gewicht van de nageboorte of de areolae bij het kenmerk betrokken zijn.

Alle levendgeboren biggen van het experiment zijn tot drie weken na geboorte elke week gewogen. De biggen van de BB moeders (dus van de kleine worpen) hadden de neiging sneller te groeien in die drie weken dan die van de AA en AB moeders. De biggen zijn tijdens de drie weken nooit overgelegd, dus het verschil zou kunnen zijn ontstaan door een verschil in concurrentie. Om dit met zekerheid te stellen zullen echter andere experimenten opgezet moeten worden. Vooralsnog lijkt het voor worpgrootte gunstige A allel het ongunstige allel voor de groei van de biggen vóór spenen (Hoofdstuk 5).

Prolactine receptor (PRLR) gen

Van de proefdieren van beide uitgevoerde experimenten is later ook nog bepaald wat voor genotype ze voor het PRLR gen hadden. Alle gemeten kenmerken zijn vervolgens getoetst op zeugniveau, d.w.z. voor de gemiddelde waarden per zeug is getoetst of ze beïnvloed zijn door PRLR genotype.

PRLR gen, dag 35 van de dracht

Van de L93 Meishan synthetic gelten van het eerste experiment is naderhand bepaald wat voor genotype ze voor het PRLR gen hadden. Vervolgens is bestudeerd of het PRLR genotype (er waren 9 AA, 25 AB en 22 BB gelten) effect had op de gemeten kenmerken (Hoofdstuk 6). De PRLR AA dieren bleken veel meer eisprongen te hebben dan de BB dieren (AB lag er tussenin). Dientengevolge was ook het aantal embryo's hoger voor de AA dieren. Dit laatste was niet statistisch significant. Toch werd voorspeld dat de AA dieren uiteindelijk de grootste worpen zouden hebben. Dit onder de aanname dat er geen verschillen in baarmoedercapaciteit tussen de verschillende PRLR genotypen zou bestaan. De baarmoederlengtes en kenmerken van de nageboorten leken de voorspelling te bevestigen. Zoals reeds beschreven past de baarmoederlengte zich aan het aantal embryo's dat er in aanwezig is aan. Als AA dieren dus echt meer embryo's dan AB en BB (en eventueel AB) dieren hebben, is een langere baarmoeder bij de AA dieren verwachten. Dit bleek inderdaad het geval: AA dieren hadden langere en zwaardere baarmoeders dan de AB en BB dieren. De AA baarmoeders hadden

dus de ruimte om meer embryo's te dragen. Embryo's van AA moeders bleken bovendien elk voor zich ook nog eens over meer ruimte te beschikken en die ook daadwerkelijk te gebruiken: Zowel het implantatieoppervlak als het gewicht van de nageboorten bleek binnen AA moeders gemiddeld groter dan binnen AB en BB moeders. Zowel AA als BB dieren bleken in staat de baarmoederlengte aan te passen aan het aantal embryo's, dit in tegenstelling tot de AB dieren. Met andere woorden, evenals BB dieren hadden AA dieren met een groter aantal embryo's langere baarmoeders dan AA dieren met kleinere worpen. De baarmoederlengte van AB dieren was onafhankelijk van het aantal embryo's.

PRLR gen, vlak na werpen

77 van de Large White x Meishan F2 gelten van het tweede experiment zijn naderhand getypeerd voor PRLR genotype. De worpgrootte van de AA gelten bleek groter dan die van de BB gelten (Hoofdstuk 7). Hiermee gepaard waren de nageboorten van de biggen van BB gelten gemiddeld iets groter dan die van de AA gelten (dus waarschijnlijk hebben het kleiner aantal foeten in een BB baarmoeder meer ruimte gehad dan het grotere aantal foeten in een AA baarmoeder, en dus ook de kans gehad om grotere nageboorten te ontwikkelen). Het gemiddelde geboortegewicht van de biggen verschilde echter niet, en dientengevolge was de efficientie van de nageboorte van biggen van AA moeders hoger dan van biggen van BB moeders.

Het PRLR genotype had ook effect op de leeftijd waarop de dieren in puberteit kwamen. De PRLR BB gelten kwamen gemiddeld 41 dagen (dus zeg maar twee cycli) eerder in puberteit dan de AA gelten. Het effect van PRLR genotype op worpgrootte en leeftijd van puberteit waren twee onafhankelijke effecten. Een derde effect van PRLR genotype was het effect op het gemiddelde aantal spenen van de biggen. Biggen van BB moeders hadden gemiddeld meer spenen dan biggen van AA moeders. Het PRLR genotype had echter geen effect op het aantal spenen van de moeders zelf.

De AB moeders zaten wat betreft alle hierboven genoemde kenmerken tussen de AA en BB moeders in.

Conclusies

In Hoofdstuk 8 zijn de hierboven beschreven resultaten bediscussiëerd. Een van de belangrijkste conclusies is dat de twee bestudeerde genotypen twee totaal verschillende processen, die op verschillende momenten van de dracht plaatsvinden, beïnvloeden. Het PRLR genotype heeft effect op het aantal eisprongen, terwijl het ESR genotype effect heeft op de foetale overleving. Dit impliceert dat er voor worpgrootte gunstige en ongunstige PRLR/ESR genotype combinaties bestaan. Het feit dat beide genotypen op verschillende momenten van de dracht hun invloed op worpgrootte uitoefenen, kan tevens verklaren waarom sommige auteurs geen verschillen in worpgrootte tussen verschillende ESR genotypen vinden. Het PRLR genotype was in die onderzoeken niet bekend en zou wel eens het ongunstige kunnen zijn geweest. Het aantal eisprongen vormt de eerste voor worpgrootte beperkende schakel (zie Figuur 3). Een worp is nooit groter dan het aantal eisprongen. Is het aantal eisprongen laag, dan heeft een grote baarmoedercapaciteit geen effect!

Tot op heden vormen beide genen een unieke mogelijkheid om op een vroeg stadium te selecteren op aantal eisprongen (PRLR gen) of op foetale overleving (ESR gen), dit ongeacht het feit of het vruchtbaarheidsgenen of merkers zijn. Zoals bij alle selecties op favoriete allelen of merkers, zal de vooruitgang die geboekt wordt bij selectie een slechts éénmalige vooruitgang zijn. Na selectie heeft het dier immers het gewenste genotype en kan men dus niet nog een keer op hetzelfde gen selecteren. Het gevaar van het volledig uitsluiten van de voor worpgrootte ongewenste allelen is bediscussiëerd in Hoofdstuk 8. Beide genen coderen voor een eiwit dat een zeer breed scala aan functies bezit, niet alleen op het gebied van vruchtbaarheid maar ook op andere gebieden als afweerstysteem, groei, en differentiatie. Het voor worpgrootte ongunstige allel kan voor een ander kenmerk dus misschien wel juist het gunstige zijn. Een voorbeeld hiervan word ook al beschreven in dit proefschrift. Het PRLR B allel is het ongunstige allel voor worpgrootte, maar het gunstige allel voor leeftijd begin puberteit, en aantal spenen van de biggen. Voorbeelden als deze vormen een waarschuwing voor het merkeronderzoek in het algemeen: een merker-allel kan tegelijkertijd positieve en negatieve effecten hebben.

Ondanks deze waarschuwing blijven de twee genen zeer uniek: Het zijn de enige twee genen die een duidelijke relatie met worpgrootte bij het varken vormen (alhoewel voor het PRLR gen meer onderzoek gewenst is om dit hard te maken

voor verschillende lijnen). Het ESR gen is het enige tot op heden beschreven gen waarvan de allelen iets zeggen over de foetale overleving bij het varken. Daar deze foetale overleving een van de laatste schakels in het draagproces vormt, is het belangrijk dat het niet de beperkende schakel is. Vandaar dat de kennis over het ESR genotype zeer waardevol kan zijn (mits bekend is welk allel het voor foetale overleving gunstige allel is bij dat varken).

De kwestie "merker of vruchtbaarheidgen" wordt zeer uitgebreid bediscussiëerd in hoofstuk 8. De resultaten van dit proefschrift doen zeer sterk vermoeden dat het ESR gen niet het vruchtbaarheidsgen zelf is, maar een merker. Uiteraard doet dit niets af aan de waarde. Het levert alleen een extra punt van voorzichtigheid bij het selecteren: als men op het gunstige ESR allel selecteert, selecteert men tevens op een gunstig allel van een ander gen. Dat andere gen is nog niet bekend, en dus kan men zich ook nog niet direct verdiepen in de mogelijke "neveneffecten" van de selectie.

BEDANKT / THANK YOU !!!

Vaak heb ik van Tette moeten horen "jij wil ook altijd alles alleen doen!" Als ik echter op een rij zet welke mensen er allemaal meegeholpen hebben aan de tot standkoming van dit proefschrift, komt er een golf van namen voorbij. Een aantal daarvan wil ik jullie niet onthouden.

Allereerst het stalpersoneel. Zonder hen zouden de proeven helemaal niet uitgevoerd hebben kunnen worden. Ben van den Top, Ries Verkerk, André Jansen, Marijke Giesbers, en ook alle anderen die met name tijdens de weekenden voor mijn varkentjes zorgden: Bedankt !! In de stallen was het heerlijk; alles kon; hadden we wat nodig, dan werd het gemaakt; een rommelhokje werd binnen een dag omgetoverd tot een laboratorium. Geen gezeur, niet eerst allerlei formuliertjes invullen, nee gewoon actie! Tijdens mijn verblijf in de stallen heb ik ontzettend veel van jullie over varkens geleerd. Ik zal mijn eerste tocht met Hannibal om 12 uur 's nachts echter niet gauw vergeten; ik was doodsbang! Twee jaar lang lagen de kraamhokken vol met mijn biggen. Een goede reden dus om af en toe, als ik het rekenen en schrijven beu was even te gaan kijken (en kletsen). Het is heerlijk ontspannend om tien minuten op je hurken naar een hoopje versgeboren slapende biggen te zitten kijken!

Piet de Groot en Henk Vos. Twee trouwe assistenten. Bloed tappen, pipetteren, berigheidscontroles en moederkoekhappen op de meest waanzinnige tijden. "En dat alles voor de wetenschap". Piet, al vanaf het prille begin betrokken bij het project. Al het voorwerk wat de proefdieren aangaat is dankzij jouw goede organisatie en registratie prima verlopen. Toen ik nog niet wist dat ik AIO zou worden, was jij al druk in de weer met het fokken van mijn proefdieren. Ook jij hebt me van alles over het varken geleerd. Ik moet je echter helaas bekennen dat het me nog steeds niet duidelijk is wat je nu met "het orenspel" bedoelde.... Henk, koeienman in hart en nieren. Ineens werd je gebombardeerd tot assistent in de varkensbusiness. Met een nimmer aflatend enthousiasme wist je menig bloedje te tappen en moederkoekje te vangen. Ook jij herinnert je je eerste tochtje met Hannibal waarschijnlijk nog wel! (Wat is zo'n hokje klein hè.) Bedankt, ook voor al je gezellige verhalen die het lange wachten op de nageboorten wat korter deden lijken. En dat moederkoekhapboek-of-records komt er nog een keertje aan!

Jascha Leenhouwers, moederkoekhapper nummer 1! Door de varkenspest zat je plotseling aan mijn proef vast. Tussen alle vitaliteitstesten in zat daardoor ook jij iedere dag 6 uur in de stallen te waken. Ik ben benieuwd of de bonsai-big er nog ooit van komt. Ook de studenten, stagiaires en tijdelijke medewerkers wil ik niet ongenoemd laten; Frans Vermeulen, Bart Spee, Baukje Vlemmix, Nicole Ng-A-Tham, en Anette van Dorland, die ons allen heel wat werk uit handen hebben genomen.

Al het practische werk werd buiten de leerstoelgroep Fokkerij en Genetica uitgevoerd. Diverse andere groepen werden zodoende "verblijd" met mijn aanwezigheid. "Die fokker van beneden" wil onder andere alle medewerkers van de toen nog vakgroep Veehouderij bedanken. Niet alleen voor het gebruik maken van hun laboratoriumruimte (hordelopen over baarmoeders, het verdragen van de geuren van nageboorten en het luisteren naar het ritmisch geklik bij het tellen van alle areolae), maar ook voor de bitterballen die ze mij tijdens hun frequente borrels toestopten. Met naam wil ik Frits Rietveld en Wouter Hazeleger noemen. Frits, dankzij jou wisten wij feilloos bloed te tappen van een gecanuleerde zeug. Als er een canule verstopt zat hoefden we maar te piepen en jij kwam ons te hulp. Bovendien zorgde je ervoor dat er steeds voldoende alcohol, stikstof, spuiten, watten, buisjes, etc. waren. (De watten die ikzelf een keertje voor nood kocht liggen er nog steeds...). Wouter, meester in het canuleren. Dankzij jou, de gastvrijheid van het Ilob en de hulp van Dick van Cleef, Truus en Adri, hadden we ook daadwerkelijk gecanuleerde zeugen. Over gastvrijheid gesproken; ook Ko van Ginkel en zijn medewerkers van het noodslachthuis in Veenendaal wil ik bedanken dat ik (met die "lelijke varkens") zelfs in de weekenden en op feestdagen bij hen de vloer plat mocht lopen. Corrie Oudenaarden, Elène Vos en Jo Erkens: Hormoonbepalingen, dat wordt dus echt nóóit mijn hobby! Henk Schipper en Ronald Booms: Nicole, Anette en ikzelf brachten vele uren in jullie histologielab door. We begrijpen met z'n allen nu nog steeds niet waarom alle monsters minstens één keer opnieuw ingebed moesten worden. Na de inbedperikelen zijn er toch nog vele coupes gesneden en gekleurd. De resultaten ervan staan uiteindelijk niet eens in het proefschrift!

Help also came from abroad, amongst others from employees of PIC group: A lot of DNA samples had to be genotyped for ESR and PRLR. When I started in 1996 I got an email-address from a Richard Wales, to who I could send all the DNA samples. Richard, I always have enjoyed all our correspondence through email, and I think it is a pity we never met. The same counts for Kerry Harvey: As far as I

know, Kerry was the one who actually did all the genotyping. When Richard got a new job, Alan Mileham was my new contact-person. Alan, once again my apologies that I did not recognize your name when I actually sat next to you during a diner! Last but not least, Hein van der Steen and Graham Plastow: I really appreciated all your very fast replies and "cheer ups" after minor or major disappointments.
Four years ago, I met Professor Ford for the first time. His presentation at the ICPR in Rolduc (The Netherlands) inspired me to do the experiment we got used to call the "moederkoekhapexperiment". By email Professor Ford guided me through the first principles of catching, labeling, fixing and staining of placenta samples. Professor Ford: After a huge experiment I was eager to discuss our results with you. Unfortunately, however, it still took some time to create all these results, since there was also another experiment that had to be finished. Therefore I am glad and honoured that you are willing to discuss them with me during my defence.
A very special word of thank to the Editorial Assistant of Theriogenology, John Patterson: Please remember, if I ever will win that Nobel price, the promised bottle of champagne will be sent to you!

Vanaf december 1998 vervaagden de grenzen tussen AIO's van verschillende leerstoelgroepen en universiteiten. Discussiegroep TOPIG werd opgericht. Alle leden en ex-leden van TOPIG: Heel hartelijk bedankt voor jullie informatie, enthousiasme en verschillende filosofieën. De diversiteit van alle besproken onderwerpen heeft bij mij zeker bijgedragen tot een bredere kijk op (het doen van) onderzoek.

Kamergenoten (Carolien de Ruyter-Spira, Erik Baaijens, Anette van Dorland, Margaret Nkomo, Pim van Hooft), AIO zusjes (aanvankelijk het ABC team Ant Vollema en Carolien, en later Annemieke Rattink en Esther Baart), en het kloppend hart van de leerstoelgroep (Ada Wiggerman en Maria Lippelt): Bedankt voor jullie oren, humor, schouders, dropjes, chocolade, zwemmen en nog veel meer!

Uiteraard wil ik ook mijn begeleidingsgroep van harte bedanken: Bas Kemp en Nicoline Soede, en de scheppers van dit project; Pim Brascamp en Tette van der Lende. Tette, de bescheiden duizendpoot met een geheugen dat omgekeerd evenredig is aan zijn geduld. Ik denk dat maar weinig mensen beseffen hoeveel jij doet. Begeleiders als jij zijn uniek, en dan niet alleen vanwege je tradities waar andere begeleiders een voorbeeld aan kunnen nemen (voortdurende aanvoer van relevante artikelen, tracteren als het eerste artikel is geaccepteerd, etc). Naast al je

reguliere bezigheden draaide je als volwaardig vierde bloedtapper alle diensten mee. De (voor velen in die tijd mysterieuze) schriftjes zijn slechts een tipje van de ijsberg van hoeveel plezier we met z'n vieren tijdens dat experiment hebben gehad. Gelukkig heb ik je ervan weten te weerhouden om tijdens het tweede experiment iéder weekend 24 uur te komen helpen (dat houdt immers niemand vol!). Tja, toen besloot je dan maar om elk tweede weekend die 24 uur te draaien.... (waarmee je de rest van het moederkoekhapteam overigens menig moment van rust bood). Ik hoop echter dat je me nu wél gelooft als ik zeg dat iets best wel veel werk zal zijn! Je hebt heel wat te stellen gehad met die eigenwijze AIO die als ze iets niet begrijpt maar door blijft drammen en die inderdaad al het reken en schrijfwerk zelf wilde doen! Echter niet om de gegevens voor zichzelf te houden, maar om ervan te leren en te ontdekken waarvoor ze al dat practische werk gedaan had. Daarnaast echter ook een beetje om anderen er van te overtuigen dat het "goed kunnen opschieten met je begeleider" niet synoniem is met "al het denkwerk overlaten aan je begeleider".

In de loop der jaren zijn een aantal sociale contacten behoorlijk verwaterd. Niet iedereen heeft immers hetzelfde dag/nacht ritme. Turbo heeft het geheel niet overleefd. Hetzelfde geldt voor de accordeonlessen en het lidmaatschap van het Wagenings Volkooren. Te vaak moest ik iets afzeggen of kon ik niks met zekerheid afspreken omdat er "wel eens een geltje berig kon worden" of "was uitgeteld". Zelfs de anders zo spontane telefoongesprekken moesten worden gepland. Gelukkig boden de post en de email uitkomst. Dus, alle trouwe schrijvers en bellers en alle anderen die niet persoonlijk in dit dankwoord genoemd worden, familie, vrienden en bekenden, die me met hun leuke en minder leuke verhalen en avonturen in de afgelopen jaren hebben blijven doen beseffen dat er meer op deze wereld is dan wetenschap: Merci!

Mamma en pappa;
Ik houd van jullie, en daarom draag ik dit proefschrift aan jullie op !!

LIST OF PUBLICATIONS

Refereed articles

Van Rens BTTM, Hazeleger W, and Van der Lende T (2000). Periovulatory hormone profiles and components of litter size in gilts with different estrogen receptor (ESR) genotypes. Theriogenology 53:1375-1387.

Van Rens BTTM, and Van der Lende T (2000). Fetal and placental traits at Day 35 of pregnancy in relation to the estrogen receptor genotype in pigs. Theriogenology 54:843-858. Erratum figures in Theriogenology 55 (2001) 1029-1032.

Van Rens BTTM, and Van der Lende T (2001). Litter size and piglet traits of gilts with different prolactin receptor genotypes. Accepted by Theriogenology.

Steerenberg PA, De Jong WH, Geerse E, Aleva BJ, Besselink CMLT, Van Rens BTTM, Rutten VPMG, Poels LG, Scheper RJ, Den Otter W, and Ruitenberg EJ (1990). Major histocompatibility complex class II antigen expression during potentiation of line-10 tumor immunity after intralesional administration of BCG. Cancer immunology immunotherapy 32:95-104.

Rombout JHWM, Van Rens BTTM, Sijtsma SR, Van der Weide MC, and West CE (1992). Effects of vitamin A deficiency and Newcastle Disease virus infection on lymphocyte subpopulations in chicken blood. Veterinary Immunology and Immunopathology 31:155-166.

Koumans-Van Diepen JCE, Taverne-Thiele JJ, Van Rens BTTM, and Rombout JHWM (1994). Immunocytochemical and flow cytometric analysis of B cells and plasma cells in carp (Cyprinus carpio L.); an ontogenic study. Fish and Shellfish Immunology 4:19-28.

Verburg- Van Kemenade BM Lidy, Groeneveld Adrie, Van Rens Birgitte TTM, and Rombout Jan HWM (1994). Characterization or macrophages and neutrophilic granulocytes from the phonephros of carp (Cyprinus carpio L.). Journal of Experimental Biology 187:143-158.

Abstracts

Van Rens BTTM, and Stroband HWJ (1989). Ultrastructural study of porcine maternal endometrium and embryo during implantation. The Netherlands Journal of Zoology 39:107-108 (abstr).

Van Rens BTTM, Van der Lende T, De Groot PN, and Van der Steen HAM (1997). The estrogen receptor (ESR) gene and components of litter size in Meishan synthetics. Proceedings of the 5th ICPR, Maastricht, The Netherlands, 2-4 June 1997, p 112 (abstr).

Van der Lende T, and Van Rens BTTM (2000). The effect of the prolactin receptor gene on ovulation rate and embryonic survival in gilts. 14th International Congress on Animal Reproduction Stockholm, 2-6 july 2000. Abstracts, volume 1, p 142 (abstr).

Van Rens BTTM, Vos H, De Groot PN, and Van der Lende T (2000). Effects of the estrogen receptor gene on the porcine placenta. 14th International Congress on Animal Reproduction Stockholm, 2-6 july 2000. Abstracts, volume 1, p 169 (abstr).

Van Rens BTTM, and Van der Lende T (2000). Effect of prolactin receptor (PRLR) gene polymorphism on litter size and placental traits in gilts. Journal of Reproduction and Fertility, Abstract Series 26, p12 . SSF Wintermeeting, Utrecht, 15-16 December 2000 (abstr).

Van Rens BTTM, and Van der Lende T (2000). Litter size and placental traits in gilts with different oestrogen receptor (ESR) genotypes. Journal of Reproduction and Fertility, Abstract Series 26, p13. SSF Wintermeeting, Utrecht, 15-16 December 2000 (abstr).

Van Rens BTTM, De Groot PN, Vos H, and Van der Lende T (2001). The effect of prolactin receptor (PRLR) gene polymorphism on reproductive traits in gilts. Proceedings of the 6th ICPR, Columbia, Missouri, USA, 2-6 June 2001, p 112 (abstr).

Van der Schans A, Van Rens BTTM, Van der Westerlaken LAJ, and De Wit AAC (1992). Bovine embryo production by repeated transvaginal oocyte collection and in vitro fertilization. Proceedings of the 12th ICPR 1992, The Hague, The Netherlands, p 1366-1368 (abstr).

Van der Lende T, and Van Rens BTTM (2000). Timing of foetal mortality in gilts on the basis of the length distribution of mummified foetuses and frequency of non-fresh stillborn piglets. Journal of Reproduction and Fertility, Abstract Series 26, p 13. SSF Wintermeeting, Utrecht, 15-16 December 2000 (abstr).

Miscellaneous

Van Rens BTTM, and Van der Lende T (1997). The estrogen receptor gene and prolificacy in the pig. Proceedings of the WIAS seminar "What have we learned from the Meishan pig?", Wageningen, 21 May 1997 (6 pages).

Van Rens BTTM, and Van der Lende T (1997). The estrogen receptor gene and prolificacy in the pig. Proceedings of National Pig Reproduction Meeting, organized by the Netherlands Foundation for the Study of Animal Reproduction (SDV), Kerkrade, dd. 5 June 1997, p41-46.

Van Rens B. Baarmoedercapaciteit heeft invloed op worpgrootte. Zoektocht naar het maximum in worpgrootte. Varkens, Vakblad voor de varkenshouderij no. 12, p 50-51 (21 september 1999).

Van Rens BTTM (2000). The porcine placenta. Proceedings of the WIAS seminar "The placenta", Wageningen, 13 April 2000 (14 pages).

Van Rens B. Betere resultaten door selecteren op genniveau. Grotere worpen vergen juiste keuze vruchtbaarheidsgenen. Varkens, Vakblad voor de varkenshouderij no 3, p 48-49 (20 maart 2001).

Van der Lende T en Van Rens B (1996). Vruchtbaarheidsgen sleutel tot grotere worpen. Gentechniek biedt kansen om produktie te verbeteren. Boerderij/Varkenshouderij 81-no. 17 (20 augustus 1996).

Van der Lende T, Van Rens BTTM, and Leenhouwers JI (2000). Biological and genetic aspects of pre- and perinatal mortality in swine. Proceedings of the Vth Seminário Internacional de Suinocultura, San Paulo, Brazil, September 27-28, 2000, 125-141.

CURRICULUM VITAE

Birgitte Theodorus Theresia Maria van Rens werd in Meijel geboren op 14 januari 1964. Nietsvermoedend snoof ze daar voor het eerst de luchten van haar toekomstige proefdieren op. Ze behaalde in 1982 haar VWO diploma aan Rijks Scholen Gemeenschap "Den Hulster" te Venlo, en begon dat zelfde jaar haar studie Biologie-nieuwe stijl aan de Landbouw Hogeschool te Wageningen.
In 1988 studeerde ze af aan Landbouwuniversiteit Wageningen. Haar afstudeervakken waren Ontwikkelings- en Voortplantingsbiologie (Ultrastructuur van uterusepitheel en embryo gedurende de implantatie bij het varken), en Gezondheids- en Ziekteleer (Invloed van vitamine A deficiëntie en/of Newcastle Disease Virus infectie op de celgebonden immuunrespons van kuikens). Bovendien volgde ze tijdens haar studie de Lerarenopleiding eerste fase en het vak Onderwijskunde II (oude stijl) aan de vakgroep Pedagogiek en Didactiek, met bijbehorende stage aan het Rhedens Lyceum te Velp. Haar praktijktijd bracht ze door op de afdeling Immunopathologie van het RIVM (Rijksinstituut voor Volksgezondheid en Milieuhygiëne) te Bilthoven, alwaar ze met behulp van lymfocytenstimulaties en de FACS onderzoek deed naar lymfocytensubpopulaties betrokken bij Line-10 tumor immuniteit van de cavia.
Van april 1989 tot september 1990 was ze aangesteld als universitair docent aan vakgroep EDC (Experimentele Diermorfologie en Celbiologie), sectie Celbiologie, van Landbouwuniversiteit Wageningen. Het onderzoeksdeel betrof met name electronenmicroscopisch en FACS-onderzoek naar leucocytensubpopulaties van de karper, en een studie naar de mogelijkheden en beperkingen van cellsorting m.b.v. FACS.
Van november 1990 tot juni 1991 volgde ze een HBO opleiding informatica, alwaar ze de ambimodulen HE0 t/m HE2 en HB1 behaalde.
Van juni 1991 tot december 1992 werkte ze als wetenschappelijk medewerker aan het IVO-DLO (Instituut voor Veeteeltkundig Onderzoek, Dienst Landbouwkundig Onderzoek), afdeling Voortplanting. Hiertoe was ze op proefbedrijf "Het Gen" te Lelystad gestationeerd. Zij deed daar onderzoek naar de optimalisatie van in vitro maturatie, fertilisatie en ontwikkeling van rundereicellen verkregen door middel van transvaginale follikelpunctie.
Van juni 1993 tot april 1994 werkte ze als medewerker studentenadministratie aan Hogeschool Diedenoort Wageningen. In de maanden februari en maart 1994 was ze daar bovendien docente Biologie.
Van mei 1994 tot januari 1996 was ze wetenschappelijk medewerker aan het Hubrecht Laboratorium te Utrecht, standplaats Vakgroep Bedrijfsdiergeneeskunde

en Voortplanting, Universiteit Utrecht. In het kader van een samenwerkingsproject met Holland Genetics, Genus (UK) en Innogenetics BV (België), voerde ze daar een studie uit naar de bruikbaarheid van een door Innogenetics BV geproduceerd synthetisch maturatie- en kweekmedium voor de verbetering van in vitro maturatie, fertilisatie en ontwikkeling van rundereicellen.
In januari 1996, tenslotte, begon ze als assistent in opleiding aan vakgroep Veefokkerij van Landbouwuniversiteit Wageningen (inmiddels omgedoopt tot leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit) aan het promotie onderzoek zoals beschreven staat in dit proefschrift. De bijbehorende opleiding genoot ze bij onderzoeksschool WIAS (Wageningen Institute of Animal Sciences).