

LH profile and advancement of ovulation after transcervical infusion of seminal plasma at different stages of oestrus in gilts

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The influence of a transcervical infusion of seminal plasma on preovulatory LH profiles and the advancement of ovulation after seminal plasma infusion for different times during oestrus were investigated using the single uterine horn infusion technique (Mariensee model), in combination with transcutaneous sonographic monitoring of the ovaries. Preparative surgery in 23 German Landrace gilts comprised the detachment of the left uterine horn from the corpus, leaving the caudal end open to the peritoneal cavity but sealing the corpus wound. In six gilts fitted with a permanent jugular vein catheter the patent horns were administered a transcervical infusion of seminal plasma ($n = 5$ cycles) or PBS ($n = 4$ cycles) immediately after the detection of oestrus by a teaser boar. In addition, 17 non-catheterized gilts received infusions of seminal plasma either 0 h ($n = 3$ gilts), 16 h ($n = 7$ gilts) or 24 h ($n = 7$ gilts) after the detection of oestrus. Seminal plasma infusion at the onset of oestrus provoked ovulation in the ipsilateral ovary of the treated horn 8.5 ± 0.9 h earlier than in the contralateral (control) ovary. Seminal plasma did not influence the LH profile compared with PBS ($P > 0.05$), but shortened the interval between the LH peak and ipsilateral ovulation to 23.4 ± 4.0 h compared with 31.8 ± 3.4 h in the contralateral ovulation ($P \leq 0.01$). Infusion 16 h after the onset of oestrus reduced the effect to 4.6 ± 3.8 h with a wide range of 0–8 h ($P < 0.01$). The effect was more pronounced in gilts with long intervals between the onset of oestrus and contralateral ovulation compared with earlier ovulation on the control ovary. Seminal plasma infusion less than 16 h before contralateral ovulation and 24 h after the detection of oestrus had no effect. It is concluded that transcervical infusion of seminal plasma early in oestrus synchronizes the variable intervals between the onset of oestrus and ovulation in sows by a locally active mechanism.

Introduction

Induction of ovulation by coitus is a widespread phenomenon in mammalian species. Some species known as spontaneous ovulators, such as cattle, sheep and pigs, may become temporarily induced ovulators for the optimal coordination of the essential steps of fertilization (Jöchle, 1975). Natural mating shortens the interval between the onset of oestrus and ovulation in gilts (Pitkjanen, 1958; Signoret *et al.*, 1972). This has been explained by the stimulatory effect of copulation (Ziecik *et al.*, 1981; Kirsch *et al.*, 1985) and by specific components of boar seminal plasma (Seglin'sh and Brutgans, 1981; Claus, 1989; Weitze *et al.*, 1990). Previous studies that used the single uterine horn infusion technique with the contralateral uterine horn as the untreated control horn (Mariensee model, Jungblut

et al., 1991) and sonographic monitoring of ovaries showed that infusion of seminal plasma at the onset of oestrus provoked ovulation in the ipsilateral ovary of the treated horn 10.7 h earlier than in the contralateral ovary. This provided evidence that a local effect of seminal plasma on the ipsilateral ovary is involved in the advancement of ovulation (Waberski *et al.*, 1995). Recently, it was shown in a sonographic study that the interval between the peak concentration of the LH surge and ovulation was relatively constant (on average 30 ± 3 h) between sows (Soede *et al.*, 1994). The aim of the present study was to investigate whether a single infusion of seminal plasma into the uterine horn influences peripheral concentrations of plasma LH in relation to the time of ovulation on ipsi- and contralateral ovaries. In addition, the influence of the time of infusion at different stages of oestrus on the advancement of ovulation by seminal plasma was investigated.

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Materials and Methods

Animals and surgery

23 German Landrace gilts with an average body mass of 95 kg were surgically prepared according to the Mariensee model (Jungblut *et al.*, 1991), as described in detail by Waberski *et al.* (1995). Surgery was carried out under general anaesthesia using Stresnil[®] (Janssen GmbH, Neuss) and Nembutal[®] (WDT, Hannover). Briefly, the left uterine horn was detached from the corpus, leaving the caudal end open to the peritoneal cavity but sealing the corpus wound. For easier sonographic distinction, the mesovarium of the left ovary was loosely attached to the abdominal wall using a synthetic yarn (Synthofil[®]; Braun, Melsungen). Animals were allowed to recover from surgery in individual pens for 10 days and were then transported to the Hannover Veterinary School. They were housed in groups of two or four with olfactory and visual contact with the boar. Ten days after ovulation in the first oestrus after transport, six gilts were fitted with permanent jugular vein catheters under general anaesthesia. Catheterized pigs were housed in individual pens to prevent perturbation of the catheters by other gilts.

Collection of blood samples

Catheterized animals were used to handling and to the blood sampling procedure before the experimental period began. Blood samples were collected into tubes containing EDTA (Monovettes[®], Sarstedt) from day 10 to day 17 after ovulation in the first oestrus, twice daily, at 08:00 and 20:00 h. Thereafter, the frequency of collection of blood samples was increased to intervals of 60 min until 24 h after the onset of oestrus. Thereafter, blood samples were collected at intervals of 4 h until ovulation on the contralateral ovary. Immediately after collection, blood samples were centrifuged at 2500 *g* for 15 min and the plasma was stored at -20°C until hormone analysis.

Hormone measurements

Luteinizing hormone was determined in duplicate in 100 μl plasma by a homologous radioimmunoassay as described by Pomerantz *et al.* (1974) and Ponzilius *et al.* (1986). The specific antiserum (UCB porcine-Anti LH, UCB, Brussels) was raised in rabbits against porcine LH. The lower limit of detection was 0.2 ng LH ml^{-1} plasma. Porcine LH, 1 mg, used for labelling and standards had a biological activity equivalent to 0.9 mg NIH standard LH-S19. Intra- and interassay coefficients of variation were 3.5% and 6%, respectively.

Seminal plasma

Semen was collected from 50 adult boars at the Artificial Insemination Center Neustadt a.d. Aisch over a period of 2 months. Samples were centrifuged at 3000 *g* for 20 min and the cell-free plasma was stored frozen at -20°C . The plasma pool was prepared by thawing the frozen samples and mixing them when fully thawed. Aliquots of 100 ml were stored at -20°C until use.

Transcervical infusions

In catheterized animals transcervical infusions of 100 ml seminal plasma or PBS into the patent uterine horns were performed by insemination catheters as soon as tolerance to mounting by the boar was detected. In three gilts the jugular vein cannula remained patent for two consecutive cycles. These gilts received transcervical infusions of seminal plasma in their second oestrus after transport followed by infusions of PBS in the third oestrus. Of the remaining catheterized gilts, two were infused with seminal plasma and one with PBS in the second oestrus after transport. In the non-catheterized animals transcervical seminal plasma infusions were performed at three different times: in three gilts immediately after detection of tolerance to the boar, in seven gilts 16 h, and in seven gilts 24 h thereafter. All non-catheterized gilts were treated in their second or third oestrus after transport. Refluxes of infused solutions were collected for 5 min after the completion of the infusion and the volumes were measured.

Assessment of the intervals between the onset of oestrus and ovulation

Oestrus was checked three times a day by introducing the gilts into the pen of a teaser boar (08:00, 16:00, 24:00 h). The onset of oestrus was defined as half of the time interval between the last rejection of mounting and the first tolerance. During oestrus, the ovaries were examined sonographically every 4 h (04:00, 08:00, 12:00, 16:00, 20:00, 24:00 h) to determine the time of ovulation. Transcutaneous sonography was performed using a 5 MHz sector scanner (Sonoline SI-250, Siemens, Erlangen), as described by Weitze *et al.* (1989). The time of ovulation was set at $t/2$ between the last detection of follicles and their subsequent disappearance. Detection of fewer follicles than in the preceding scan followed by a total disappearance in the subsequent examination was taken as sign of ongoing ovulation. In this case, the time at which a reduction in number of follicles was registered was taken as the time of ovulation. The effect of an infused solution on the advancement of ovulation in a gilt was calculated from the time difference between ovulation in the ipsilateral (treated horn) and contralateral (control horn) ovaries.

Statistical analyses

Statistical analyses were made using the GLM procedure from SAS (SAS/STAT, 1989). In gilts with a positive response to seminal plasma infusion, the interval from the onset of oestrus to contralateral ovulation was found to have a significant ($P \leq 0.01$) influence on the time differences between ipsi- and contralateral ovulations and was therefore considered as a covariate. The volume of reflux (0–52 ml) and the duration of oestrus (48–72 h) did not have a significant ($P > 0.05$) influence on the time difference between ipsi- and contralateral ovulations and were therefore not considered as covariates. The onset and the end of the preovulatory LH surge were determined as the first and the last sample in which the LH concentration was higher than the mean basal LH concentration plus one SD. The basal LH concentration was the mean LH concentration in blood samples collected between day 10

Table 1. Characteristics of oestrus, LH surge, ipsilateral (patent uterine horn) and contralateral (detached uterine horn) ovulation after infusion of seminal plasma ($n = 5$ cycles) or PBS ($n = 4$ cycles) into a patent uterine horn of gilts

Parameter	Seminal plasma		PBS	
	Mean \pm SD	Range	Mean \pm SD	Range
Oestrus and ovulation				
Oestrus duration (h)	60.8 \pm 4.4	56–64	58.0 \pm 4.0	56–64
Interval from onset of oestrus to ipsilateral ovulation (h)	33.6 \pm 0.9 ^{ax}	32–34	44.5 \pm 6.0 ^b	36–50
Interval from onset of oestrus to contralateral ovulation (h)	42.0 \pm 0 ^y	42–42	44.5 \pm 6.0	36–50
Difference between ipsi- and contralateral ovulation (h)	8.4 \pm 0.9 ^a	8–10	0 \pm 0 ^b	0–0
LH characteristics				
Basal concentrations (ng ml ⁻¹)	0.6 \pm 0.2	0.3–0.7	0.7 \pm 0.3	0.4–1.1
Maximum concentration (ng ml ⁻¹)	6.6 \pm 1.5	4.5–8.4	7.6 \pm 1.8	5.3–9.4
Rise in concentration from basal to maximum (%)	1334 \pm 494	900–2000	1133 \pm 319	806–1516
Time from onset of LH surge to LH-maximum (h)	12.6 \pm 6.3	6–22	10.8 \pm 3.5	7–15
Duration of the LH surge (h)	35.2 \pm 9.4	23–47	34.3 \pm 16.9	22–59
Timing of LH surge relative to oestrus and ovulation				
Interval from onset of oestrus to onset of LH surge (h)	- 2.8 \pm 6.7	- 12–5	2.8 \pm 4.6	- 2–9
Interval from onset of oestrus to peak LH concentration (h)	10.2 \pm 3.4	5–14	13.5 \pm 6.8	5–21
Interval from onset of LH surge to ipsilateral ovulation (h)	34.0 \pm 6.0 ^x	29–44	41.8 \pm 3.0	38–45
Interval from onset of LH surge to contralateral ovulation (h)	44.4 \pm 6.0 ^y	37–52	41.8 \pm 3.0	38–45
Interval from peak LH concentration to ipsilateral ovulation (h)	23.4 \pm 4.0 ^{ax}	18–29	31.0 \pm 2.2 ^b	29–34
Interval from peak LH concentration to contralateral ovulation (h)	31.8 \pm 3.4 ^y	28–37	31.0 \pm 2.2	29–34

Results are from nine cycles of six gilts. Three gilts received infusions of seminal plasma and PBS in two consecutive cycles.

^{a,b} Values within rows with different superscripts are significantly different ($P \leq 0.01$).

^{x,y} Values within columns with different superscripts are significantly different ($P \leq 0.01$).

and day 17 of the oestrous cycle. The relative rise in LH per gilt was calculated by dividing the concentration by basal LH concentration and multiplying the result by 100. The comparison of adjusted means was made using the Student's *t* test.

The relationship between the intervals from infusion to contralateral ovulation and the time difference between ipsi- and contralateral ovulation was described by the non-linear regression model $y = C1 \cdot \arctan(C3(x - C4)) + C2$. The coefficients C1 and C2 were first estimated by the arctan function. The coefficients C3 and C4 were calculated using the non-linear procedure of SAS. The coefficients C1 and C2 were then corrected using the linear regression procedure of SAS. The resulting model was $y = 3.47 \cdot \arctan(0.2(x - 22.2)) + 4.08$. The confidence interval for the coefficient C3 was between 0.05 and 0.35. The confidence interval for the coefficient C4 was between 19.06 and 25.35.

Results

Effect of seminal plasma infusions at the onset of oestrus on LH profile and ovulation

In gilts infused with seminal plasma at the onset of oestrus, the intervals between the beginning of oestrus and ovulation were 8.4 h (8–10 h) shorter ($P \leq 0.01$) in the ipsilateral than in the contralateral (control) ovaries (Table 1). Infusion of PBS had no effect on ovulation in the ipsilateral ovary. LH concentrations and the duration of the LH surge, the interval from the onset of oestrus to the onset and peak of LH surge, and the interval from the onset and peak of LH surge to

ovulation in the contralateral ovary were not significantly different ($P > 0.05$) between the groups treated with seminal plasma and those treated with PBS. Seminal plasma shortened the interval from the onset of LH surge to ovulation in the ipsilateral ovary to 34.0 h (29–44 h) compared with 44.4 h (37–52 h) in the contralateral ovary ($P \leq 0.01$). In the group treated with seminal plasma, the interval from the LH peak to ipsilateral ovulation was reduced to 23.4 h (18–29 h) compared with 31.8 h (28–37 h) in the contralateral ovary ($P \leq 0.01$). Three gilts received infusions of seminal plasma and PBS in two consecutive cycles. In these gilts both the LH profile and the time of ovulation corresponded to the results obtained for all gilts: the maximum concentration of LH and the duration of the LH surge in the seminal plasma cycles were 7.0 ± 1.3 ng ml⁻¹ and 35.3 ± 5.9 h, and in the PBS cycles 8.4 ± 1.8 ng ml⁻¹ and 32.0 ± 7.2 h, respectively. In the same three gilts the intervals from the onset of LH surge and the peak LH concentration to ipsilateral ovulation in the seminal plasma cycles were 36.7 ± 6.4 h and 21.7 ± 3.5 h, and in the PBS cycles 40.7 ± 2.5 h and 31.3 ± 2.5 h, respectively. All six catheterized gilts showed synchronous ovulation on both ovaries after infusion of PBS and advanced ovulations on ipsilateral ovaries after infusion of seminal plasma. There was no difference between the intervals from onsets and peaks of LH surges and contralateral ovulations between the groups ($P > 0.05$). The time of infusion relative to the onset of the LH surge and day of oestrous cycle is shown for individual gilts in the seminal plasma group (Fig. 1). Gilts entered oestrus between day 18 and 20 after the previous ovulation. All infusions in both seminal plasma and PBS groups were performed at the onset or during the LH surge.

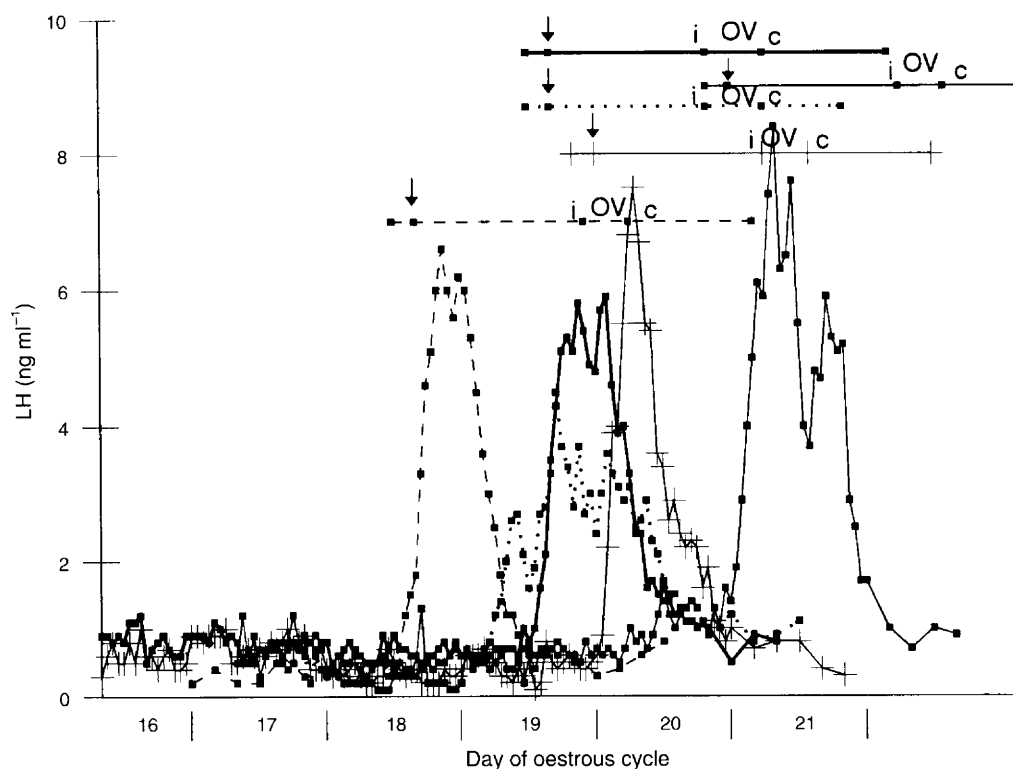


Fig. 1. LH surges relative to oestrus duration (horizontal lines), time of seminal plasma infusions into single-uterine horns (arrows) and ipsi-(i) and contralateral (c) ovulations (OV; marked in horizontal lines), ($n = 5$ gilts).

Table 2. The advancement of ipsilateral ovulation compared with contralateral ovulation after single uterine horn infusion of seminal plasma at different times in oestrus in gilts

Time of infusion after the detection of oestrus	0 h		16 h		24 h	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Number of gilts	8*		7		7	
Parameter	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Oestrus duration (h)	57.0 \pm 9.0	40–64	58.3 \pm 8.9	48–72	56.0 \pm 8.0	48–64
Interval onset of oestrus to ipsilateral ovulation (h)	32.5 \pm 1.8	30–34	37.7 \pm 8.5	24–50	40.8 \pm 6.4	34–50
Interval onset of oestrus to contralateral ovulation (h)	41.0 \pm 1.5	38–42	42.3 \pm 10.8	24–58	40.8 \pm 6.4	34–50
Interval infusion to ipsilateral ovulation (h)	28.5 \pm 1.8	26–30	17.7 \pm 8.5	4–30	12.8 \pm 6.4	6–22
Interval infusion to contralateral ovulation (h)	37.0 \pm 1.5	34–38	22.3 \pm 10.8	4–38	12.8 \pm 6.4	6–22
Difference between ipsi- and contralateral ovulation (h)	8.5 \pm 0.9 ^a	8–10	4.6 \pm 3.8 ^b	0–8	0 \pm 0 ^c	0–0

*Five gilts with permanent jugular vein catheters are included.

^{a-c} Values with different superscripts are significantly different ($P < 0.01$).

Effect of the time of seminal plasma infusion on the time between the onsets of oestrus and ovulation

Infusions at 24 h after the detection of oestrus did not result in earlier ipsilateral ovulation (Table 2). Infusions of seminal plasma at 16 h after the detection of oestrus reduced ($P < 0.01$) the time difference between ipsi- and contralateral ovulation to an average of 4.6 h with a range of 0–8 h, compared with the infusion immediately after the detection of oestrus (8.5 h). The two gilts in this group that showed no difference between ipsi- and contralateral ovulation had short intervals from the onset

of oestrus to ovulation (24 and 34 h, respectively). Infusions at less than 22 h before ovulation failed to advance ovulation on ipsilateral ovaries (Fig. 2). Infusions at times longer than 32 h before ovulation led to an advancement of ovulation of at least 8 h (8–10 h). Infusions at times in between showed a variable effect from 2 h to 8 h.

Discussion

In accordance with earlier observations using the single uterine horn infusion technique in combination with sonographic

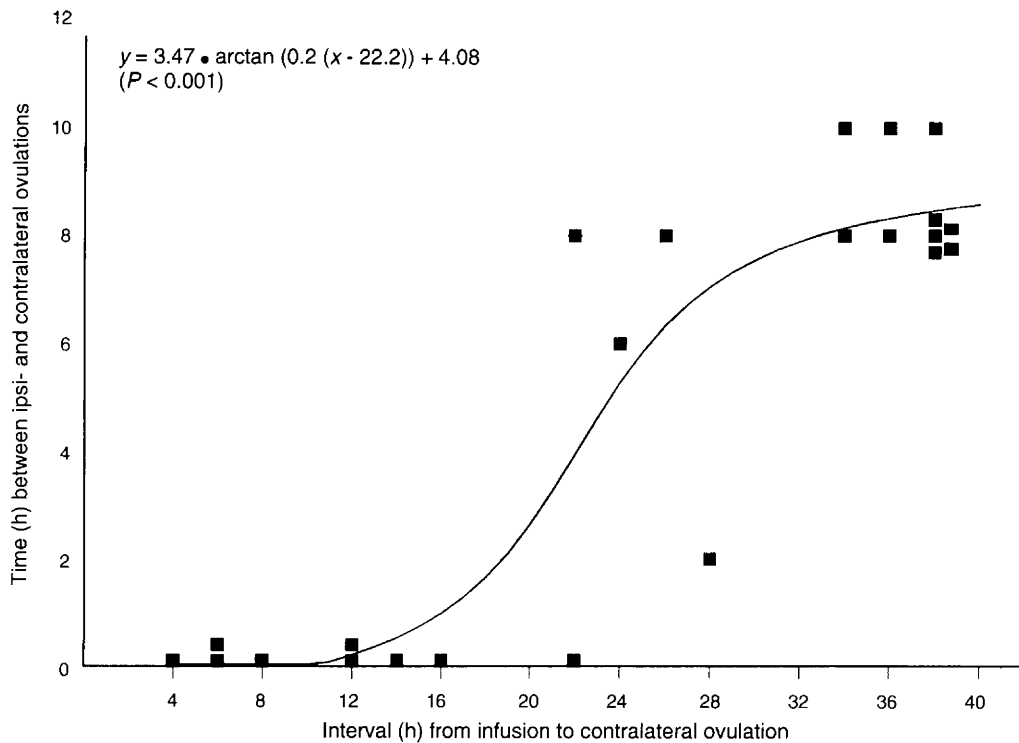


Fig. 2. Relationship between the intervals from seminal plasma infusion to control (contralateral) ovulations and the time differences between ipsi- and contralateral ovulations ($n = 22$ gilts).

detection of ovulation (Waberski *et al.*, 1995), the present study has shown that seminal plasma advances ovulation in the ipsilateral ovary of the treated horn by 8 to 10 h in comparison with the contralateral (control) ovary. Since the amount of active seminal plasma components such as oestrogens varies widely between boars and season (Claus *et al.*, 1983), a pool of seminal plasma was used to exclude ejaculate specific influences. The Mariensee model used in both studies requires few animals and allows for the distinction between local and systemic effects, since the times of ovulation are compared between ovaries at the treated and the untreated side in each gilt. In 23 cycles of 13 gilts of a previous study (Waberski *et al.*, 1995) and in 11 cycles of 11 gilts in the present study, the single uterine horn infusion of ineffective media or of seminal plasma at a late oestrous stage was followed by synchronous ovulation on both ovaries at regular intervals after the onset of oestrus. Gilts in which an infused medium was found to be ineffective showed ipsilaterally advanced ovulation after the infusion of seminal plasma or an active component thereof. The loose attachment of the left mesovarium at the abdominal wall for better sonographic distinction of left and right ovaries, therefore, did not influence the time of ovulation. Here, we confirmed our previous conclusion that the ovulation advancing effect of seminal plasma is based on local phenomena, since LH concentrations and LH surge profiles were not influenced by the infusion of seminal plasma compared with PBS infusion. This is in accordance with the report of Ziecik *et al.* (1981) who found no difference in the LH profiles of inseminated compared with non-inseminated gilts. However, Kirsch *et al.* (1985) suggested an earlier rise of preovulatory LH surge in both mated and inseminated gilts compared with control animals,

and Claus (1989) suggests that oestrogens in seminal plasma may trigger a preovulatory LH surge. The present study cannot rule out influences of the insemination procedure and of the distension reflex on LH release. However, a specific effect of seminal plasma on LH profile was not seen. This finding suggests that the proposed enhancement of LH release by seminal plasma with subsequent ovulation in camels (Xu *et al.*, 1985; Pan *et al.*, 1992) is governed by a different mechanism.

In the study reported here, the interval from the LH peak to ovulation in the ovary adjacent to the untreated uterine horn was in accordance with recent observations using transrectal sonography for the detection of ovulation in sows (Soede *et al.*, 1994; Mburu *et al.*, 1995). LH profiles may not differ between gilts and sows (Tilton *et al.*, 1982; Blair *et al.*, 1994). The model used in this study clearly established that seminal plasma shortens the interval between the LH peak and ipsilateral ovulation to 23.4 h, on average, compared with LH peak ovulation intervals of 31.8 h in the contralateral ovary. The minimal interval between LH peak and ovulation was 18 h, which is considerably longer than the minimal interval of 8 h in untreated multiparous sows, as described in the sonographic study of Dalin *et al.* (1995).

The timing of the preovulatory peak of LH varies in pigs from 32 h before to 22 h after the onset of oestrus (Helmond *et al.*, 1986). In the present study, LH peaks were found between 5 and 21 h after the onset of oestrus. This more synchronized pattern can be explained by the exposure of the gilts to the boar (Claus, 1989). Therefore, all catheterized gilts were infused at the onset of or during the LH surge. The infusion of seminal plasma at 16 h after the detection of oestrus failed to advance ipsilateral ovulation when the time interval

between infusion and ovulation was greater than 16 h. In addition, seminal plasma administered 24 h after the detection of oestrus, at the time when first insemination is usually performed, also had no effect on the time of ovulation. However, the effects of seminal plasma on fertilization other than those promoting ovulation may be effective at this time (Waberski *et al.*, 1996). As reported by Waberski *et al.* (1995), the ovulation advancing effect of seminal plasma infused at the onset of oestrus was more pronounced in gilts exhibiting long intervals between the onset of oestrus and ovulation in the control ovary, than those with shorter intervals. It is concluded that natural mating early in oestrus is a useful physiological mechanism for synchronizing the widely varying intervals between the onset of oestrus and ovulation in sow herds as described by Weitze *et al.* (1994) and that the seminal plasma is instrumental in this process. Since the success of fertilization is strongly related to short intervals between insemination and ovulation (Waberski *et al.*, 1994; Soede *et al.*, 1995), seminal plasma infusions early in oestrus are likely to enhance fertilization chances by advancing ovulation. The mechanism by which a yet unknown peptide component together with oestrogens in boar seminal plasma (Waberski *et al.*, 1995) shortens the LH surge–ovulation interval and the consequences for the oocyte maturation stage, however, remain to be elucidated.

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