Detailed motility evaluation of boar semen and its predictive value for reproductive performance in sows

Voorspellende waarde van spermabeweeglijkheid bij de beer voor de reproductieresultaten bij zeugen

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ABSTRACT

Reliable estimates of boar fertility potential from semen evaluation could be a valuable tool for boar selection. The aim of this study was to investigate the morphology and the detailed motility parameters of diluted boar semen and to relate these to their predictive value concerning conception and farrowing rate, litter size and the number of live born piglets. In addition, the optimal time for evaluation of the motility of preserved semen with respect to its predictive effect on fertility was determined. One hundred ejaculates from 38 boars were evaluated morphologically by eosin-nigrosin staining and different motility characteristics were assessed using Computer Assisted Semen Analysis (CASA). The motility was determined at 15, 45 and 120 minutes after incubation at 37°C. The conception rate, farrowing rate, litter size and number of live born piglets were registered from 276 sows inseminated with these ejaculates. Different regression models were used to evaluate the predictive value of the semen characteristics on these fertility parameters, taking into account the effect of herd, parity and weaning to estrus interval.

The motility characteristics of the spermatozoa varied significantly during the 15 to 120 minutes of incubation. The longer the incubation time, the more the velocity parameters along the actual cell path decreased, while the parameters of straightforward movement increased. The predictive value of individual semen parameters on conception and farrowing rate was very small. The predictive value of certain associations of different semen parameters, on the other hand, was significant.

The percentage of motile spermatozoa had a significant ($P<0.05$) and positive effect on the total number of piglets born (litter size) and on the number of live born piglets, independent of the time of measurement (X^2 0.38-1.00 and 0.41-1.00, respectively). Accurate evaluation of the motility of a semen dose is therefore imperative for estimating its predictive value relating to fertility.

In conclusion, since the time of evaluation after warming the samples significantly influences the motility parameters, CASA measurement should be done when the cells are completely acclimatized to 37°C. On the basis of the available data, a 45 min incubation period appeared to be sufficient. The percentage of motile spermatozoa, as assessed by CASA on diluted semen, offers detailed predictive information regarding litter size, irrespective of the time of measurement.

SAMENVATTING

Een betrouwbare inschatting van het bevruchtend vermogen van een beer aan de hand van het sperma zou zeer waardevol zijn bij de selectie van beren. De doelstelling van deze studie was om de morfologie en de bewegingskarakteristieken van verdund berensperma te onderzoeken en hun voorspellende waarde voor de reproductieparameters van zeugen na te gaan. Daarenboven werd het optimaal tijdstip voor de evaluatie van de beweeglijkheid van verdund sperma met het oog op de voorspellende waarde voor fertiliteit nagegaan. Honderd ejaculaten van 38 verschillende beren werden geëvalueerd op morfologie aan de hand van een eosine-nigrosinekleuring en op beweeglijkheid via Computer Assisted Semen Analysis (CASA). De beweeglijkheid werd bepaald op 15, 45 en 120 minuten na incubatie bij 37 °C. De conceptie, het afbigpercentage, de worpgrootte en het aantal levend geboren biggen werden geregistreerd bij 276 zeugen die met deze ejaculaten geïnsemineerd werden. Het voorspellend effect van de spermaeigenschappen op de fertiliteitparameters werd bepaald via verschillende regressiemodellen waarbij het effect van het bedrijf, de pariteit en het interval spenen-bronst in rekening gebracht werden.

De bewegingskarakteristieken van de spermatozoa verschilden significant in de tijdspanne van 15 tot 120 minuten na het opwarmen tot 38°C. De snelheidsparameters langs het traject van de cel daalden, terwijl de parameters voor een progressieve beweging toenamen bij een toenemende incubatie.

Kleine voorspellende waarden van spermakarakteristieken werden verkregen voor het dracht- en afbigpercentage waarbij vooral de associaties tussen parameters significant waren. Op elk tijdstip had het percentage bewegende cellen daarentegen een significant ($P<0,05$) en positief effect op de worpgrootte en het aantal levend geboren biggen (X^2 respectievelijk 0,38-1,00 en 0,41-1,00).

Aangezien het tijdstip van de evaluatie na het opwarmen de bewegingskarakteristieken significant beïnvloedt, dienen CASA-metingen van vloeibaar berensperma bewaard bij 17°C te gebeuren wanneer de cellen volledig geacclimatiseerd zijn bij 38 ±0,5°C. Op basis van deze data lijkt een incubatieduur van 45 minuten voldoende. Het percentage bewegende cellen bepaald via CASA bij verdund sperma geeft voorspellende informatie over de worpgrootte, onafhankelijk van het tijdstip van beoordeling.

INTRODUCTION

Relating sperm characteristics assessed *in vitro* to field fertility has been a challenge in several studies dealing with different animal species (Rodriguez-Martinez, 2003). Pre-selection of males or ejaculates tends not only to improve the conception rate and, in some species, the litter size, but additionally it increases the economic profit for the farmer. The outcomes of studies relating sperm characteristics to fertility have been variable and the correlations have not always been obvious (Budworth *et al.*, 1988; Tardif *et al.*, 1999; Gadea, 2005). Several difficulties were encountered in these studies (Gadea, 2005). First, the semen quality only partially reflects the fertility outcome. Not only is the interaction of the semen with the oocyte important, but also the interaction between the female genital tract and the sperm cells and the embryos is important, and this is more difficult to assess. Secondly, the pre-selection of ejaculates with good quality decreases the variation in sperm characteristics and makes it more difficult to find a significant correlation with subsequent fertility (Gadea *et al.*, 2004). Factors related to the sow and the technical expertise concerning optimal estrus detection and time of insemination strongly affect fertility results. Finally, the method used for assessment of the sperm characteristics plays a pivotal role.

A negative association between morphological anomalies and fertility was observed in several studies (Xu *et al.*, 1998; Waberski *et al.*, 1994; Alm *et al.*, 2006). The predictive effect of motility on fertility outcome was less straightforward. In most studies, easy to perform but very subjective methods, such as the visual motility examination, have been used to assess the sperm characteristics. Although these methods are used in practice (Vyt *et al.*, 2007) and correlate to more objective, computer-assisted motility measurement (Vyt *et al.*, 2004), visual estimates of motility are not sufficiently accurate to be used as predictors for fertility (Amann, 1989; Larsen *et al.*, 2000). To complicate the interpretation of the importance of semen parameters for predicting fertility, subpopulations of spermatozoa with slightly different motility characteristics have been described in boar semen (Abaigar *et al.*, 1999; Pena *et al.*, 2005). Computer-assisted semen analysis (CASA) systems provide detailed information on sperm motility because they can analyze many different motility characteristics of sperm cells simultaneously. Possible relationships between these detailed motility data with fertility results have been found in bulls (Budworth *et al.*, 1988), swine (Holt *et al.*, 1997) and humans (Larsen *et al.*, 2000). On the other hand, the detailed results make these systems sensitive to alterations in the system settings and demand accuracy and handling competence of the operators (Comhaire *et al.*, 1992; Verstegen *et al.*, 2002).

The predictive effect of semen motility in boars determined visually or by CASA on spermatozoa separated from the extender solution in laboratory conditions, has been the subject of several studies (Holt *et al.*, 1997; Tardif *et al.*, 1999; Gadea *et al.*, 2004). Data on the predictive effect relating to fertility of motility parameters determined by CASA directly on diluted boar semen, which can be done in practice, are not available.

The aim of the present study was to investigate the extent to which boar sperm morphology and sperm motility characteristics as determined by CASA on diluted semen can be used to predict the reproductive performance of sows in commercial pig herds. CASA measurement was done directly on diluted semen without prior recovery of sperm cells to maximize extrapolation of these data to routine practice. In addition, the motility parameters were assessed at three time points after incubation at 37°C to determine the optimal time of investigation regarding the predictive effect on fertility outcome.

MATERIALS AND METHODS

Semen collection

One hundred semen doses were obtained from a commercial AI center (Hypor, Zulte-Olsene, Belgium) between June 2004 and February 2005. The semen was collected using the gloved hand technique and diluted to $3x10^9$ spermatozoa per dose ($30x10^6$/ml) in a BTS extender (Minitüb, Tiefenbach, Germany, art 13525/1020) using a photometer (Colorimeter 252 Ciba-Corning, The Netherlands). The semen doses were stored at 17°C. One dose was sent to the laboratory for motility examination, while the remainder of the doses were used in five sow herds participating in

the breeding program. Semen doses were obtained from 38 different boars from selected lines: Hypor C-line (n=18), Hypor D-line (n=13), Hypor DG-line (n=4), beau-pi (n=1) and Piétrain (n=2).

Semen examination

When the semen doses arrived at the laboratory (10 hours post-collection), their morphology was examined using an eosin-nigrosin stain. The average of three counts of 100 cells was taken into account. The motility was assessed objectively using CASA analysis (Hamilton Thorne semen analyser, HTR Ceros 12.1, Hamilton Thorne Research, Beverly, USA). The semen samples were warmed at 37°C in an incubator and their motility was assessed at 15, 45 and 120 minutes after warming. Each motility assessment consisted of five measurements of at least 500 cells. For the regression models, the mean of these five measurements was taken into account. The software settings for the HTR Ceros 12.1 were those recommended by the manufacturer for the analysis of boar sperm, namely: frames per second (Hz): 60, number of frames: 45, minimum contrast: 18, minimum cell size (pix): 7, cell size (pix): 9, cell intensity: 125, slow-static cells with average path velocity (VAP) cut-off (µm/s): 20 and straight-line velocity (VSL) cut-off (µm/s): 5, minimum static intensity gates: 0.5, maximum static intensity gates: 2.5, minimum static size gates: 0.65, maximum static size gates: 2.6, minimum elongation gates: 20 and maximum elongation gates: 85. Different motility parameters were determined: VAP: average path velocity (µm/s), VSL: straight-line velocity (µm/s), VCL: curvilinear velocity (µm/s), STR: straightness (%), LIN: linearity (%), MOTILE%: percentage motile spermatozoa, PROGR%: percentage progressively moving spermatozoa (VAP> 45µm/s and STR > 45%), RAPID%: percentage rapidly moving spermatozoa (VAP > 45 µm/s) and STATIC%: percentage static spermatozoa (not moving during the analysis).

Reproductive parameters

The analyzed semen doses were used in 276 sows from six herds participating in the Hypor breeding program (Table 1). Double inseminations were performed at 18-36 h intervals, with a standard semen dose of $3x10^9$ spermatozoa per dose. Pregnancy testing was performed approximately 4 weeks after insemination. The conception and farrowing rates were calculated by dividing the number of sows that were tested pregnant or that had farrowed by the number of inseminated sows, respectively. At farrowing, the total number of piglets and the number of live born piglets were registered. Data on sow parity, date of insemination, non-return rate and litter size were collected.

Statistical analyses

The motility characteristics at the three different time points were compared using plots of agreement (Bland and Altman, 1986), repeated measures analyses of variance and Pearson correlation coefficients. The predictive effect of semen parameters (morphology, motility) on reproductive performance was analyzed using different regression models. Logistic regression models were used to analyze the conception and farrowing rates, whereas linear regression models were used to examine the effect on the total number of pig-

Table 1. Data on sows incorporated in the study.

Herd	Herd size	Sows in the study			WOI (mean ± DS)
		All parities	Parity 1-6	Parity >6	
A	415	55	43	12	6.1 ± 7.1
B	135	12	9	3	16.1 ± 27,0
C	400	93	87	6	8.3 ± 13.3
D	155	60	60	0	7.7 ± 10.0
E	185	40	39	1	5.7 ± 6.2
F	215	16	14	2	10.2 ± 14.5
Total		276	252	24	

WOI: weaning to estrus interval (±SD)

Table 2. Mean (±SD) motility parameters of boar semen (n = 100 ejaculates) as determined by Hamilton Thorn at 15, 45 and 120 minutes following incubation at 37°C. Values with different superscript are significantly different (P<0.05).

Time (min)	VAP	VSL	VCL	ALH	BCF	STR	LIN	MOTILE%	PROGR%	RAPID%	MEDIUM%	SLOW%	STATIC%
15'	88.2^{a}±7.5	61.2^{a}±7.6	161.0^{a}±16.5	6.1^{a}±0.8	37.1^{a}±2.8	68.7^{a}±6.8	39.4^{a}±6.6	86.4^{a}±5.8	62.9^{a}±12.1	77.6^{a}±8.8	8.8^{a}±4.1	8.6^{a}±4.5	4.9^{a}±5.1
45'	81.8^{b}±9.7	65.7^{b}±10.1	133.1^{b}±18.2	5.0^{b}±1.0	37.3^{a}±3.6	78.8^{b}±6.3	51.3^{b}±9.0	85.2^{b}±6.4	68.3^{b}±9.9	74.6^{b}±9.3	10.5^{b}±4.9	10.3^{b}±5.4	4.5^{a}±4.3
2h	79.3^{c}±11.4	67.7^{c}±10.6	119.1^{c}±20.9	4.4^{c}±1.0	36.7^{a}±3.4	83.8^{c}±5.4	59.4^{c}±9.5	83.9^{c}±6.1	67.2^{b}±10.2	71.4^{c}±10.1	12.4^{c}±5.6	11.6^{c}±5.1	4.4^{a}±4.4

For abbreviations of motility parameters see “Materials and Methods”.

Table 3. Pearson correlation coefficients for the motility parameters of pig semen (n=100) measured at different time points after incubation at 37°C. P-values were lower than 0.01 for all correlation coefficients.

Time points	VAP	VSL	VCL	ALH	BCF	STR	LIN	MOTILE%	PROGR%	RAPID%	MEDIUM%	SLOW%	STATIC%
15-45min	0.70	0.80	0.64	0.84	0.76	0.70	0.73	0.82	0.63	0.83	0.68	0.74	0.64
15-120 min	0.55	0.64	0.35	0.64	0.49	0.32	0.40	0.74	0.37	0.65	0.41	0.81	0.77
45-120 min	0.65	0.75	0.54	0.80	0.78	0.69	0.71	0.81	0.68	0.70	0.44	0.73	0.70

For abbreviations of motility parameters: see "Materials and Methods".

lets born (litter size) and the number of live born piglets. To build the final multivariable models, herd and parity were included as random variables and the weaning-to-estrus interval (WOI) was included in the model as a fixed parameter. Generalized linear mixed models were used to account for between herd variability (Verbeke and Molenbergs, 2000). A backward selection procedure was used to reduce the complex model into a small subset of factors that relate (significance level of $p<0.05$) to the outcome. During the model building process, all individual parameters and possible combinations of parameters were taken into account, and the deviance and Chi-square test were used to assess the goodness of fit of the logistic and linear models, respectively. Different models were performed with the semen data of each time point separately, and an overall model including the semen data from all time points was also performed to evaluate the evolution of the semen parameters over the incubation period examined (15 – 120 minutes). Regression analysis was performed using SAS 9.1.3.

RESULTS

Sperm parameters

The mean percentage (min-max) of spermatozoa with normal morphology was 92.2 (38.0-100.0). Two semen samples had less than 70% normal cells. The mean (min-max) percentage of sperm cells with abnormal morphology were: abnormal head 0.6 (0.0-5.0), proximal droplets 2.8 (0.0-17.6), distal droplets 2.4 (0.0-19.0) and abnormal tails 2.0 (0.0-44.0).

The motility parameters for the different time points are shown in Table 2, including the velocity parameters along the actual tract of the cell (VCL, VAP), as well as ALH, decreased with incubation time. The parameters STR, LIN and VSL, on the other hand, increased with incubation time. Plots of agreement (Figure 1) showed that the differences in most parameters were homogeneously distributed according to the mean of the two measurements at the time points examined. For low VAP values, the difference between the two time points tended to be larger. For VSL, on the contrary, the largest differences between the two measuring points were noted at high VSL. Samples with low BCF tended to have a larger difference between the measurements. This was most obvious when comparing the measurements at 15 and 45 minutes.

The correlations ($P<0.01$) between the motility characteristics at 15 and 45 minutes were higher than the correlations between those at 15 and 120 minutes and 45 and 120 minutes, respectively (Table 3).

Reproductive parameters

Of the 276 inseminated sows, 227 (82.2%) farrowed with a mean gestation length of 114.8 ± 3.4 days. Twenty-one sows (8%) returned to estrus and were inseminated again with semen of boars not included in this study, while 28 sows (10%) were culled for several reasons. Six of them were culled during pregnancy. The conception rate was 84.4%. In total, 2785 piglets were born, with a mean litter size of 12.27 (2785/227). The number of piglets born alive was 11.44 per litter (2596/227), while 0.83 piglets per litter (189/227) were stillborn. The piglets were weaned on average 23.3 days after farrowing and the mean number of weaned piglets per litter was 9.93. Of the 227 sows that farrowed, 26 had been inseminated a second or third time after weaning due to return to estrus.

A considerable number of semen characteristics were significantly associated with conception or farrowing rate (Table 4). For many of them, the associations with other variables were also significant, a fact which made the interpretation of the results more difficult. ALH and MOTILE% were positively and significantly associated with the farrowing rate at 15 min (ALH) and at 45 and 120 minutes (MOTILE%), respectively.

A positive influence of the percentage of motile spermatozoa on the total number of piglets (litter size) was observed (Table 5). According to the model, the litter size increased by 0.14 piglet per 1% increase of MOTILE%. The percentage of motile spermatozoa also had a positive influence on the percentage of live born piglets, irrespective of the time of measurement. Considering all time points, next to the evolution in MOTILE%, the evolution in WOI, the percentage of proximal droplets and the percentage of progressively moving spermatozoa all significantly influenced the number of live born piglets (Table 6). No significant influence of herd or parity was observed on either litter size or number of live born piglets.

DISCUSSION

In the present study, the morphology and motility characteristics of fresh, diluted boar semen were evaluated for their predictive effect on *in vivo* fertility. In

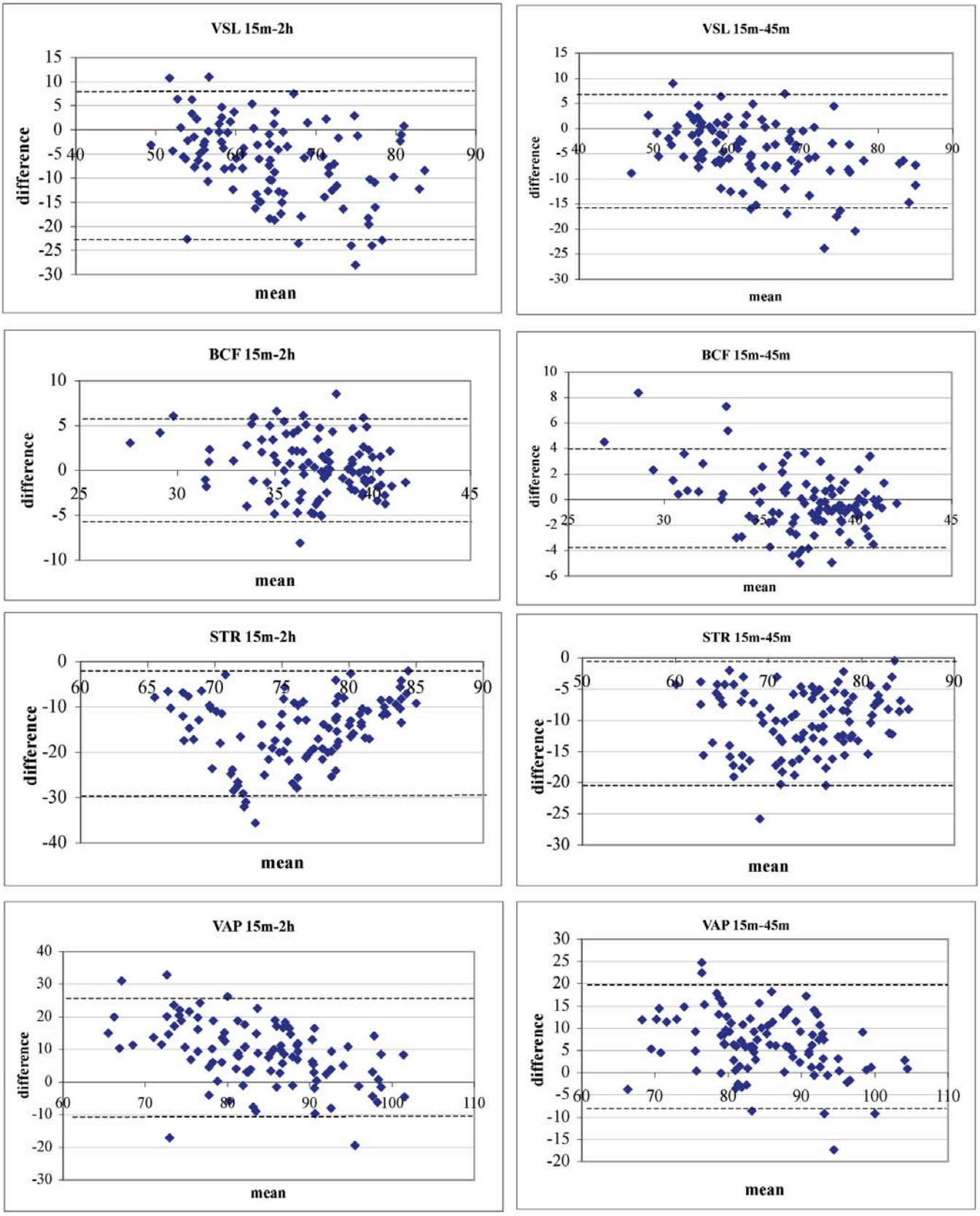

Figure 1. Plots of agreement for several motility parameters determined by HTR between two time points (15– 45 minutes and 15– 120 minutes). BCH: beat cross frequency; STR: straightness; VAP: average path velocity; VSL: straight-line velocity. Limits of agreement ($d \pm 2s_{diff}$) are indicated as dotted line.

addition, the optimal time for evaluation of motility characteristics was determined by motility assessments at three time points after warming the sample to 37°C.

The motility characteristics were clearly different, depending on the duration of incubation at 37°C prior to measurement. With increasing length of incubation, the cells were slowing down and, more in particular, the movement was evolving to a more straightforward pattern. In general, CASA measurements are performed at physiological temperature (i.e. 37°C), but the duration of semen incubation at 37°C before measuring varied considerably between studies: one minute (Iguer-ouada and Verstegen, 1999) in dog semen, 10 (Holt *et al.*, 1997) to 30 minutes (Vyt *et al.*, 2004) in boar semen and 20 minutes in humans (Larsen *et al.*, 2000). Verstegen *et al.* (2002) mentioned a hyperactivated motility of spermatozoa after a long period of incubation at low temperature. When the motility of the cells is observed visually during incubation at 37°C after a conservation period at 17°C, a vigorous, circular movement is initially seen, which evolves into a smooth, more linear motion after 5 to 10 minutes (data not shown). This observation indicates that bringing the sperm cells up to physiological temperature after a period of conservation at 17°C affects the motion parameters after a short incubation time at 37°C. In a study of boar semen (Schmidt and Kamp, 2004), hyperactivity was defined as a condition characterized by VCL > 97µm/s and ALH > 3.5 µm. Applying these criteria to the data in this study, the spermatozoa were hyperactive at all time points. Since other parameters in the present study (STR, LIN, BCF) were not concordant with the definition of hyperactivity by Schmidt and Kamp (2004), we conclude that the observed motion after warming was not hyperactivity, but was due rather to the acclimatization of the cells to their new environmental temperature. Since a measurement is preferred when the cells are fully acclimatized, the influence of the incubation conditions (incubator versus water bath) and the influence of the thermal conductivity and the diameter of the recipient – all of which influence the temperature of the semen aliquot and therefore the motion characteristics – are to be expected.

Table 4. Parameter estimates of the final multivariable logistic regression models for conception and farrowing rates, at 3 time points after warming the sample to 37°C (15, 45 and 120 minutes) and considering the evolution over all time points. Deviance 0.9275-0.9460 (farrowing) and 0.7631-0.7752 (conception); OR, odds ratio; 95% CI.

Conception Time	Parameter	OR	95%CI	P-value
15 minutes	Norm*LIN	1	0.99-1.00	0.0074
	Norm*VCL	1	0.99-1.00	0.0178
	LIN*VCL	1	1.00-1.00	0.0004
45 minutes	VCL	1.06	1.00-1.11	0.0271
	VCL*Norm	1	0.99-1.00	0.0126
	VCL*LIN	1	0.99-1.00	0.0087
120 minutes	VCL*PROGR%	1	0.99-1.00	0.0047
	VCL	1.05	1.02-1.07	<0.0001
All	Time	0.8	0.06-9.58	0.8593
	Norm	0.93	0.87-0.98	0.0079
	LIN	1.14	1.01-1.27	0.0225
	Time*VAP	0.97	0.94-1.00	0.0862
	Time*VCL	1.02	1.00-1.02	0.0163
	VAP	0.98	0.83-1.15	0.8288
	VCL	1.03	0.96-1.11	0.3658
Farrowing Time	**Parameter**	**OR**	**95%CI**	**P-value**
15 minutes	ALH	1.28	1.22-1.34	<0.0001
45 minutes	MOTILE%	1.02	1.01-1.02	<0.0001
120 minutes	MOTILE%	1.02	1.01-1.02	<0.0001
All	Time	30.52	2.39-389.04	0.0085
	Norm	0.95	0.91-0.98	0.0073
	Abn Tail	0.92	0.84-0.99	0.0422
	ALH	1.81	1.23-2.66	0.0053
	Time*ALH	0.7	0.54-0.89	0.0053
	LIN	1.08	1.02-1.14	0.0023
	Time*LIN	0.97	0.94-0.98	0.0044

Abn Tail: percentage abnormal tails; ALH: amplitude of lateral head displacement; LIN: linearity; MOTILE%: percentage motile cells; Norm: percentage spermatozoa with normal morphology; PROGR%: percentage progressively moving cells; VAP: average path velocity; VCL: curvilinear velocity

The incubation conditions in the present study reflect the semen examination after a temperature decrease in the extended semen. The issue as to whether the same movement pattern can be detected at natural service, where the semen temperature and semen concentration is not altered between ejaculation and service, was not examined in this study. Natural service is seldom applied anymore in most commercial swine herds. The present data emphasize the necessity of a standardized working protocol for CASA measurement of boar semen to obtain comparable results between laboratories. The optimal incubation time, if any, would be the time it takes for the spermatozoa to become acclimatized to their environment, which, according to our data, is more than 15 minutes. Therefore, we propose an incubation time of 45 minutes at 37°C before the CASA measurement is taken. Whether it is more advantageous to perform the CASA measurement after 120 minutes of incubation at 37°C could not be concluded from the present data. The time of measurement after warming the sample was important for obtaining standardized and comparable results.

In this study, morphological characteristics had only a small predictive value relating to fertility. The

Table 5. Parameter estimates of the final multivariable regression models for total number of piglets (litter size) at 3 time points after warming the sample to 37°C (15, 45 and 120 minutes) and considering the evolution over all time points. X^2: chi-square test for goodness of fit.

Time	Parameter	Estimate	Standard error	P-value	X2
15 minutes	MOTILE%	0.1475	0.0072	<0.0001	0.5555
45 minutes	MOTILE%	0.1495	0.0070	<0.0001	0.6143
120 minutes	MOTILE%	0.1531	0.0063	<0.0001	1.0000
All	MOTILE%	0.1479	0.0069	<0.0001	0.3800

MOTILE%: percentage motile cells

Table 6. Parameter estimates of the final multivariable regression models for number of live born piglets at 3 time points after warming the sample to 37°C (15, 45 and 120 minutes) and considering the evolution over all time points. C^2: chi-square test for goodness of fit.

Time	Parameter	Estimate	Standard error	P-value	X^2
15 minutes	MOTILE%	0.1292	0.0044	<0.0001	0.4128
45 minutes	MOTILE%	0.1329	0.0035	<0.0001	0.7723
120 minutes	MOTILE%	0.1350	0.0040	<0.0001	0.6249
All	Dist	0.1549	0.0413	0.0002	1.0000
	WOI	0.0330	0.0111	0.0030	
	MOTILE%	0.1312	0.0102	<0.0001	
	PROGR%*PROGR%	-0.0002	0.0001	0.0095	

Dist, distal droplets; MOTILE%: percentage motile cells; PROGR%: percentage progressively moving cells; WOI: weaning to oestrus interval

small negative effect of the percentage of normal spermatozoa on conception and farrowing rate (OR=0.93 and 0.95, respectively, in the overall model; Table 4) was therefore surprising when compared to the data in the literature (Xu *et al.*, 1998; Alm *et al.*, 2006). The pre-screening of ejaculates at the AI center, which resulted in a lower variation in the percentage of normal cells (Alm *et al.*, 2006) and the good fertility results of ejaculates with high numbers of cytoplasmic droplets, may explain this result. The negative influence of the percentage of abnormal tails on the farrowing rate can be explained by the importance of motility as expressed by the positive correlation with the percentage of normal cells at 45 and 120 minutes.

When evaluating the predictive value of the sperm motility parameters on sow conception, it became obvious that only a few parameters were significant, and this was mostly in association with other parameters. The predictive value of individual parameters was rather low. The absence of semen parameters with unequivocal predictive value for conception has also been observed in other studies (Holt *et al.*, 1997; Gadea *et al.*, 2004). Not only estrus management and sow factors, but also differences in defining conception, and farrowing vs 60 days non-return rate, may partly explain the difficulty of predicting conception from monitoring sperm morphology and motility parameters.

In the present study, the percentage of motile spermatozoa was significantly associated with litter size (total number of piglets) as well as with the number of live born piglets, irrespective of the time of measurement. The slightly lower predictive effect on the number of live born piglets compared to litter size is probably due to sow or environmental influences on fetal survival and it depends on the accurate determination of the number of piglets that die before birth of shortly after. Even though significant differences in absolute values for MOTILE% at the different time points were observed (Table 2), its significant positive association with litter size and number of live born piglets at each time point underlines the importance of this parameter in predicting fertility. Combinations of CASA measurements at different time points were found helpful in predicting fertility in an earlier study (Holt *et al.*, 1997), although these authors used capacitating conditions before measurement. The evolution of the percentage of motile cells during the 15 to 120 minutes of incubation at 37°C prior to measurement was also significantly associated both with litter size and with the number of live born piglets. On the basis of the data in this study, a comparison between the different time points is not necessary to obtain a predictive value from the percentage of motile spermatozoa. Several studies in swine have described the relation between sperm motility and litter size by assessing motility visually (Tardif *et al.*, 1999; Gadea *et al.*, 2004) or in laboratory conditions, with the recovery of sperm cells from the diluted sample (Holt *et al.*, 1997). Since individual boar differences cause variability in the relation between semen quality and fertility estimates (Popwell and Flowers, 2004), objective motility assessment by CASA is a powerful tool for evaluating the fertilizing capacity of an ejaculate or a boar. The detailed motility measurement using CASA in this study provides more insight into this relation and, since it was performed directly on diluted semen, the results are directly applicable in commercial AI centers.

Standardization of the time of incubation at 37°C before measurement is imperative, not for the evaluation of the predictive effect relating to fertility, but to obtain comparable results. Although the optimal time point for an accurate absolute measurement of motility parameters is difficult to determine from this study, an incubation time of 45 min is recommended, since the spermatozoa have definitely become acclimatized after that period of time. The data in this study indicate the importance of the percentage of motile spermatozoa as a predictive criterion.

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