# Effects of semen components on ovulation and fertilization

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In pigs, transcervical infusion of seminal plasma at the onset of oestrus advances ovulation and thus improves the chance of gametes meeting during their full fertilizing competence. An animal model that allows single uterine horn infusion was used in combination with transcutaneous sonographic monitoring of ovaries. Preparative surgery involved the detachment of one uterine horn from the corpus, leaving the caudal end open to the peritoneal cavity but sealing the corpus wound. Transcervical infusion of 100 ml seminal plasma immediately after the detection of oestrus advanced ovulation by between 8 and 14 h on the ipsilateral ovary adjacent to the infused horn compared with the contralateral ovary. In addition, the seminal plasma infusion did not influence the LH profile compared with uterine infusion of PBS. This finding indicates that the effect is mediated by a local mechanism in the female genital tract. The advancement of ovulation depends on the time of infusion early in oestrus and is more pronounced in gilts exhibiting a long interval between the onset of oestrus and spontaneous ovulation compared with early ovulators. At 24 h after the detection of oestrus, seminal plasma was ineffective. Apparently, seminal plasma does not affect maturation and fertilizing competence of oocytes. The activity resides in a low molecular mass protein fraction and, to a far lesser extent, in seminal oestrogens. Further characterization of the active components may allow a practical application in AI.

# Induced Ovulation in Spontaneously Ovulating Species

Success of insemination depends on the precise timing of the meeting of fully fertilizing competent gametes in the oviduct. Consequences of ageing of either spermatozoa or eggs in the female genital tract in pigs have been shown extensively (Hunter, 1967; Waberski et al., 1994; Soede et al., 1995). Furthermore, using spermatozoa of low quality or of low number may lead to high fertility when insemination is performed only a few hours before ovulation (Waberski et al., 1994). There is growing evidence that the essential steps that the spermatozoa have to undergo before fertilization, such as capacitation and ascent towards the eggs, are under the control of the periovulatory events in the female genital tract (Hunter, 1995). However, extended storage time of spermatozoa in the lower isthmus invariably leads to irreversible loss of fertilization capacity. This is especially pronounced in so called spontaneously ovulating species, such as pigs and horses, in which intervals between the onset of oestrus and ovulation extend for several days. In these species, repeated inseminations are usually required to ensure high fertility.

Coitus-induced ovulation is a known physiological mechanism that regulates the timing of the fertilization process. Some species known as spontaneous ovulators, such as cattle, sheep and pigs, may become temporarily induced ovulators for the improved coordination of the essential steps of fertilization (Jöchle, 1975). In pigs, natural mating shortens the interval between the onset of oestrus and ovulation (Pitkjanen, 1958; Signoret et al., 1972). This has been explained by the neurohormonal stimulation associated with the presence of a boar and with copulation (Ziecik et al., 1981; Kirsch et al., 1985) and specific components of boar semen (Seglin'sh and Brutgans, 1981; Claus, 1989; Weitze

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et al., 1990). The present paper summarizes our current knowledge on active substances and mechanisms involved in the advancement of ovulation by boar seminal plasma and the consequences for fertilization.

#### Local Effect of Seminal Plasma on the Time of Ovulation

Since boar seminal plasma contains a remarkably high oestrogen content (up to 11.5 µg per ejaculate), it was originally considered to be an active component regulating the function of the female genital tract. It was thought that prostaglandins released by the endometrium in response to seminal oestrogen might promote passive sperm transport and induce ovulation (Claus *et al.*, 1987, 1989). A vascular countercurrent pathway between uterine vein and ovarian arteries (Einer-Jensen 1988; Krzymowski *et al.*, 1990) provides a local pathway by which the prostaglandins might travel to the ovary. In addition, some authors (Kirsch *et al.*, 1985; Claus, 1989) suggest that seminal plasma may also influence the time of ovulation systemically by advancing the preovulatory LH surge.

The suspected influence of transcervical infusions of seminal plasma on the time of ovulation has been confirmed by the use of transcutaneous sonography for the detection of ovulation in gilts (Weitze et al., 1990). However, in this study an infused oestrogen solution was less effective than whole seminal plasma in shortening the interval between the onset of oestrus and ovulation. Pigs show a high physiological variation in their intervals from the onset of oestrus to spontaneous ovulation, ranging from 20 to 120 h (Weitze et al., 1994). Therefore, studies on the effect of a given solution (seminal plasma or a component thereof) require the investigation of a large number of oestrous cycles. We tested the hypothesis that the seminal plasma effect is mediated by a local pathway using an animal model described by Jungblut et al. (1991). The use of the animal model in combination with the sonographic detection of ovulation also allowed for a reduced number of investigated cycles since it provides a treated uterine horn and an untreated control horn within the same animal. Briefly, one uterine horn was detached from the corpus, leaving the caudal end open to the peritoneal cavity but sealing the corpus wound. Thus, a transcervically infused medium only has access to one uterine horn (Fig. 1) (Waberski et al., 1995). The infusion of 100 ml seminal plasma at the onset of oestrus shortened the interval from the onset of oestrus to ovulation by 8-14 h on the ipsilateral ovary adjacent to the patent horn compared with the contralateral ovary on the non-infused control side (Waberski et al., 1995, 1997). Seminal plasma pools were used in all studies since the concentration of steroid hormones is known to vary widely between ejaculates of different boars and between ejaculates of the same boar at different seasons (Claus et al., 1983). These results provide evidence that seminal plasma advances ovulation by a locally active mechanism. This does not necessarily exclude an additional systemic effect of seminal plasma on the time of ovulation. Therefore, in a recent study using the same animal model, the measurement of LH was included. LH concentrations and LH surge profiles were not influenced by the infusion of seminal plasma compared with PBS infusion into a single uterine horn (Waberski et al., 1997). An influence of the insemination procedure and the infused volume on LH release and, therefore, on ovulation cannot be ruled out. However, this was not considered as our experiments were designed to study the specific effect of seminal plasma. As revealed by infusion of PBS, a local volume effect on the time of ovulation was not detectable in the animal model. In addition, the intervals from the onset of oestrus to ovulation on the control ovary were not any shorter in gilts receiving an infusion of seminal plasma compared with non-infused gilts. We, therefore, concluded that the observed effect of seminal plasma is a local phenomenon.

The advancement of ovulation by seminal plasma via a local mechanism consequently shortens the interval from the LH increase to ovulation. Since LH also triggers the resumption of meiosis in follicular oocytes, the question arises whether seminal plasma would affect oocyte maturation. Recent studies indicate that fertilization competence of oocytes is not diminished after uterine infusion of seminal plasma (D. Waberski, R. Classen, P. W. Jungblut, E. Kallweit and K. F. Weitze, unpublished). This seems plausible since there is no reason to assume that mating early in oestrus followed by an additional mating close to ovulation negatively affects fertility.

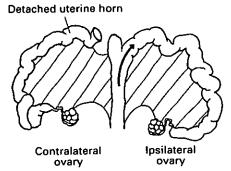


Fig. 1. Schematic design of uterus and ovaries in the animal model. The arrow indicates the flow of an infused solution. (Reproduced from Waberski *et al.*, 1995.)

## Time Dependency of the Effect of Seminal Plasma on Ovulation

The advantages of a mating-induced ovulation via the stimulus from copulation and specific effects of seminal plasma are evident. We studied the ovarian response to seminal plasma in relation to the duration from the onset of oestrus to 'spontaneous' ovulation and in relation to different times of infusion in the oestrous cycle (Waberski et al., 1997). In the first experiment, seminal plasma was infused into single uterine horns immediately after the onset of oestrus, which was detected by tolerance to the mounting of a teaser boar. The interval from the onset of oestrus to ovulation on the control ovary was recorded and related to the ovulation-inducing effect of seminal plasma as calculated from the time difference between ipsi- and contralateral ovulation. In the second experiment, using three groups of gilts prepared according to the same animal model, the effect of the time of seminal plasma infusions was tested at the onset of oestrus (0 h), 16 h and 24 h later. These studies showed that the seminal plasma effect is more pronounced in gilts with long oestrus-ovulation intervals and at an early time of infusion in oestrus (Fig. 2). Infusion of seminal plasma at 24 h after the onset of oestrus showed no advancement of ovulation. When calculated retrospectively, it was shown that seminal plasma was ineffective when the interval between infusion and contralateral ovulation was less than 20 h (Waberski et al., 1997). In several farm studies it was shown that irrespective of the duration of oestrus, which varies between 33 and 153 h, ovulation occurs fairly constantly at the beginning of the last third of oestrus (Weitze et al., 1994; Soede et al., 1995). Since seminal plasma is less effective in sows with early spontaneous ovulation compared with late ovulators, seminal plasma seems to have a synchronizing effect on the time of ovulation.

## Active Components in Seminal Plasma

Using the animal model, we confirmed our previous result (Weitze *et al.*, 1990) that seminal oestrogens are effective only to some extent. Advancement of ovulation was observed on average only for 3.3 h after infusion of 10  $\mu$ g oestradiol into a single uterine horn, while infusion of a steroid-depleted seminal plasma fraction resulted in ovulations occurring 7.3 h earlier on the ipsilateral than on the contralateral ovary. Addition of 10  $\mu$ g oestradiol to the steroid-depleted fraction restored the effect to that seen with whole seminal plasma (10.7 h on average). Further characterization provided evidence that the proteinaceous 1–10 kDa fraction of seminal plasma plays a key role in eliciting ovulation. It is noteworthy that transcervical infusions of 250  $\mu$ g of either prostaglandin  $F_{2\alpha}$  or prostaglandin  $F_{2\alpha}$  in 100 ml PBS were ineffective (Waberski *et al.*, 1995). Boar seminal plasma contains only very low concentrations of prostaglandins (Poyser, 1974, Waberski *et al.*, 1995), which are unlikely to have biological activity. However, prostaglandins released from the endometrium were suspected to induce ovulation (Claus, 1989). The rise of intrafollicular prostaglandins a few hours

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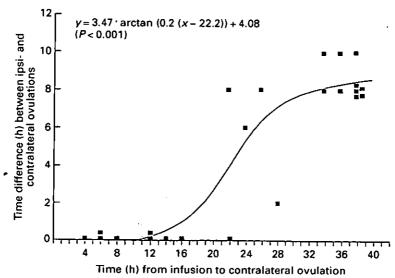


Fig. 2. Time dependency of the seminal plasma effect on the time of ovulation in 22 gilts. (Reproduced from Waberski *et al.*, 1997.)

before ovulation is widely accepted as stimulating the cascade leading to follicular rupture (Espey and Lippner, 1994). However, the rise of intrafollicular prostaglandins in response to the infusion of oestrogens as measured by Weiler and Claus (1991) is likely to occur too early and not in sufficient quantity to contribute to the induction of ovulation. It is still not known whether seminal plasma components act directly on the ovary or whether the effect is mediated by a signal from the female genital tract after the exposure to seminal plasma. As candidates for a direct effect on ovulation, proteins of low molecular mass with a possible ovulation inducing-effect were tested in the animal model. However, neither uterine infusion of relaxin nor GnRH as a substitute for GnRH-like peptides affected ipsilateral ovulations (D. Waberski, R. Classen, P. W. Jungblut, E. Kallweit and K. F. Weitze, unpublished). Further progress in the characterization of the active fraction requires sophisticated separation methods that do not influence the activity of the relevant seminal plasma components.

## Practical Application for AI

The question arises as to whether the dilution of a boar ejaculate and division into at least 20 portions for use in AI might diminish the benefits of seminal plasma in a single AI dosage as compared with the whole ejaculate. As mentioned, at 24 h after the onset of oestrus, when the first AI is commonly performed, seminal plasma does not affect the time of ovulation. However, when given at an advanced stage of oestrus, seminal plasma may promote passive sperm transport in the female genital tract (Viring and Einarsson, 1980; Claus et al., 1989). However, in a previous study, we did not find evidence that the seminal plasma plays a major role in the success of fertilization under well balanced reproductive management conditions (Waberski et al., 1996). Since in this study, high fertility was achieved even after a single insemination with a very few spermatozoa  $(0.5 \times 10^9)$ , the experimental AI condition may not be representative of on-farm conditions. Nevertheless, the major effect in terms of the reproductive biological interaction between male genital secretions and the female genital tract is in the advancement of ovulation after an intrauterine infusion of seminal plasma early in oestrus.

#### Conclusion

In conclusion, an intrauterine infusion with active components at the onset of oestrus may facilitate the timing of the subsequent insemination close to ovulation and, thereby, enhance the chance of fertilization. Since availability of boar seminal plasma is limited to AI centres, and for hygienic reasons, the use of active substitutes is desirable. As a prerequisite for the application of the effect of seminal plasma, further studies should consider individual variance of boar seminal plasma and further characterization of the active component should be undertaken.

The support of the German Association of Pig Production (ZDS, Bonn) and the Dr. Dr. h.c. Karl Eibl Foundation (Neustadt a.d. Aisch) is gratefully acknowledged.

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