Serum concentrations of **anti-Müllerian hormone and estradiol** in gilts and their age at puberty

In several species, AMH blood levels are an indirect measure of the number of ovarian follicles, which can indicate the female's fertility potential.

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In the present study, AMH levels were determined in immature sows of various ages and were related to potential measures of future fertility, including age at puberty, the number of ovarian follicles at puberty, and the response of gilts to ovarian stimulation after puberty.

Two experiments were performed in the study:

Experiment one

Blood samples were drawn from 200 sows at 90, 120, 150, 180, and 200 days of age. The serum concentrations of anti-Müllerian hormone (AMH) and estradiol (E2) were determined in samples from the 30 sows that showed signs of heat earliest (166.1 ± 0.7 days), the 30 that showed these signs the latest, up to a maximum of 200 days (198.8 ± 0.6 days), and 18 gilts that had remained in anestrus after 200 days. Gilts that became pubescent earlier, had higher AMH levels ($p < 0.05$) than those that went into puberty later, and both groups had higher levels of AMH than the anestrous gilts ($p < 0.05$). Regardless of age, serum E2 was higher ($p < 0.05$) in the gilts that reached puberty than in those that

These results add weight to the explanation that the blood levels of AMH reflect the future fertility of gilts.

remained in anestrus. Pubertal age had no effect on the number of preovulatory ovarian follicles when spontaneous pubertal heat was detected.

Experiment two

An intramuscular (IM) injection of 400 IU eCG and 200 IU hCG (PG600®, MSD Animal Health) was administered to 152 prepubertal sows who were then exposed to a boar so that the onset of estrus could be detected. Serum AMH concentrations were higher ($p < 0.05$) in the first 25 gilts to show puberty than in the last 28, and additionally, the former also produced more preovulatory follicles ($p < 0.0001$).

Taken together, these data point to an association between serum AMH concentrations, the degree of physiological maturity, and ovarian follicular development in reared gilts.


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Introduction

An important measure of sow performance and thus, the potential profitability of the farm, is the number of piglets produced per sow over their lifetime, a metric that depends on the litter sizes and number of litters produced (that is, parity at the time of their elimination). Several management or environmental factors can affect parity at the time of removal, but the primary factor is the quality of the gilts selected to form part of the sow herd[1,2]. However, while the selection criteria for robustness and, by extension, potential longevity are known, the indicators of potential fertility remain limited. According to a review by Patterson and Foxcroft[2], management protocols can be implemented to accelerate the onset of puberty in gilts and to identify those that are maturing earlier and are therefore potentially more fertile.

However, the availability of an indicator to determine future potential fertility at an earlier age (before the final selection of replacements) would be more beneficial to the industry.

Female fertility is thought to be influenced by several factors, including the size of the ovarian follicular reserve[3,4]. Ovarian reserve is a term that refers to both the number of follicles in the follicular reserve and their quality thus, the age-associated decline in fertility in sows is related to decreased ovarian reserve[4]. AMH, which in most species, occurs only in the granu-

AMH as a marker of the size of the ovarian reserve

However, AMH expression has also been detected in cells of the preovulatory follicular theca of the ovary and in the corpora lutea in sows[8]. There is also limited evidence that the serum AMH concentration in young sows at the time of weaning is associated with a better response during estrus to subsequent boar exposure[9], although no age-related differences in the serum concentration of AMH was detected in 60, 80, or 100-day-old sows. In addition, it was shown in heifers that higher concentrations of AMH were associated with younger age at puberty[10].

The following study was carried out to determine if there are associations between serum levels of AMH and E2, as well as responses to estrus in reared gilts. Thus, we tested the hypothesis that circulating levels of AMH are indicative of the reproductive potential of gilts, as indicated by differences in age at puberty and their response to exogenous gonadotropins.

losa cells of growing follicles, is currently considered a marker of the size of the ovarian reserve[3,5–7].

One of the suggested functions of AMH is the limitation of both the initial and cyclic recruitment of follicles, thereby inhibiting the expression of LH receptors and aromatase induced by follicle stimulating hormone (FSH)[11].

Influence of AMH on aromatase activity

Because AMH influences aromatase activity, we can assume that it also affects estrogen production. Indeed, an inverse relationship between serum estrogen and AMH concentrations has been documented[12,13].

Materials and methods

Experiment one

For experiment one, 200 Landrace × Yorkshire sows aged 90 days old and with a minimum body weight of 25 kg were selected. The gilts were housed in groups of 6 in an open housing system with 2.0 m^2 per head from the time of selection to detection of their first estrus. They were group-fed a diet formulated to provide 3,200 kcal of digestible energy (DE)/kg and 15% crude protein, which allowed them to gain up to 2.5 kg/pig/day; they were also provided water *ad libitum* via nipple drinkers. From 150 to 200 days of age, the gilts were directly exposed to mature boars for 15 min/day to stimulate the onset and facilitate the detection of pubertal estrus. Blood samples were drawn from all the gilts at 90, 120, 150, 180, and 200 days of age by jugular venipuncture. The serum of the 30 gilts who showed the earliest and latest heats, respectively, by 200 days, and the 18 gilts that had remained in anestrus at 200 days were analyzed to determine the blood concentrations of AMH and estradiol. The gilts underwent an ovarian examination by real-time transcutaneous ultrasound to determine the number of preovulatory follicles (> 6 mm) when their first estrus was detected.

Experiment two

In experiment two, 152 Landrace × Yorkshire gilts were selected. Blood samples were taken from them all at approximately 165 days (weighing around 100 kg) and were processed as described for experiment one to determine progesterone and AMH levels and confirm their non-cyclic status. All the gilts subsequently received an intramuscular injection of 400 IU eCG and 200 IU hCG (PG600®, MSD Animal Health). From day 2 after the injection, the gilts were exposed to mature males daily until day 10 to facilitate the detection of estrus. Gilts showing signs of heat at 7 days were examined by ultrasound to determine the number of ovulatory follicles, as described in experiment one. Serum progesterone concentrations were determined by ELISA assays[14].

The study was carried out in a 600 sows at a commercial facility in eastern Thailand.

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Results

Experiment one

In experiment one, the mean ages at puberty of the 30 most precocious and 30 least precocious sows were 169.3 ± 0.6 days and 195.2 ± 0.9 days, respectively. As shown in *table 1*, the serum AMH concentrations of the youngest group at 90, 120, and 150 days of age were higher than those of the less precocious group or those in anestrus ($p < 0.05$), but these differences were not evident at 180 days and 200 days. Sows in anestrous had lower serum AMH values than the younger and older groups at all the age timepoints ($p < 0.05$; *table 1*). There were significant negative correlations between the age at onset of puberty and serum AMH concentrations at 90, 120, and 150 days of age (*table 2*).

In the younger sows, serum E2 concentrations were stable at 90 and 120 days, were significantly higher at 150 days, and then remained stable until 180 days (*table 1*). A similar pattern was observed for the gilts that went into heat later, although the increase was not evident until 180 days (*table 1*), resulting in a significant correlation between age at puberty and serum E2 levels on day 150 (*table 2*). Compared with the gilts that reached puberty, serum E2 concentrations were lower ($p < 0.05$) in those in anestrus at all the ages studied (*table 1*).

The pubertal age of the replacement gilts had no effect on the number of preovulatory follicles in gilts that showed more or less precocious estrus, respectively (16.5 ± 2.2 versus 15.1 ± 0.64 in).

Serum hormonal levels	Age (days)	Earlier (n = 30)	Later (n = 30)	Anestrus (n = 18)
AMH (ng/ml)	90	12.5 ± 1.1^{a,x}	9.8 ± 1.2^{b,x}	7.6 ± 1.2^{c,x}
	120	12.6 ± 1.2^{a,x}	9.8 ± 1.1^{b,x}	7.9 ± 1.1^{c,x}
	150	12.9 ± 1.1^{a,x}	10.1 ± 1.1^{b,x,y}	8.4 ± 1.1^{c,x}
	180	13.1 ± 1.3^{a,x}	12.8 ± 1.2^{a,x,y}	9.1 ± 1.2^{b,x}
	200	13.8 ± 1.2^{a,x}	13.0 ± 1.4^{a,y}	9.1 ± 1.4^{b,x}
E2 (pmol/l)	90	95.4 ± 11.2^{a,x}	89.2 ± 8.3^{a,x}	65.2 ± 6.2^{b,x}
	120	100.2 ± 8.5^{a,x}	94.5 ± 5.2^{a,x}	68.4 ± 9.1^{b,x}
	150	122.3 ± 12.1^{a,y}	95.4 ± 8.1^{b,x}	69.3 ± 11.2^{c,x}
	180	130.2 ± 9.5^{a,y}	124 ± 10.2^{a,y}	70.6 ± 6.7^{b,x}
	200	129.4 ± 14.2^{a,y}	126.5 ± 11.2^{a,y}	72.8 ± 9.2^{b,x}

$^{a, b, c}$ The rows with different superscripts significantly differed $p \leq 0.05$;
$^{x, y}$ The columns with different superscripts significantly differed $p \leq 0.05$.

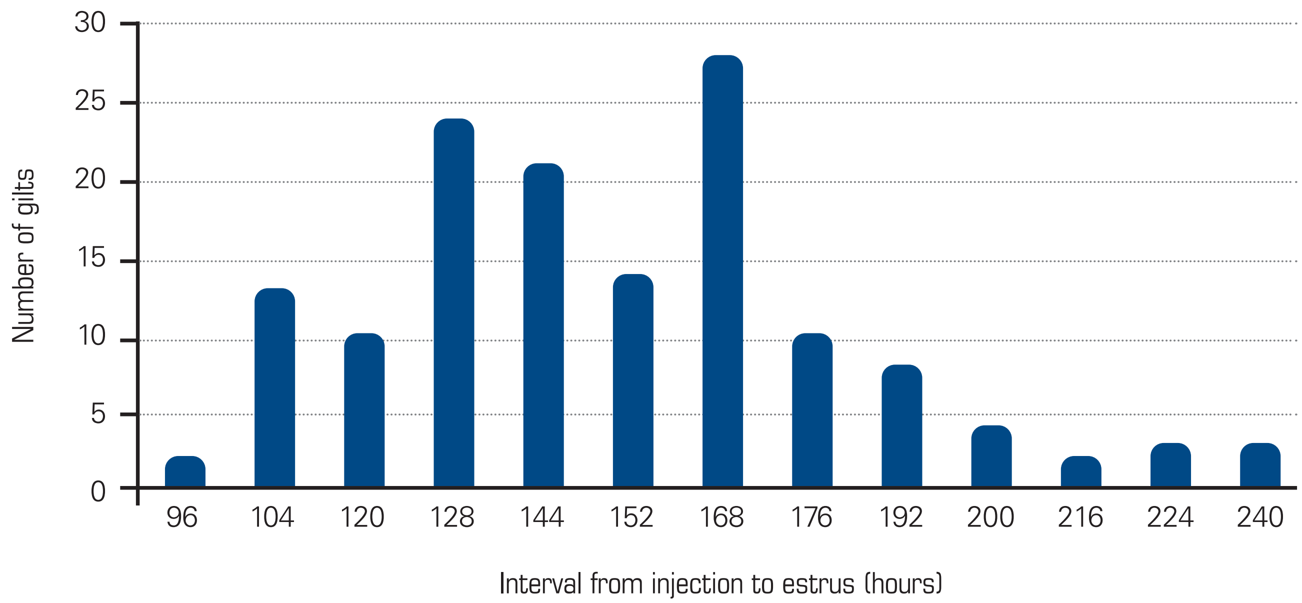
Table 1. Serum anti-Müllerian hormone (AMH) and estradiol (E2) concentrations of reared gilts at 90, 120, 150, 180, and 200 days of age.

Serum hormonal levels	Age (days)	*r*	*p*-value
AMH (ng/ml)	90	−0.81	0.005
	120	−0.67	0.002
	150	−0.64	0.001
	180	−0.41	0.07
	200	−0.32	0.12
E2 (pmol/l)	90	0.31	0.18
	120	0.41	0.09
	150	−0.74	0.02
	180	0.32	0.16
	200	−0.51	0.08

Table 2. Correlations between the age of onset of puberty and serum hormone levels at 90, 120, 150, 180, and 200 days of age ($n = 60$).

Experiment two

In the second experiment, all the gilts were considered prepubescent at the beginning of the study. The first 25 gilts reached puberty between 96 and 120 hours and the last 30 took 176 to 240 hours (as shown in the *figure*); 10 gilts remained in anestrus. The mean serum AMH concentration in the first 25 sows to show heat was higher ($p < 0.02$) than in the last 30 sows (13.6 ± 1.2 versus 11.8 ± 1.1 ng/ml). The first 25 gilts also had more ($p < 0.001$) preovulatory follicles than the last 30 (13.3 ± 1.3 versus 11.4 ± 1.2).



Distribution of the intervals for the detection of heat in the gilts after injection of 400 IU of eCG and 200 IU of hCG (PG600®, MSD Animal Health).

Discussion

The results demonstrated a negative association between serum AMH concentrations and puberty onset age in replacement gilts. Furthermore, it has been suggested that gilts that mature earlier are innately more fertile or may experience more estrous cycles prior to breeding, resulting in improved fertility. It has also been observed that, although aspects of the immunolocalization of AMH in porcine ovaries are comparable to those of other species, there are some differences[8]. However, it is currently reasonable to assume that the concentration of circulating AMH in prepubertal reared gilts reflects the size of their set of antral follicles.

These data indicate that, in gilts that mature relatively earlier, circulating levels of AMH remained largely unchanged between 90 and 200 days of age. This lack of change supports previously published data in which no obvious differences were documented in the levels of circulating AMH in gilts between days 60 and 180[12] or between days 80 and 160[13].

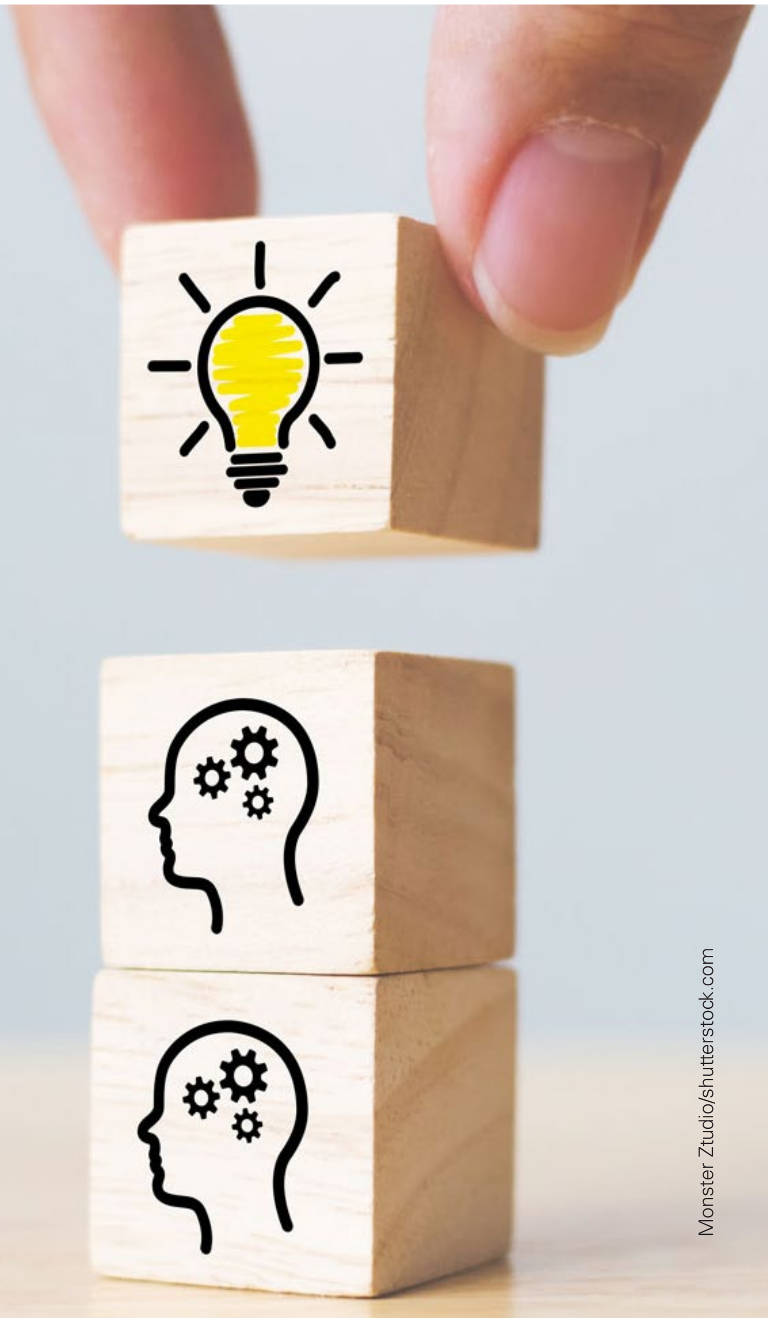
Serum AMH was lower in the late-maturing gilts in this study than in the early-maturing ones, until levels increased starting from day 180. A remarkably similar pattern was observed for the serum E2 concentration: gilts that reached puberty at an earlier age showed elevated E2 from 150 days, while those that were older when they reached puberty showed an elevation at 180 days.

Increased estradiol

Notably, these increases in serum E2 occurred at similar intervals before the mean age of puberty. In addition, the increase in serum E2 detected at 180 days was associated with an increase in AMH concentrations observed in the gilts that matured earlier. This may suggest that the inhibitory effects of AMH on FSH activity[11], and by extension, on the production of E2, decrease in the peripubertal period in gilts.

Thus, although speculative, it appears that a yet undefined permissive degree of ovarian maturity must be achieved before puberty occurs in gilts. Regardless, the number of preovulatory ovarian follicles was not influenced by age at puberty.

These data suggest a link between prepubertal and later circulating AMH concentrations in gilt fertility.



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Key points

1

The fact that the circulating concentrations of AMH and E2 were uniformly lower in the sows that remained in anestrus at 200 days of age suggest that they probably had fewer antral follicles, resulting in a failure to reach the ovarian maturity levels required to start showing estrous cycles.

2

Thus, data from experiment two support the association between circulating AMH concentrations and ovarian activity. Injection of 400 IU of eCG and 200 IU of hCG (PG600®, MSD Animal Health) in gilts at 165 days of age induced estrus.

According to commercial standards, the response to estrus was particularly good, with 142 of the 152 treated gilts (93.4%) showing signs of estrus. Nevertheless, the intervals from the time of injection until heat detection were variable and were influenced by the circulating concentrations of AMH.

The influence of anti-Müllerian hormone

The gilts that responded more quickly had higher levels of AMH. This indicates that higher AMH levels are associated with a more responsive set of ovarian follicles, a suggestion supported by the higher number of pre-ovulatory follicles observed in the gilts in which estrus was detected more quickly after injection. The impact of higher serum AMH levels and increased ovarian response to exogenous stimulation on gilt fertility remains to be determined.

Conclusion

These data support the hypothesis that circulating AMH levels are indicative of the reproductive potential of replacement gilts, as signaled by the age differences in spontaneous puberty, estrus, and ovulatory responses to exogenous gonadotropins. These data refer only to this specific study population and so generalization of a specific selection threshold is inappropriate. More work on swine genetics, herds, and management will be needed to further develop this as a useful measurement. However, if successful, its application would be invaluable to the global swine industry.

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