Semen handling in porcine AI centers: the Belgian situation

Spermabehandeling in kunstmatige inseminatiecentra voor varkens in België

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ABSTRACT

In the present study, the practice of semen examination and semen processing in Belgian porcine artificial insemination (AI) centers was evaluated by means of a written questionnaire. The questionnaire was sent to 50 AI centers and 38 filled it in and returned it (response rate 76%). The size of the herds in the responding centers varied between 4 and 165 boars, with a mean of 45. In 80% of the AI centers, the semen doses contained at least 3 billion spermatozoa. Sperm morphology and motility were considered the most important parameters for sperm quality evaluation. Semen morphology was systematically examined in 71% of AI centers. A lower limit of 80% normal spermatozoa was used in 66% of the AI centers. Motility was monitored systematically in every ejaculate in 94% of AI centers. A lower limit of 70% motility was used. Motility assessment was performed visually in all but one of the AI centers.

Ninety-seven percent of Belgian AI centers used a short-term semen extender (storage for 3 days). Since semen transport times are short in Belgium, there is no necessity for long-term storage of diluted porcine semen. In 50% of the AI centers, long-term extenders (storage for 7 days) were used on a part of the collected semen, mainly for storage over the weekend. In some AI centers, extender solutions at room temperature are added to the ejaculate, apparently without negative effects on fertility. Antibiotics are seldom supplemented to the extended porcine semen. The semen doses contained in most cases 100 ml extended semen.

The present study shows that the Belgian porcine AI centers generally incorporate semen quality examinations in their daily routine, especially for motility assessment. In a minority of AI centers, especially the smaller ones, the semen morphology examination should be done more systematically. This focus on semen quality, together with the consistent use of a sufficient number of spermatozoa per semen dose, reflects the concern of Belgian AI centers to produce quality semen doses. In some AI centers, improvements in semen handling could be made, especially concerning the temperature of the extender and its preservation conditions.

SAMENVATTING

In deze studie werden de methodiek van het spermaonderzoek en de spermabehandeling in kunstmatige inseminatie (KI) centra voor varkens in België geëvalueerd aan de hand van een schriftelijke enquête. Daarvoor werd een vragenlijst gestuurd naar 50 KI-centra waarvan er 38 een ingevulde enquête terugzonden (respons rate 76%). De grootte van de bedrijven die antwoordden, varieerde van 4 tot 165 beren. In 80% van de KI-centra bevatte een spermadosis minstens 3 miljard zaadcellen. De belangrijkste criteria voor de beoordeling van de spermakwaliteit waren de morfologie en de beweeglijkheid. De spermamorfologie werd systematisch onderzocht in 71% van de KI-centra. Een minimum van 80% normale spermacellen werd als criterium aangehouden in 66% van de KI-centra. De beweeglijkheid werd in 94% van de KI-centra systematisch onderzocht bij elk ejaculaat. Een minimum van 70% beweeglijkheid werd als ondergrens in acht genomen. Op één na onderzochten alle KI-centra de beweeglijkheid visueel.

Zevenennegentig percent van de KI-centra gebruikte een kortetermijnverdunner. In België wordt verdund sperma slechts over kleine afstanden getransporteerd waardoor er weinig behoefte is aan langetermijnbewaring. In 50% van de KI-centra werd een deel van het sperma verdund met langetermijnverdunners, vooral voor de bewaring tijdens het weekend. In sommige KI-centra werd de verdunner op kamertemperatuur aan het sperma toegevoegd, klaarblijkelijk zonder negatieve gevolgen voor de spermakwaliteit. Antibiotica werden slechts zelden aan het verdund sperma toegevoegd.

Dit onderzoek toont aan dat de Belgische KI-centra bij het spermaonderzoek, vooral de beweeglijkheid in de dagelijkse routine opnemen. In een minderheid van de KI-centra, vooral de kleinere, zou de controle van de morfologie meer systematisch moeten gebeuren. De aandacht voor de spermakwaliteit in combinatie met een consistente aanwezigheid van een voldoende aantal zaadcellen per dosis, weerspiegelt het streven van de Belgische KI-centra om spermadosissen van hoge kwaliteit te produceren. Wat betreft de spermaverwerking zijn in sommige gevallen verbeteringen mogelijk, meer specifiek met betrekking tot de temperatuur van de verdunner en de bewaring ervan.

INTRODUCTION

Artificial insemination (AI) is common practice in swine producing countries. The purchasing of semen from specialized AI centers has advantages compared to on-farm sperm collection. These advantages include the availability of superior genetic lines (Robinson and Buhr, 2005) and a separate place, usually called a laboratory, for semen processing (Koninklijk Besluit 6-10-2006; 90/429/EEG). In this laboratory, the semen parameters can be examined routinely and the semen doses are packaged. To obtain optimal fertility results, most AI centers monitor semen quality to some extent in order to provide ready-to-use diluted semen of consistent quality. Besides the number of spermatozoa in a semen dose, the motility and morphology of the spermatozoa are most frequently evaluated as semen quality parameters since they are known to influence fertility in vivo (Tardif *et al.*, 1999; Alm *et al.*, 2006).

Although several techniques have been described for assessing these parameters in detail, fast and inexpensive methods are most popular in commercial AI centers (Martin Rillo *et al.*, 1996). In practice, morphology is assessed by eosin-nigrosin staining and motility is assessed visually. Visual motility assessment generally correlates well with more detailed, computer assisted semen analysis (CASA) (Vyt *et al.*, 2004a) and is sufficiently accurate to assess sperm function if performed properly by an experienced person (Woelders, 1991). However, little data is available on the methodology used or on the frequency of semen examination in commercial AI centers.

Besides the intrinsic quality of the ejaculate, dilution and storage conditions are equally important for maintaining semen quality (Johnson *et al.*, 2000). Although differences in semen quality preservation between semen extended in short-term and long-term extenders have been found under laboratory conditions (Vyt *et al.*, 2004b) and in field trials (Johnson *et al.*, 1988), little is known about the dilution techniques and the storage conditions of the diluted semen used in practice. Although numerous studies exist on semen examination and semen processing, no data is available on the actual application of these literature findings in practice, where economic considerations are also taken into account.

The purpose of this study was to collect detailed information on the semen handling and semen examination procedures used in commercial AI centers and to compare this with findings in the literature.

MATERIALS AND METHODS

A questionnaire was sent to all porcine AI centers in Belgium (n=50) in April 2006. Forty-seven AI centers were located in the Flemish part and 3 in the French-speaking part of the country. To enhance the response rate, a French translation of the questionnaire was sent to the latter AI centers. The questionnaire was pre-tested in one of the AI centers before the study. The questionnaires were sent either by e-mail or fax (n=33), or else by conventional mail (n=17). Fourteen AI centers returned the questionnaire within three weeks. The remaining AI centers were contacted by phone. In 13 cases, reception of the completed questionnaire was obtained after one telephone contact, while in 11 cases, two or three telephone reminders were necessary. Twelve AI centers (24%) did not return the questionnaire for the following reasons: refusal to cooperate (n=1), no time (n=3), unknown reason (n=8).

The questionnaire was composed of 25 closed questions. The questions were divided in different topics: 1) herd size and semen dose production (3 questions), 2) semen examination (10 questions), and 3) semen dilution (12 questions).

RESULTS

A total of 38 questionnaires were received (response rate 76%). These 38 AI centers are representative for 94.6% of all the AI boars in Belgium and represented a production of 2017281 semen doses in 2005 (data Flemish government). No problems regarding the ambiguity of the questions were mentioned. Some AI centers that returned the questionnaire did not respond to all questions. Especially the number of semen doses produced annually was frequently left unanswered (10/38, or 26%).

Data on continuous variables are presented in Table 1. The mean number of boars present in the responding AI

Table 1. Data on continuous variables concerning herd size and semen production in 38 commercial artificial insemination (AI) centers in Belgium.

Variable	Response (n)*	Positive (n)**	Mean (min - max)
number of boars present	38		45 (4 - 165)
number of doses produced / year	28		42136 (2000 -195000)
doses / boar year	28		625 (323 - 1443)
kind of recipient	36		
plastic bottles (% of production)		29	32 (0.1 - 100)
blisters (% of production)		29	71 (0.1 - 100)
tubes (% of production)		7	60 (10 - 100)
Gedis^{R} (% of production)		4	30 (1 - 100)
straws (frozen) (% of production)		1	10 (10 - 10)

*Response: number of AI centers answering the question
**Positive: number of positive responses

Table 2. Data on categorical variables concerning semen examination in 38 commercial artificial insemination (AI) centers in Belgium.

Variable	Response (n)	Number of AI-centres	% of AI-centres
Concentration determined by	37		
photometer		27	73.0
counting chamber		4	10.8
other		6	16.2
Number of spermatozoa / dose	36		
> $3*10^9$		13	36.1
$3*10^9$		16	44.4
2.5 - <$3*10^9$		5	13.9
2 - <$2.5*10^9$		2	5.6
Dose volume	37		
> 100 ml		2	5.4
100 ml		24	64.9
between 80 and 100 ml		6	16.2
80 ml		5	13.5
Morphology examination	37		
by accurate count		29	78.4
by estimation		8	21.6
Minimum % of normal cells	36		
60		2	5.6
70		9	25.0
80		24	66.7
85		1	2.7
Visual motility assessment	35		
by scoring system		23	65.7
by estimation		12	34.3
Minimal % of motile cells	34		
60		2	5.9
70		12	35.3
80		18	52.9
90		2	5.9

Response: number of AI centers answering the question.

centers was 45. At five AI centers, one hundred or more boars were present, while at 10 centers less than 20 boars were present. Diluted semen was mainly distributed as blisters (29/36 with a mean of 71% of their production), followed by plastic bottles (29/36, mean 32%) and tubes (7/36, mean 60%).

Semen examination

The data on semen examination is presented in Table 2. The concentration of spermatozoa in the ejaculate was determined by using a photometer (73%), by using a counting chamber (11%), by visual inspection (5.5%), by using an automated semen analyzer (5.5%), by using a CASA system (2.5%) and by an external person without details on the methodology (2.5%). Small AI centers mostly assessed concentration by using a counting chamber or by macroscopic interpretation of turbidity. In most cases (80%), a semen dose of ≥ $3*10^9$ sperm cells was used in a volume of 100 ml (65%). Larger AI centers (> 100 boars) tended to use slightly lower semen doses (min. 2-2.5 billion sperm cells per dose). Five AI centers had experienced problems with determining the concentration in the past. These problems were due to photometer aberrations and did not lead to altered fertility results.

Examination of semen morphology was done routinely on every ejaculate in 71% of the AI centers, mostly by means of an accurate count on eosin-nigrosin staining. The absence of assessment of morphology (n=11) and motility (n=2) was more frequent in AI centers with less than 30 boars. The criterion for percentage of normal spermatozoa was at least 80% according to 66% of the answers. One AI center accepted a minimum of 55% normal cells as lower limit. Standard evaluation of sperm

Table 3. Data on categorical variables concerning semen handling procedures in 38 commercial artificial insemination (AI) centers in Belgium.

Variable	Response (n)	Number of AI-centres	% AI-centres
Extender	38		
one		21	55.3
more than one		17	44.7
Type of extender			
short-term		37	97.4
only long-term		1	2.6
Temperature of extender (°C) before addition	35		
>35-37		26	74.3
>30-35		4	11.4
>22-30		0	0.0
>10-22		4	11.4
<10		1	2.9
Time of preparation of extender before mixing with semen	38		
< 1h		26	68.4
1-3 h		7	18.4
3-6 h		2	5.3
6-12 h		1	2.6
12-24 h		2	5.3
> 24 h		0	0.0
Preservation of the extender	34		
in open recipient		9	23.7
in closed recipient		25	65.8
Air volume in closed recipiënt of the extender	25		
> 50%		5	20.0
25-50%		3	12.0
10-24%		5	20.0
<10%		12	48.0
Time period for use of the extender	37		
<12 h		19	51.4
12-24 h		11	29.7
> 24 h		7	18.9
Water used to prepare the extender	38		
demineralized		30	78.9
destilled		7	18.4
deinoised		1	2.6
Water source	37		
bought as commercial product		30	81.1
own production		7	18.9

Response: number of AI centers answering the question

motility was performed in 94% of AI centers, usually visually by means of a personal scoring system. Two centers used a semen analyzer, while one AI centre used a CASA system. A motility of at least 70% was considered as minimum in 94% of the AI centers. Two centers used a lower limit of 90% motility.

Semen handling

An overview of the results concerning semen dilution and semen handling is given in Table 3. An extender for short-term preservation (max. 3 days) was used in 97% of the AI centers. Fifty percent used a long-term extender. The latter were nearly always used for sow-line boars whose semen was used for several days and for semen prelevations on Fridays. One center (with < 10 boars) exclusively used a long-term semen extender. Extenders were kept at 35-37°C before adding to the semen in 74% of the AI centers. In four AI centers, the extender was kept below 21°C, and in one other AI center even at 4°C. Extenders were usually prepared shortly before semen collection (68% less than 1 hour before) and stored in closed

recipients. Extender solutions were used for less than 12 hours in half of the centers, and for more than 24 hours in 19% of the AI centers. Most extenders used in Belgian AI centers are supplemented with antibiotics. Although the exact composition of several extenders is not released by the producers, gentamycin was the most frequently supplemented antibiotic. Additionally, four out of 38 AI centers added antibiotics to the extender before mixing with the semen. Only three centers reported problems with bacterial proliferation in the past. One of them was still adding antibiotics to the extender. No other substances (oxytocin, prostaglandins, vitamins) were added to the extender or to the extended semen itself, although one AI center advised to give prostaglandins via the insemination pipette before the semen was introduced.

DISCUSSION

In the present study, data on semen handling and semen quality assessment was obtained by means of a questionnaire. The majority of AI centers participated in the study (response rate 76%, representing 94.6% of the AI boars in Belgian AI centers), thus rendering the data obtained representative for the situation in Belgian AI centers.

Part of the variation in semen examination and semen handling can be explained by the heterogeneity in production capacity of the AI centers. The use of a counting chamber or macroscopic interpretation of turbidity to assess sperm concentration, as is typical of smaller AI centers, is an indication that they make less financial investments in automated systems. The determination of semen concentration by macroscopic estimation of turbidity is a very crude method that no longer fits in a modern AI center. Semen quality was assessed less routinely in AI centers with less than 30 boars, while larger centers tended to examine morphology and motility of every ejaculate. Although the rate of disease introduction with subsequent influence on semen quality can be expected to be lower in smaller AI centers with low replacement rate (Glossop, 1998), routine examination of semen in small AI centers can be an important monitoring tool for disease introduction, since small AI centers often have additional activities in the swine industry.

When looking at the different semen evaluation procedures, concentration was mostly assessed using a photometer. This procedure is less time consuming and easier to perform than using a counting chamber. Nevertheless, in five AI centers (13%) concentration assessment of the ejaculate had been a problem in the past, indicating the importance of regular control of a photometer. No problems regarding the determination of the concentration were recorded with the use of counting chambers or CASA, although differences between these methods have previously been described (Vyt *et al.*, 2004a). Large AI centers used the lowest numbers of spermatozoa per dose, resulting in the highest number of doses annually produced by each boar. The lower the number of spermatozoa in each dose, the more important it is that a stringent control of morphology and motility (Tardif *et al.*, 1999) be carried out since suboptimal quality results more quickly in decreased fertility at lower semen dose. In all AI centers with lower numbers of spermatozoa per dose included in this study, at least the semen motility was assessed routinely, although it would have been better to assess the semen quality as accurately as possible. In addition, estimating the morphology and motility of the semen makes it possible to adjust the sperm number in a semen dose (Johnson *et al.*, 2000) when these parameters are suboptimal. This can be an advantage in AI centers using a lower semen doses. Most AI centers used a criterion of at least 80% morphologically normal spermatozoa, whereas in the literature 70 % is proposed (Britt *et al.*, 1999). The latter criterion is followed by 94% of Belgian AI centers, a fact which indicates their concern for good quality semen.

Concerning motility control, only one AI center worked with a CASA system, which resulted in detailed motility data. In practice, visual motility assessment is used most frequently since it is easy to perform and does not require large investments (Woelders *et al.*, 1991). Most AI centers used more strict minimum motility requirements than the 60% criterion in the literature (Britt *et al.*, 1999). The strict minimum criteria for morphology and motility in commercial AI centers, together with the choice for a higher semen dose, which is not critical for the fertility outcome, indicate that Belgian AI centers consider good fertility results more important than maximizing semen dose production.

The data on semen processing showed that all AI centers except one used a short-term semen extender. The short distances for semen transportation due to the high pig herd density in Belgium and the geographical concentration of AI centers enables daily delivery of fresh semen to nearly all sow farms. Therefore, long-term extenders are only used if the semen has to be kept for several days. An additional motivation for some AI centers to use short-term extenders is the lower cost.

A surprising finding in this study was the substantial variation in temperature of the extender added to the ejaculate among AI centers. Although the detrimental effect of rapid cooling of fresh boar ejaculates is known (Johnson *et al.*, 2000), several AI centers do not make any attempt to minimize the temperature difference between extender and the ejaculated semen, apparently without negative effects on fertility results. Pre-dilution of the ejaculate by adding a small portion of extender prior to the final dilution, the higher number of spermatozoa in a dose and the strict criteria on motility may mask a possible negative effect of cold shock in these cases. Nevertheless, since adjusting the temperature of the extender is easy to perform, the possible deleterious effect of cold shock should be avoided in all AI centers.

In some AI centers, the preparation of extender solutions more than one day in advance, the use of open recipients or recipients containing a large air volume and the use of prepared extenders for more than a day may negatively influence semen quality. The pH of extenders is influenced by contact with ambient air containing low amounts of CO_2 and, as such, negatively influences sperm

motility (Vyt *et al.*, 2007). These semen handling procedures can be optimized in some AI centers.

Only three AI centers (8%) mentioned problems with bacterial contamination, decreased motility and decreased shelf life, which indicates that this is not a common problem in commercial AI stations, although bacteriospermia is frequently encountered in freshly diluted semen (Althouse and Lu, 2005). Four AI centers added antibiotics to the diluted semen, although only one of them had experienced problems with bacterial growth in diluted semen in the past.

The data obtained in this study are representative for semen examination and semen handling procedures applied in Belgian commercial AI centers. It appeared that economic considerations clearly influence semen examination and semen processing since inexpensive methods rather than more objective, automated procedures are preferred. In general, motility is the most frequently used criterion to assess sperm quality, and the semen doses are sufficiently high. Extender temperature and contact with ambient air can and should be optimized to avoid possible negative effects on semen quality.

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