Influence of SLA haplotype on ovulation rate and litter size in miniature pigs*

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Summary. Systemic blood was collected from and surgery performed on sows of 3 strains of miniature swine bred for specific SLA (swine MHC) haplotypes (a, c and d) from Day 2 to Day 6 after mating (first day of mating = Day 0). Ovulation rate was determined by counting corpora lutea and embryos were flushed from the uterus. Progesterone, oestradiol-17 β and oestrone were quantitated in blood plasma and uterine flushings by RIA. SLA^{d/d} females had a higher ovulation rate than SLA^{a/a} or SLA^{c/c} females (11.50 ± 0.87 vs 9.11 ± 0.68 and 8.17 ± 0.83 , respectively; $P < 0.01$). Oestrone was higher than oestradiol-17 β in systemic plasma (56.5 ± 6.4 vs 33.0 ± 4.7 pg/ml, $P < 0.01$) while oestradiol-17 β was higher than oestrone in uterine flushings (19.8 ± 1.4 vs 14.9 ± 1.5 pg/horn, $P < 0.10$). Systemic progesterone concentration was correlated with day after mating ($r = 0.93$, $P < 0.01$). There was no effect of haplotype on any of the hormone concentrations measured. Litter size was analysed from 99 matings amongst SLA^{a/a}, SLA^{a/c}, SLA^{a/d}, SLA^{d/c} and SLA^{d/d} sires and dams. Litter size from -/d and d/d sows or from d/d boars were larger ($P < 0.05$) than for all other matings. Although ovulation rate was higher in SLA^{d/d} sows, the significant effect of sire SLA genotype on litter size suggests an additional effect of the d haplotype on embryonic survival.

Keywords: pig; major histocompatibility complex; litter size

Introduction

Successful reproduction in mammals is dependent on many biological phenomena including the rates of ovulation, fertilization and conceptus survival. Relatively little is known about the genetic control of these processes, but there has been increasing interest in the possible influence of genes of the major histocompatibility complex (MHC) on these and other aspects of reproduction (Warner, 1986). The MHC genes code for cell surface proteins which mediate cell–cell interactions in the immune response and allow the immunological recognition of self from non-self. The developing fetus expresses surface antigens foreign to the maternal system and it has been suggested that this histoincompatibility may provide a stimulus which is beneficial to fetal survival (Lewis *et al.*, 1986; Rodger & Drake, 1987). Pig conceptuses express β_2 -microglobulin which may reflect the presence of MHC antigens as early as Day 12 after mating (Meziou *et al.*, 1983) and it has been reported that presensitization of sows to paternal antigens increases litter size (Murray *et al.*, 1983). These observations suggest that immunological

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reactions between developing pig embryos and the dam may influence subsequent embryonic survival.

The identification of the MHC system in pigs (SLA, swine leucocyte antigen complex; Vaiman *et al.*, 1970) has allowed the investigation of more specific interactions among the sire, dam and conceptuses with respect to their degree of histocompatibility and how this ultimately affects reproduction (Koch, 1985). For instance, Vaiman & Renard (1980) reported that SLA heterozygous offspring are more common than predicted for some matings, based on the known SLA haplotypes of the parents. The development of distinct genetic lines of miniature pigs with specific SLA haplotypes (Sachs *et al.*, 1976) has aided research in this area.

The present paper focusses on data collected from SLA inbred miniature pigs. SLA type is determined by both the maternal and paternal genotype. To separate the influence of the maternal uterine environment from that of the genes of the embryo itself on embryonic survival, one can examine the effect of paternal type on litter size. The present study reports the analysis of litter records of matings involving miniature sows and boars homozygous and heterozygous for several SLA haplotypes. In addition, ovulation rate was determined and concentrations of oestradiol-17 β , oestrone and progesterone were quantitated in systemic blood and/or uterine flushings after embryo recovery from Day 2 to 6 of gestation in miniature sows homozygous for three SLA haplotypes.

Materials and Methods

Ovulation rate and hormone analyses. Miniature sows homozygous for the haplotypes SLA^{a/a} (N = 9), SLA^{c/c} (N = 6) and SLA^{d/d} (N = 8) were utilized for the embryo-collection phase of this experiment. Food and water were withheld for 24 h before surgery which was performed between Days 2 and 6 after mating (Day 0 = first day of oestrus). Before surgery, a blood sample was withdrawn from the anterior vena cava, and plasma was collected and frozen (–20°C) for hormone analyses. Anaesthesia was induced with sodium thiamylal (Surital: Parke-Davis Laboratories, Morris Plains, NJ; 667 mg/kg body wt) and maintained with a mixture of oxygen and halothane (fluothane: Ayerst Laboratories, New York, NY) as previously described (Magness & Ford, 1982). The reproductive tract was exposed through a midventral incision. Corpora lutea (CL) were counted and embryos were flushed from each uterine horn with 20 ml Medium 199 (Gibco, Grand Island, NY). The flushings were collected at the uterotubal junction through a funnel-tipped glass tube inserted through the uterine wall and firmly secured in the lumen with surgical silk. On Days 2 or 3 after mating, 20 ml medium were flushed from the infundibulum through the oviduct to the uterotubal junction using a blunt-tipped 12-gauge needle and a 20-ml glass syringe. From Day 4 to Day 6, each uterine horn was flushed twice with 20 ml medium from the base towards the uterotubal junction by insertion of a sterile 18-gauge 3.8 cm needle through the uterine wall. Embryos flushed from the uterus were then counted utilizing a dissecting microscope. From the first flush of each horn, 5 ml medium were saved and frozen (–20°C) for steroid analyses. Blood plasma and flushing media were analysed for progesterone, oestradiol-17 β and oestrone as previously described and validated in this laboratory (Magness & Ford, 1982). The sensitivities of the assays were 50–80 pg progesterone, 2 pg oestradiol-17 β and 2 pg oestrone. Within-assay variabilities for plasma and flushing medium were, respectively, 5.8 and 5.5% for progesterone, 9.2 and 8.4% for oestradiol-17 β and 5.4 and 6.2% for oestrone. Inter-assay variabilities for plasma and medium were, respectively, 12.1 and 10.2% for progesterone, 15.1 and 12.3% for oestradiol-17 β and 11.6 and 14.1% for oestrone.

Data were evaluated by analysis of variance, and by correlation and linear regression analyses (Statistical Analysis System, 1985).

Litter size. Miniature pigs were housed at the Iowa State University pig breeding farm at Madrid, Iowa, and maintained under conditions of management similar to those used for commercial pigs. These miniature pigs with 3 different haplotypes were derived from a single mating (Sachs *et al.*, 1976) and therefore are highly related. Our herd of miniature swine was established by importation from NIH in 1982 and retains inbreeding coefficients in excess of 0.50. On average, genetic relationship among animals exceeds 75% and therefore these animals offer a unique resource not found in most livestock. The description of litter data collected from 1982 to 1987 is shown in Table 1. The 99 litter records resulted from matings of 7 different SLA genotypes for both sires and dams, including one sire for which the SLA genotype was not determined. These data were evaluated by analysis of variance procedures using the Statistical Analysis System (1985) programs. The effect of SLA genotype on litter size was analysed by using a model which included the effect of presence of a particular parent SLA genotype (sire or dam) and the effect of sire or dam nested within each genotype. The data were first analysed by sire SLA genotype and then separately again by dam SLA genotype.

Table 1. Number of litters from matings listed by SLA genotype of sires (N = 17) and dams (N = 35)

SLA genotypes		No. of litters
Sire	Dam	
a/a	a/a	16
	a/d	1
	c/c	2
	c/d	1
	d/d	2
a/c	a/c	5
	c/d	4
a/d	a/a	3
	a/d	12
	c/c	1
	d/d	1
	a/a	2
c/c	a/c	4
	a/d	2
	c/c	17
	c/d	1
d/d	a/a	2
	a/d	5
	c/d	1
	d/d	3
	g/d	1
g/d	g/d	8
g/g	g/g	1
N.D.*	a/a	1
	a/c	3

*Not determined.

Results

There was a significant correlation between jugular venous progesterone concentration and day ($r = 0.93$; $P < 0.01$, Fig. 1) across all SLA genotypes. No SLA genotype or day effects or interactions of these factors were noted with regard to concentrations of oestradiol-17 β or oestrone in jugular venous plasma or uterine flushings (Fig. 2). However, oestrone concentrations were higher in systemic blood than were those of oestradiol-17 β (56.5 ± 6.4 vs 33.0 ± 4.7 pg/ml respectively, $P < 0.01$) while in uterine flushings, oestradiol-17 β was the predominant oestrogen (19.8 ± 1.4 vs 14.9 ± 1.5 pg/horn respectively, $P < 0.10$, Fig. 2).

Ovulation rate, as determined by CL number, was greater ($P < 0.01$) in SLA^{d/d} dams than in SLA^{a/a} or SLA^{a/c} dams in which ovulation rates were similar (Table 2). There was no significant effect of day of gestation (2–6 days) or SLA genotype on the number of embryos recovered during flushing, although numbers of embryos recovered from SLA^{d/d} dams tended to be greater (Table 2). Stage of embryonic development ranged from 1 to 2 cells on Day 2 to blastocysts on Day 6.

Overall, significant effects of SLA genotype of the parent on litter size was seen for both sire and dam ($P < 0.05$, Table 3). Litters born to sows with the SLA^{-/d} (SLA^{a/d} or SLA^{c/d}) or SLA^{d/d} haplotype or sired by boars with the SLA^{d/d} haplotype were significantly larger than those of other

Fig. 1. Systemic progesterone concentration during Days 2–6 of gestation (Day 0 = 1st day of mating) of SLA^{a/a}, SLA^{c/c} and SLA^{d/d} miniature pigs.

Fig. 2. Oestrone and oestradiol-17β concentrations (mean ± s.e.m. for the no. of pigs given in parentheses) in systemic blood and uterine flushings of SLA^{a/a}, SLA^{c/c} and SLA^{d/d} miniature pigs. Means within a site of sampling with different letters differ significantly ($P < 0.05$).

Table 2. Maternal differences in ovulation rate, number of embryos recovered and percentage recovery rate from Day 2 to Day 6 after mating in pigs

SLA genotype	No. of females	No. of CL/female	No. of embryos recovered/flush	% recovery rate*
a/a	9	9.11 $\pm$ 0.68 ^a	7.11 $\pm$ 0.79	76.78 $\pm$ 6.62
c/c	6	8.17 $\pm$ 0.83 ^a	5.00 $\pm$ 0.73	62.73 $\pm$ 8.05
d/d	8	11.50 $\pm$ 0.87 ^b	7.88 $\pm$ 1.14	67.95 $\pm$ 6.89

*Embryos recovered as a percentage of total CL number.
Different superscripts within a column differ ($P < 0.01$).

Table 3. Least squares means of litter size by SLA genotype of parent

SLA genotype classified by	Sire or dam SLA genotype		
	SLA ^{-/-}	SLA ^{-/d}	SLA ^{d/d}
Sire only	5.57 $\pm$ 0.37 ^a (<i>n</i> = 58)	6.49 $\pm$ 0.63 ^{a,b} (<i>n</i> = 25)	7.38 $\pm$ 0.68 ^b (<i>n</i> = 12)
Dam only	5.43 $\pm$ 0.35 ^a (<i>n</i> = 57)	6.58 $\pm$ 0.43 ^b (<i>n</i> = 36)	7.87 $\pm$ 1.00 ^b (<i>n</i> = 6)

Different superscripts within a row differ ($P < 0.05$). Means within a column are not significantly different.

matings. No significant difference was noted between SLA^{-/d} and SLA^{d/d} haplotypes of boars or sows with regard to litter size.

Discussion

Profiles reported in this study of progesterone, oestradiol-17 β and oestrone, in systemic blood and uterine flushings from Day 2 to Day 6 after mating, are similar to those reported previously for miniature and commercial strains of pigs (Henricks *et al.*, 1972; Howard *et al.*, 1983). No differences in steroid concentrations were noted among SLA genotypes, suggesting that differences in litter size do not arise as a result of marked differences in ovarian function.

Ovulation rate of animals in this study was lower than for commercial breeds of pigs (Day, 1968) and was similar to that observed in other miniature and feral swine (Hagen & Kephart, 1980; Howard *et al.*, 1983). In addition, a significantly higher ovulation rate was noted in SLA^{d/d} compared to either SLA^{a/a} or SLA^{c/c} dams (Table 2). A report by Rothschild *et al.* (1984) indicated that selection exclusively for ovulation rate in an experimental herd of commercial pigs also indirectly selected for some SLA haplotypes. The results reported by Rothschild *et al.* (1984) and those presented here suggest an association between the genes controlling ovulation rate and the MHC of pigs.

Sire effects on litter size might occur as a result of effects on fertilization rate or embryo survival. In domestic pigs fertilization rate is known to exceed 95%. An influence of genotype on boar fertility sufficient to alter litter size would also be reflected in overall conception rates (Rahnefeld & Swierstra, 1970). No differences in conception rates among genotypes were obvious in this herd that would have indicated a selective advantage in fertility of d/d boars. In addition, no significant

genotype differences were found in percentage embryos recovered (no. of embryos/ovulation rate), suggesting that paternal genotype effects on litter size seen in these miniature pigs may be the result of differences in embryo survival rather than number of ova fertilized. The relatively low embryo recovery rates in this study (63–77%) are consistent with those recently reported by Diehl *et al.* (1986) for the same strain of miniature pig. Although undefined SLA-associated genotypic effects may have contributed to the observed differences between SLA genotypic strains, the high inbreeding co-efficient maintained in this herd suggests that an effect at the SLA locus is most likely.

Significant effects of SLA genotype on litter size were seen for both sire and dam (Table 3). The equivalence of these effects suggests no major maternal SLA haplotype effect on embryo survival. This agrees with the hormone profiles being equivalent for all three haplotypes. Litters from matings involving sires or dams with the SLA^{-d} or SLA^{d/d} genotypes were of similar size. Due to the limited sample size in this study, it is premature to reach conclusions about the additive or dominant gene effects of the SLA^d haplotype on litter size. However, the probability that an embryo survives, and hence litter size is larger, appears to increase with the probability that the embryo received an SLA^d haplotype from either parent.

In mature domestic pigs, embryonic mortality appears to be independent of fertilization rate, maternal oestrogen or progesterone concentrations or uterine capacity before Day 30 (Pope & First, 1985). Instead, several observations suggest that the rate of embryonic development may affect survival. Wilmut *et al.* (1985) observed enhanced survival of more developed pig embryos over their less developed littermates and Pope & First (1985) demonstrated that Day-7 embryos transferred to Day-6 recipient sows survived better than Day-6 embryos transferred to Day-7 recipients. A link has been established between the rate of embryonic development and the MHC complex in mice (Verbanac & Warner, 1981; Goldbard *et al.*, 1982; Goldbard & Warner, 1982; review by Warner, 1986). These researchers demonstrated the existence of a gene controlling the rate of preimplantation embryonic development (Ped) which is located within the H-2 complex (mouse MHC). It has been suggested that the rate of embryonic development in mice may be linked to the expression of certain MHC antigens (Warner *et al.*, 1987).

The present study detected no obvious differences in developmental stage of embryos amongst the three strains of SLA homozygous miniature pigs. However, since cleavage divisions are discrete events, it is possible that more subtle differences in the rate of development amongst embryos of different genotypes may exist; this would require a more thorough investigation of the rate of early development of pig embryos. Differences in rate of development of pig embryos might also become apparent at stages later than Day 6, the latest stage examined in this study. Variation in developmental stages of commercial pig embryos are easily observed within a uterine horn at 7 or 8 days of gestation and are exaggerated by Day 10–12 (Anderson & Parker, 1976). Many major physiological changes occur at this time in the embryo and uterus (Ford, 1985), including the synthesis of oestrogens by expanding blastocysts on Day 12 (Perry *et al.*, 1976). Pope & First (1985) demonstrated an embryocidal effect of exogenous oestrogen on Day-9 or -10 embryos but not on Day-12 or -13 embryos, suggesting that the onset of oestrogen synthesis by more developed embryos might jeopardize the viability of those less developed. Little is known about the mechanism(s) controlling the onset of oestrogen synthesis by pig embryos. Enzymes of the cytochrome P₄₅₀ complex, which are involved in steroid biosynthesis, have been mapped within the MHC complex in the mouse and in man (White *et al.*, 1985). Since MHC-associated antigens have been demonstrated on Day 12 pig conceptuses (Meziou *et al.*, 1983), and since Day 12 is also the day that pig embryos begin to synthesize oestrogen, it is possible that the two events are related.

The results of the present study suggest an association of SLA genotype and embryo survival and lend support to the concept that genes expressed in early pig embryos may affect their survival.

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