Comparisons of endocrinology and behavioural events during the periovulatory period in Meishan and Large-White hybrid gilts

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Attainment of puberty, cycle lengths, ovulation rate and endocrinology during the periovulatory period were studied in Meishan (MS) and European Large-White hybrid (LW) gilts. The mean age at onset of puberty of 115 days in MS ($n = 20$) gilts was younger ($P < 0.001$) than the 235 days in LW ($n = 23$). In the MS population studied, ovulation rate was not different ($P > 0.1$) during the third and fourth oestrous cycles, nor were there differences ($P > 0.1$) in the mean cycle length over the first three cycles. Overall changes in plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH) and oestradiol did not differ significantly ($P > 0.1$) between the breeds (MS, $n = 6$; LW, $n = 5$) during the periovulatory period, but plasma inhibin concentrations were significantly ($P < 0.05$) higher in the MS. The time intervals from the oestradiol peak concentration and the onset of the LH surge until the onset of behavioural oestrus were significantly different ($P < 0.005$) between the breeds, with oestrus occurring earlier in the MS. However, no difference ($P > 0.1$) was found between the groups when the intervals from the peak oestradiol concentration to the onset of the LH surge were compared. These results indicate differences between the breeds, particularly in terms of the age of attainment of puberty and the timing of the onset of behavioural oestrus relative to the oestradiol and LH surges.

Introduction

Chinese Meishan pigs are generally characterized by sexual precocity and enhanced prolificacy when compared with European breeds (Bazer *et al.*, 1988). A major component of the prolificacy is increased prenatal survival (Bolet *et al.*, 1986; Bazer *et al.*, 1988; Bidanel *et al.*, 1989). However, little is known about the endocrine mechanisms mediating such differences in reproductive performance. Preliminary studies by Ellendorff *et al.* (1988), with a population of Meishan (MS) gilts derived from an importation into France in 1979, indicated that not only did MS pigs display a longer duration of oestrous behaviour than their Large-White hybrid (LW) counterparts, but also that concentrations of LH during the preovulatory surge were higher in the MS. Other studies with pigs from the same population have also shown a longer period of behavioural oestrus in MS pigs when compared with European breeds, and a longer interval from the onset of behavioural oestrus to ovulation; cycle length was, however, shorter (Bazer *et al.*, 1988). There are conflicting reports with regard to ovulation rate; a lower ovulation rate was reported in the MS than in the European breeds (Bazer *et al.*, 1988; Bolet *et al.*, 1986), whereas others (Ashworth *et al.*,

1990, 1992; Haley and Lee, 1990) reported a higher ovulation rate in a population of MS gilts imported to the UK in 1987.

Several of these earlier comparisons appear to be confounded by comparing MS and other breeds of gilts at different physiological ages. The present study was carried out to compare age at puberty, cycle length and ovulation rate between MS and LW pigs that had experienced a similar number of oestrous cycles, and to determine oestradiol, LH, FSH and inhibin secretion during the periovulatory phase in MS and LW pigs and relate these changes to the onset of behavioural oestrus.

Materials and Methods

Animals

Twenty prepubertal MS gilts and three MS boars were transported from the Edinburgh Station of the Institute of Animal Physiology and Genetics Research (IAPGR) at 7–8 weeks of age. These were from a population derived from the importation from China in 1987. Twenty-three prepubertal Landrace $\times$ (Landrace $\times$ Large White) gilts (LW) and three LW boars were transported locally from the University of Nottingham herd at the age of 20–24 weeks. On acquisition all

gilts were checked daily for oestrous behaviour with mature vasectomized boars of the appropriate breed, and the day of onset of puberty and subsequent cycle lengths were recorded.

Ovulation rates were determined in MS ($n = 13$) and LW ($n = 8$) during the luteal phase of the third cycle by laparoscopy or laparotomy, and at the end of the fourth cycle by counting the number of corpora albicantia present when the ovaries (MS, $n = 19$ gilts; LW, $n = 19$ gilts) were recovered on predicted day 20 of the cycle for the follicular study described by Biggs *et al.* (1990, 1993).

Cannulation and blood sampling

From the original pool of animals, five LW and four MS gilts were surgically fitted with indwelling jugular cannulae under general anaesthesia three days before the predicted onset of fourth oestrus; owing to the availability of animals, two additional MS gilts were cannulated before the third and sixth oestrus.

Blood samples were withdrawn at 1 h (3 ml for determination of LH, FSH and inhibin) and 8 h (5 ml for determination of oestradiol in addition to the above) intervals over a 4 day period, with a 3 h frequent sampling period on day 2 of the sampling regimen when the samples (3 ml for LH and FSH) were taken at intervals of 10 min. The blood was collected into heparinized tubes and centrifuged at 1500 g for 20 min. Plasma was stored at -20°C until analysed for LH, FSH, inhibin and oestradiol as described below. After the withdrawal of each blood sample, cannulae were flushed with 1 ml of sterile, heparinized (10 iu ml^{-1}) saline.

During the sampling period, gilts were checked hourly for onset of behavioural oestrus by testing for a lordosis response to back pressure.

Hormone radioimmunoassays

Plasma LH concentrations were determined in duplicate 200 μl samples by a homologous double-antibody radioimmunoassay using a goat antiserum (GRF-G81-1), described by Cosgrove *et al.* (1991). The purified LH preparation IVO ($0.77 \times \text{NIH ovine LH-S1}$) was used as a reference standard. Intra- and interassay coefficients of variation were 6.2 and 9.3%, respectively, and the limit of sensitivity of the assay was 17.0 pg per tube.

Plasma FSH concentrations were determined in duplicate 100 μl samples by a homologous double-antibody radioimmunoassay using a rabbit antiserum (USDA-398-04P, USDA Animal Hormone Program, Reproduction Laboratory, Beltsville, USA), described by Cosgrove *et al.* (1991). Purified pig FSH was used as a reference standard (USDA porcine FSH-B1, $0.98 \times \text{NIH ovine FSH-S1}$). Intra- and interassay coefficients of variation were 7.6 and 18.7%, respectively, and the limit of sensitivity was 0.55 ng per tube.

Plasma oestradiol concentrations were determined in duplicate 200 μl samples by a radioimmunoassay according to the extraction method of Webb *et al.* (1985) using the same antibody (raised in rabbits against oestradiol-11 β -succinyl-bovine serum albumin) and conjugated hormone for iodination (oestradiol-11 α -succinyl-tyrosinemethylester). The assay procedure was validated

for porcine plasma by the extraction of known amounts of oestradiol from charcoal stripped pig plasma and there was no effect when different sample volumes were assessed. The slope of the extracted samples was parallel to the standard curve with a correlation $r = 0.92$. The range of the standard curve was 0.125–32 pg per tube and the limit of sensitivity was 0.32 pg per tube. Intra- and interassay coefficients of variation were 11.1 and 19.2%, respectively.

Plasma inhibin concentrations were determined in duplicate 50 μl samples by a radioimmunoassay using a rabbit antiserum (R-150), described by McNeilly *et al.* (1989), which was directed against a synthetic fragment of the 1–26 amino acid sequence of the amino terminus of the α chain of 32 kDa porcine inhibin (the free α -subunit). This inhibin peptide fragment was also used as the standard. The intra- and interassay coefficients of variation were 9.4 and 12.6%, respectively, and the limit of sensitivity was 5 pg per tube.

Statistical analyses

The following analyses were performed using the GENSTAT statistical package (GENSTAT 5 Committee, 1989). Student's t test was used to compare age at onset of puberty, initial cycle lengths and ovulation rate between the breeds. Endocrine profiles were standardized to the time of onset of the LH surge. The duration of the LH surge, FSH surge and the interval from the onset of behavioural oestrus to the onset of the LH surge and peak oestradiol concentration were compared using Student's t test. The frequent sampling period was used to establish basal LH and FSH concentrations (except in the case of one LW whose LH surge occurred during the frequent sampling period and thus basal concentrations were established over ten of the previous hourly samples) and the surge onset in each case was defined as a sustained increase of two standard deviations above the mean basal values. The duration was defined as the interval over which the surge was sustained two standard deviations above the basal mean.

The following endocrine characteristics were compared between the breeds by an analysis of variance and the pooled variance estimate used to calculate the standard error of the difference (SED), although for clarity, individual standard errors of the mean (SEM) are shown. Surge release of LH and FSH were compared by an analysis of the area under the curve. Peak surge values for LH, FSH and oestradiol were defined as the highest concentration (+ one sample either side of this value) observed for each hormone. Basal concentrations of LH and FSH were analysed using mean concentrations over the 36 h before onset of surge. Inhibin concentrations were analysed using mean concentrations throughout the periovulatory period from 36 h before to 34 h after onset of LH surge. The data were also split into pre- and post-LH surge onset and compared between the breeds and within a breed for both periods.

Results

Onset of puberty, cycle length and ovulation rate

First oestrus (puberty) was observed at an earlier age ($P < 0.001$) in MS than in LW gilts (Table 1), but oestrous cycle

Table 1. Mean and SD of the age at onset of puberty (days) and duration of the first three oestrous cycles (days) in Meishan (MS) and Large-White hybrid (LW) gilts

	MS		LW	
	Age at onset of puberty (days)	Cycle length (days)	Age at onset of puberty (days)	Cycle length (days)
Mean	115	20.0	235	20.4
SD	20.1	1.2	21.7	1.4
n	20	59	23	64

Fig. 1. Mean and SD of ovulation rate at third and fourth oestrous cycles for Meishan (□) ($n=13$ and 19 for third and fourth cycle, respectively) and Large-White hybrid (■) ($n=8$ and 19 for third and fourth cycles, respectively) gilts.

length was not different between breeds during the first three cycles ($P > 0.1$). Ovulation rates during the third and fourth oestrous periods were similar ($P > 0.1$): 14.9 versus 14.0 in MS and LW, respectively, during the third and 14.1 in both breeds during the fourth cycles (Fig. 1).

Time of onset of behavioural oestrus

MS gilts demonstrated oestrus earlier ($P < 0.005$) than their LW counterparts (Fig. 2). The mean intervals between the onset of the LH surge to the onset of oestrus were 0.5 ± 12.4 h (with a range of -15 to $+14$ h) for MS and 32.4 ± 10.6 h (with a range of $+14$ to $+40$ h) for LW. A difference ($P < 0.005$) between the breeds was also noted when the interval from the peak concentration of oestradiol in plasma until the time of onset of behavioural oestrus was compared (4.2 ± 15.0 versus

33.6 ± 7.6 h for MS and LW, respectively). However, no difference ($P > 0.5$) was found in the interval from the peak concentration of oestradiol until the mean onset of the LH surge (3.7 ± 5.7 versus 1.2 ± 8.0 h for the MS and LW, respectively).

Number of animals sampled

The blood sampling regimen was defined as a four day period designed to commence two days before the onset of oestrus. However, owing to the variability in the precise time of onset of oestrus, the number of animals represented in each part of this sampling window did not remain constant. From 36 h before the onset of the LH surge until 34 h after the onset of the surge, numbers of animals were six for MS and five for LW; from 35–40 h after LH surge onset, the numbers of animals declined to two MS and four LW gilts.

Periovulatory endocrine changes

There were no significant differences ($P > 0.1$) between the breeds in terms of mean basal LH and FSH concentrations (0.25 versus 0.23 ng ml $^{-1}$, SED = 0.28 for LH, and 7.33 versus 4.87 ng ml $^{-1}$, SED = 3.56 for FSH in MS and LW, respectively), or peak LH (1.44 versus 1.21 ng ml $^{-1}$, for MS and LW, respectively; SED = 0.21) and FSH (34.4 versus 28.5 ng ml $^{-1}$ for MS and LW, respectively; SED = 7.9) surge concentrations or the area under the curve for LH and FSH (Fig. 2). The duration of the LH surge (31.6 ± 8.6 versus 35.0 ± 7.8 h for MS and LW, respectively) and the FSH surge (18.8 ± 2.8 versus 22.3 ± 3.95 h for MS and LW, respectively) were also similar between breeds ($P > 0.1$). Both breeds exhibited a second rise in FSH concentration after the first peak, but because of the low number of animals sampled during this period, no analysis was performed.

Oestradiol peak values (41.8 versus 32.1 pg ml $^{-1}$ for MS and LW, respectively; SED = 3.7) and area under the curve (27.8 versus 22.0 pg ml $^{-1}$ h $^{-1}$ for MS and LW, respectively; SED = 4.7) were not significantly different ($P > 0.1$) between the breeds.

The concentration of inhibin was significantly ($P < 0.05$) higher in the MS than in the LW when analysed over the entire periovulatory period (0.57 versus 0.47 ng ml $^{-1}$ for MS and LW, respectively; SED = 0.04). Inhibin concentration was significantly higher ($P < 0.05$) in the MS after the LH surge, but before the onset of the surge this was only a trend ($P = 0.09$). There was no difference ($P > 0.1$) in the circulating inhibin concentration for either MS or LW when values before and after the onset of the LH surge were compared within a breed.

Discussion

The observation that MS gilts achieve puberty at a significantly earlier age than their LW counterparts confirms previous observations on the population of MS gilts imported into France in 1979 (Bolet *et al.*, 1986; Bazer *et al.*, 1988). The age at onset of pubertal oestrus in the MS of 115 days in the present study was similar to the mean of 114.7 days reported by Haley *et al.*

Fig. 2. Endocrine profiles showing the mean and SEM for (a) oestradiol, (b) inhibin, (c) FSH and (d) LH synchronized at about the onset of the LH surge for Meishan (MS) (○; $n = 6$) and Large-White hybrid (LW) (●; $n = 5$) gilts. The onset of behavioural oestrus is indicated for each gilt, MS (▽) and LW (▼).

(1990) in the population of MS imported into the UK in 1987. Although the MS females studied in France have been reported to have shorter oestrous cycles (Bazer *et al.*, 1988), this was not supported by our present observations, nor did we detect any difference in length of the first, second or third oestrous cycle. The reason for these discrepancies is unclear, although the MS cycle was reported by others to be shorter by only one day and a larger number of animals and a more frequent oestrous detection regimen may be required to detect these differences, if they exist.

The comparison of ovulation rate at the third and fourth cycle indicated no differences between the breeds. There is some disagreement in the literature on ovulation rate; a lower

ovulation rate was reported in the population of MS pigs imported into France than in European breeds (Bolet *et al.*, 1986; Bazer *et al.*, 1988). In contrast, Haley and Lee (1990) and Ashworth *et al.* (1990) observed a higher ovulation rate in the population of MS pigs imported into the UK; they also suggested that the ovulation rate in the MS may be underestimated unless the corpora lutea were dissected from the ovary, since some corpora lutea are buried in the ovary, and not visible by superficial inspection. However, this does not account for the discrepancy in results from the gilts studied in the present experiment and those published by others investigating the British population of Meishans, as our estimates of ovulation rate at the fourth cycle were determined by dissection of the ovaries. We suggest

that the results obtained in previous reports and in the different groups have been confounded by age, environmental factors, number of oestrous cycles experienced, parity and also the European breed used for comparison. In our study, we compared the breeds at a similar oestrous cycle number, as did Bazer *et al.* (1988) and Bolet *et al.* (1986). Although Haley and Lee (1990) observed large numbers of Meishan pigs, many of these were sows that would have experienced several oestrous cycles prior to breeding and probably a greater number of cycles than the LW of similar parity with which they were compared. It is well known that cycle number and parity affect ovulation rate (Anderson, 1987). Similarly, many of the results reported by Ashworth *et al.* (1992) were obtained from sows, although they did report a higher ovulation rate in MS gilts that were investigated after second or third oestrus. More recent investigations of our population of MS gilts at the seventh oestrous cycle have shown that these animals have a higher ovulation rate (17.4 ± 0.5 , $n = 5$) than those examined at third or fourth cycle (L. S. Faillace, C. Biggs and M. G. Hunter, unpublished). It is suggested that a proportionately greater increase in ovulation rate occurs in MS after they have experienced pregnancy than with European breeds (R. K. Christenson, personal communication). The early onset of puberty in the MS can be achieved at a bodyweight as low as 15.8 kg (Faillace *et al.*, 1991) and the production of a large litter at this body size might not be advantageous.

The duration of oestrus has been reported to be longer in MS than in LW pigs (Bolet *et al.*, 1986; Bazer *et al.*, 1988), and the mean time of ovulation after onset of oestrus was subsequently reported as approximately 44–49 h for MS and 33–38 h for LW females (Terqui *et al.*, 1990; Faillace *et al.*, 1991; Wilmut *et al.*, 1992). However, the relationship between the onset of oestrus and the LH surge, and the LH surge and ovulation was not known. The present study provides evidence that the LH surge occurred later in the MS relative to the onset of behavioural oestrus than in LW pigs, and suggests that this accounts for the large difference in time of ovulation from oestrus onset. In the present study there was a large variation within the breeds for the interval between the onset of oestrus and the onset of the LH surge and variation in this interval has been reported by previous workers (Hughes and Varley, 1980; Tilton *et al.*, 1982; Anderson, 1987). Subsequent work (Faillace *et al.*, 1991) demonstrated that the interval from behavioural oestrus to ovulation is similar to that reported by Wilmut *et al.* (1992) and Terqui *et al.* (1990).

Since circulating oestradiol concentrations were not significantly different between the breeds, the MS appear to be more sensitive to oestradiol in terms of initiating a behavioural response, but not in terms of positive feedback, as the interval from peak oestradiol to the onset of the LH surge was similar in both breeds. A greater interval between the onset of oestrus and LH surge discharge has been reported in prolific breeds of sheep (Land *et al.*, 1973) and it is possible that this has arisen from a difference in oestrogen secretion patterns or alternatively from a different sensitivity to oestrogen feedback control of oestrous behaviour.

In contrast to the initial work by Ellendorff *et al.* (1988), there were no significant differences in the gonadotrophin and oestradiol profiles between the two breeds; however, the mean concentration for each hormone was higher in the MS than in

the LW. The variation between the animals may have prevented us from establishing a significant difference between the breeds. There was no evidence of a fall in inhibin concentrations coincident with the FSH surge as observed by Mukai *et al.* (1989) and Hasegawa *et al.* (1988) in the cyclic gilt, but inhibin concentrations were significantly higher in the MS than in the LW during the periovarulatory period. This was despite the fact that concentrations in follicular fluid from individual preovulatory follicles in animals at a similar reproductive age (Biggs *et al.*, 1993) tended to be lower in the MS than in the LW. The higher concentration of inhibin in the plasma of the MS suggests that either the smaller nonovulatory follicular population was secreting inhibin in appreciable quantities, as in ewes (Campbell *et al.*, 1991), or non-ovarian sources of inhibin (Knight, 1991) were making a significant contribution to circulating concentrations or both of these occurred. Although inhibin concentrations were raised in the MS, there was no apparent reduction in circulating FSH concentrations. It is possible that not all the inhibin measured in the plasma was bioactive, as the radioimmunoassay directed towards the amino terminus of the 32 kDa α -subunit of inhibin will also measure any free α -subunit in the plasma, which is thought to be biologically inactive. It is not known how abundant the free α -subunit of inhibin is in pigs, but Hasegawa *et al.* (1988) demonstrated that their radioimmunoassay which is directed towards the amino terminus of the 32 kDa α -subunit of porcine inhibin correlated well with the pattern of bioactive inhibin in porcine follicular fluid. Another conclusion could be that there was decreased sensitivity to feedback of inhibin on FSH secretion in MS gilts; this is supported by evidence that treatment with charcoal-stripped porcine follicular fluid was less effective in reducing FSH in castrate MS than in castrate LW gilts (Tilton *et al.*, 1991). It is also interesting to note that there have been reports of a higher circulating concentration of inhibin in prolific breeds of sheep during the late follicular phase (Tsonis *et al.*, 1988) and also of a lower follicular inhibin concentration in Booroola ewes (Cummins *et al.*, 1983; Driancourt *et al.*, 1991).

In conclusion, these studies have confirmed the earlier onset of puberty in the MS. The difference in timing of the onset of behavioural oestrus relative to the LH surge explains previous observations of a longer interval from oestrus to ovulation in the MS and suggests that this is a consequence of an earlier onset of behavioural oestrus in this breed. In the study reported here ovulation rate and the pattern of circulating LH, FSH and oestradiol during the periovarulatory period were similar between breeds. However, the inhibin concentration was higher in the MS and this requires further investigation.

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