USE OF BOAR SPERMATOZOA FOR ARTIFICIAL INSEMINATION
II. FERTILIZING CAPACITY OF FRESH AND FROZEN SPERMATOZOA IN GILTS INSEMINATED EITHER AT A FIXED TIME OR ACCORDING TO WALSMETA READINGS

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Summary

A fertility trial was conducted comparing two methods of defining the optimum time to inseminate frozen boar semen. One hundred sixty-four gilts were inseminated, one-half at a fixed insemination time of 32 to 34 h after calculated onset of estrus and the remainder were inseminated on the basis of changes in the vaginal mucosal electrical resistance. Inseminations were made when the instrument (an inversely scaled ohmmeter) reading fell between 54 to 64 on a scale of 0 to 100. Both fresh and frozen boar semen were used. Pregnancy rates and live embryos/gilts from inseminations made at the fixed time and inseminations made on the basis of ohmmeter readings did not differ significantly for either fresh or frozen-thawed spermatozoa. Pregnancy rates and number of live embryos/gilt were significantly higher for gilts inseminated with fresh spermatozoa than for those inseminated with frozen-thawed spermatozoa (82%, 11.6 vs 61%, 8.2, respectively). Inseminations were conducted from January to September. Pregnancy rates were significantly higher for inseminations made for the first quarter of the year (January, February and March) than for inseminations made for the third quarter (July, August and September). No benefit was derived from using an ohmmeter to time insemination with frozen-thawed boar spermatozoa.

(Key Words: Artificial Insemination, Frozen Semen, Ohmmeter, Swine.)

Introduction

The necessity for efficient and convenient utilization of frozen boar semen to enhance the propagation of germ plasm of high genetic potential is well known. The inability to store boar semen extended in the liquid form for more than 2 d without a significant reduction in fertility (Johnson et al., 1981) severely limits the utilization of artificial insemination of swine in the USA. Artificial insemination of frozen boar spermatozoa under usual management conditions results in lowered fertility and a reduction in litter size (Johnson et al., 1981; Johnson, 1980). One of the factors responsible for lower fertility of frozen boar semen is the reduced survival time of spermatozoa in the uterus, uterotubal junction and oviducts compared to the survival of fresh spermatozoa (Purcel et al., 1978). These results agree with the conclusions of Larsson (1976) and Purcel and Johnson (1975) that frozen semen should be inseminated close to the time of ovulation to obtain maximum fertilization. Because of these findings and the experience of numerous other researchers, artificial insemination of frozen boar semen is recommended to take place as close to the estimated time of ovulation as is feasible. Measurement of vaginal mucosal electrical resistance (Edwards and Levin, 1974) to predict the most advantageous time of

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insemination with fresh or stored, but not frozen semen, has been reported. Utilization of an inversely scaled ohmmeter to measure vaginal mucosal electrical resistance in sows has also been reported (Pinkert et al., 1977). Significant numbers of these instruments are currently being used under management conditions throughout Western and Eastern Europe; no one has tested their effectiveness with frozen semen.

This study was designed to determine the effectiveness of the ohmmeter for pinpointing the assumed optimum time to inseminate gilts with frozen semen, and to ascertain the fertilizing capacity of frozen boar semen across seasons; i.e., January through September.

**Materials and Methods**

In this study, 164 Dutch Landrace crossbred gilts [Dutch Landrace (DL) X Dutch Large White (DLW)] were used. Gilts were 7 to 9-mo-old, and their live weight ranged from 100 to 130 kg. They were purchased from local producers held for approximately 35 d and observed for estrus. Gilts were housed, six/pen, in a confinement facility bedded with straw at the Research Institute for Animal Husbandry Experimental Farm, Zeist, The Netherlands. Gilts received 12 h of artificial light daily (200 to 250 lux at pig eye level).

Twelve boars (8 DL and 4 DLW) in routine artificial insemination (AI) service were selected for the study and provided semen on a twice/week collection schedule. Semen was collected and evaluated for sperm motility. An aliquot of semen was removed for freezing ($18 \times 10^9$ sperm). The remainder of the ejaculate was diluted with Kiev extender ($3 \times 10^9$ sperm—$100 \text{ ml/inseminate}$) and used for routine AI by the AI Center. The aliquot taken for freezing was split equally into three tubes and maintained at room temperature for 2 h, centrifuged, diluted in Beltsville freezing extender (BF-5), cooled, rediluted and frozen in pellets on dry ice and stored in liquid nitrogen at $-196^\circ\text{C}$. After freezing and storage one pellet was removed, thawed and evaluated for sperm motility and acrosome morphology. Minimum requirements for keeping the frozen semen were 35% progressively motile sperm and 45% normal apical ridges (NAR); otherwise all the frozen semen from that ejaculate was discarded.

Procedures described above are given in more detail in a previous report (Johnson et al., 1981).

Vestibular mucosal electrical resistance was determined with a Walsmeta instrument on all gilts three times daily (0700, 1200, and 1700 h) beginning at the first detection of standing estrus. The instrument is designed to measure physiological changes in vaginal vestibular mucosa. The Walsmeta is an inversely scaled ohmmeter with a scale of 0 to 100 (i.e., the higher the reading the lower the electrical resistance). A standardized procedure based on manufacturer's instruction was followed for all gilts. The instrument was first calibrated to a line at the 50 mark. Before insertion of the probe (containing five electrolytically treated electrodes) the lips of the vulva were spread apart. The probe was inserted into the vagina to the depth of the white circular ring on the probe (about 5.5 cm) with the five electrodes facing the left side of the vagina. Pressure was exerted to ensure contact with the vestibular membrane. The probe was moved slightly to locate areas of lowest reading. A reading was taken by pressing the test button. After the reading, the probe was withdrawn, cleaned in ethyl alcohol and dried. Readings were delayed at least 15 min if the gilt urinated while the probe was in place.

Estrus was checked twice daily with a teaser boar and by the back pressure test at 0630 and 1630 h. Periods of estrus were classified according to the scheme described by Willemse and Boender (1966). The scheme is based on the premise that the length of the period of estrus during which the inseminator is capable of provoking the standing reflex comprises two-thirds of the total estrus period. The remainder of the estrus period is classified as boar periods. Therefore, the estrus period was subdivided into six segments comprising B1 (stand only to boar); I1; I2; I3; I4 (stand to both boar and inseminator) and B2 (at the end of estrus, standing only to boar). For the purposes of this study the six segments will be referred to as the stage of the estrus and numbered consecutively from 1 to 6.

Gilts were inseminated with $5.2 \times 10^9$
frozen-thawed spermatozoa (55 ml) or 3.0 x 10^9 fresh spermatozoa (100 ml). Three inseminators were used over the course of the experiment and thawed the frozen spermatozoa according to procedures described in a previous report (Johnson et al., 1981) just prior to insemination. Inseminations were made with a Melrose Catheter. Gilts to be inseminated were assigned at random across treatments to the various boars, to ensure maintaining equality among treatments. Fresh semen was collected, extended and shipped as needed over the 9 mo period by the AI Center and was also assigned at random to gilts across treatments. No gilts were inseminated before expression of their second estrus. One-half of the gilts were inseminated at a fixed time, 32 to 34 h after the calculated onset of estrus (midway between first expression of estrus and the last time the gilt was checked for estrus). The other one-half of the gilts were inseminated at some time greater than 30 h after calculated onset of estrus when (1) the Walsmeta reading fell between 54 to 64 on a scale of 0 to 100, and (2) the gilt exhibited the standing reflex. The use of 54 to 64 as the time to inseminate in the Walsmeta group is based on manufacturer's recommendations described as the time most advantageous for obtaining optimum fertilization. Gilts not returning to estrus after insemination were slaughtered at 26 to 32 d, and embryos were recovered and examined for normalcy and viability. Gilts expressing estrus by 21 d after the first insemination were reinseminated. Gilts that were reinseminated were automatically slaughtered 26 to 32 d after the second insemination.

Statistical Evaluation. Pregnancy rates were tested by fitting the data to a linear model (Grizzle et al., 1969). Other variables were analyzed by the General Linear Models procedure of the Statistical Analysis System (Barr et al., 1979).

Results and Discussion

Pregnancy rates and number of embryos (alive or dead) recovered per gilt did not differ between gilts inseminated at a fixed time and those inseminated on the basis of a Walsmeta reading (table 1). Walsmeta readings were conducted on all gilts regardless of treatment group. Walsmeta readings for gilts in the fixed time group were lower than those in the Walsmeta group (table 1; P<.005). Such a

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<th>Spermatozoa</th>
<th>Time of insemination determined by:</th>
<th>Mean no. embryos</th>
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<td>Frozen</td>
<td>Walsmeta reading</td>
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<tr>
<td>Fixed time</td>
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 Different from mean for fresh spermatozoa (P<.001).
difference was expected, however, since a minimum reading of 54 was required before gilts in the Walsmeta group could be inseminated. These results suggest that setting the time of insemination according to the time of onset of estrus results in fertility equal to or better than fertility obtained using a Walsmeta instrument. This conclusion is applicable for both fresh and frozen semen, if estrus detection methods include using a teaser boar at least twice/day to check estrus.

The pregnancy rate and number of live embryos recovered/gilt were lower (P<.05 and P<.001) for gilts inseminated with frozen-thawed spermatozoa than for gilts inseminated with fresh spermatozoa (table 1). These results are similar to the farrowing data obtained in a companion experiment (Johnson et al., 1981). In that experiment semen from the same 12 boars was used. There was a difference of 32 percentage points in farrowing rate (79 vs 47%) and 2.8 pigs/litter (10.6 vs 7.4%) between fresh and frozen-thawed spermatozoa. Allowing for prenatal death between 32 and 115 d (previous study) gestation, the 21% difference in pregnancy rates and 3.4 embryos/gilt achieved in this study is comparable.

Reduced fertility with the use of frozen-thawed spermatozoa can be attributed at least partially to reduced viability of spermatozoa. Sperm motility averaged 48% in frozen samples, about 30% below motility of fresh sperm. Acrosome morphology was also substantially lower in frozen sperm, 53% NAR for frozen-thawed spermatozoa vs 91% for fresh spermatozoa. However, similar numbers (calculated) of motile sperm and sperm with NAR were inseminated for fresh ($2.4 \times 10^9$ and $2.7 \times 10^9$ sperm) and for frozen ($2.5 \times 10^9$ and $2.8 \times 10^9$). However, survival time of frozen-thawed spermatozoa in the uterus and oviducts is reduced (Pursel et al., 1978). This may indicate that even though many frozen-thawed spermatozoa are motile and possess undamaged acrosomal membranes, some other factor essential for normal viability is affected by freezing and thawing.

Pregnancy results grouped according to four categories of Walsmeta readings (below 54, 54 to 60, 61 to 64 and above 64) are presented in table 2. There were no significant differences for any of the parameters between the categories except the expected difference in mean Walsmeta reading.

Time of insemination in relation to the stage
of estrus inseminated affected the pregnancy results (table 3). Among gilts inseminated with frozen-thawed spermatozoa, the pregnancy rate and number of embryos recovered/gilt were significantly higher for gilts inseminated in stages 4, 5 and 6 than for those inseminated in stage 3 (table 3). The pregnancy rate for gilts inseminated in stage 3 based on Walsmeta readings gave the poorest results (21% vs 70% for fixed time gilts). No reason is readily apparent to account for the wide difference. It appeared from the data that the stage of estrus and the number of hours from onset of estrus to insemination and Walsmeta readings may have been interrelated. Statistical evaluation did not confirm this, however.

Fertility and characteristics of estrus classified by the quarter of the year in which the gilts were inseminated are given in table 4. Pregnancy rate was significantly higher in the first than in the third quarter of the year. Frozen spermatozoa used in the first quarter had been stored for less than 50 d; frozen spermatozoa used in July, August and September (3rd quarter) had been stored for more than 100 d. Whether age of semen affected pregnancy rate is not known. However, in a recently completed study with 340 gilts (J. G. Aalbers et al., unpublished data), there was no difference in pregnancy rate between gilts inseminated with frozen spermatozoa stored for 1 to 15 d and gilts inseminated with frozen spermatozoa stored for 90 d or more. Fertility in sows and gilts is generally lowest in the hot months of July and August, and results of a recent survey of farms involving more than 11,000 sows showed that fertility was lowest in July and August and highest from November to March (Hurtgen et al., 1980). These results would tend to agree with that survey. With respect to the fresh semen inseminations (only 44 in this experiment), there were no significant differences between quarters of the year. Pregnancy rates for gilts inseminated with fresh semen in quarters 1, 2 and 3 were 76, 76 and 90%, respectively.

Embryo survival in gilts inseminated with frozen-thawed semen paralleled pregnancy rate (table 4); however, the differences were not significant. Walsmeta readings for the third quarter of the year were lower than for the first and second quarters (P<.001). However, this had no apparent impact on the results.

The rate of pregnancy was higher among gilts inseminated with frozen spermatozoa from DLW boars than among those inseminated with frozen spermatozoa from DL boars (73 vs 55%); however, the number of live embryos/gilt was lower for gilts inseminated with frozen DLW spermatozoa (DL, 8.4; DLW, 7.8). Differences were not significant, but the trend toward a higher pregnancy rate at 26 to 32 d for gilts inseminated with DLW frozen boar spermatozoa agreed with earlier field trial results using the same boars (Johnson et al., 1981).

Electrical resistance of the vaginal mucous membrane decreases during estrus and is generally lowest when conception is highest (Babicheva and Mozgov, 1971; Edwards and Levin, 1974). This is the basis for the operation

<table>
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<tr>
<th>Treatment group</th>
<th>Stage of estrous period</th>
<th>Pregnancy rate, %</th>
<th>No. live embryos recovered/pregnant gilt</th>
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*Number of gilts in parenthesis.

bDifferent from pregnancy rates among gilts inseminated in stages 4, 5 and 6 (P<.05).
of the Walsmeta, although the instrument is scaled inversely to electrical resistance. From the results of this study it is apparent that no advantage can be gained over fixed time insemination when adequate estrus detection methods are used. However, this is not to say that under certain conditions (i.e., estrus detection without teaser boar, etc.) the Walsmeta may not be useful.

The Walsmeta's usefulness for frozen semen does not seem to be warranted since so much care must be taken to ensure a “close to ovulation” insemination, and this can be attained with less investment of labor and capital through the use of twice/day estrus detection and close observation of the animal.

### Literature Cited