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Seminal factors influencing return to estrus in female pigs following artificial insemination

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Abstract

Tests were applied to extended, chilled boar semen to further define those factors associated with return to estrus in inseminated female pigs (sows and gilts). Females were each inseminated twice with the same batch of extended chilled single-sire semen that was concurrently assessed at the Charles Sturt University Andrology Laboratory (CSUAL) in Australia. Semen traits tested were pH, clump score and temperature while sperm morphology assessment included abnormal heads, acrosomes, midpieces, tails and retained cytoplasmic droplets. Sperm motility and concentration were tested using a computerized sperm analyser (CASA) system. Female return types were categorised as early, early regular, early irregular, late regular and late. Depending on the type of variable of interest, statistical analyses used linear mixed models or generalised linear models. Terms included in the models were dam line, sire line, parity, insemination season, return type, individual boars and inseminators. Of 1205 inseminated females, 894 (74.2%) farrowed, 3 (0.2%) aborted and 308 (25.6%) showed different types of return to estrus. The fixed variables dam-line, sire-line and parity were significantly ($P < 0.05$, $P < 0.05$ and $P < 0.001$ respectively) associated with female return type, although inseminator and insemination season were not. Of the semen/sperm traits tested, the acrosomal defects that were significantly associated ($P < 0.05$) with female return-type were morphologically abnormal acrosomes and percent intact acrosomes while cytoplasmic droplets, normal morphology and bacterial score also influenced return type ($P < 0.05$). There were also correlations between sperm factors such as abnormal sperm tails, motility and velocity and sow parity. In conclusion, sperm morphologic assessment, in particular of the acrosome region, was useful in predicting female returns to estrus.

Keywords: boar fertility, return to estrus, sperm morphology.

Introduction

Subfertility or infertility in female pigs, as represented by returning to estrus following insemination, represents a large source of economic loss for pig producers in Australia and worldwide. Returns to service following insemination and subsequent failure to farrow are major factors in culling sows from the breeding herd (Koketsu *et al*., 1997). Sow return rates have been reported as high as 12% in Brazil (Vargas *et al*., 2009) and 38% in the USA (Lucia *et al*., 1996). Previous studies on causes of sow returns post-insemination (either naturally or artificially) have focused on breeding strategy (Elbers *et al*., 1995), ovarian structures (Kauffold *et al*., 2004; Vargas *et al*., 2009), genetic factors (Holm *et al*., 2005), lactational feed intake (Koketsu *et al*., 1997) and seasonal infertility (Bertoldo *et al*., 2009). These studies have included considerations such as previous lactation length, age at first service, nutrition, parity and body condition score. Potential male influences on sow returns, apart from boar infertility per se, appear to have received relatively little attention.

A major factor contributing to return to estrus in inseminated female pigs is early pregnancy loss (EPL); a phenomenon which occurs in all livestock species and one in which failure of fertilized ova to reach the blastocyst stage is common (Betts and King, 2001). However, development can also proceed past the blastocyst stage with subsequent failure to attach to the endometrium (Grimard *et al*., 2006). Work with other livestock species shows that males vary in their contribution to EPL (Bulman, 1979; Parinaud *et al*., 1993) despite comparable *in-vitro* fertilization (IVF) rates (Courot and Colas, 1986; Maxwell *et al*., 1992). In pigs it is also considered that sperm factors contribute to EPL and subsequent return to estrus or failure to farrow (Chenoweth, 2007).

Abnormal sperm DNA and/or chromatin integrity can adversely influence not only fertilization but also subsequent viability of the conceptus (Lewis and Aitken, 2005; Chenoweth, 2007). Diff-Quick, a staining procedure used routinely in clinical settings, has shown promise as a simple, inexpensive method to depict sperm DNA/chromatin damage (Van Steirteghem, 2009; Chenoweth *et al*., 2012).

Thus the aim of this study was to further define the effects of a number of factors, including genetic, seasonal and sperm/semen traits, on females returning to estrus following insemination.

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Materials and Methods

Animals

A total of 1205 females housed at a commercial pig stud (PIC) in Australia were each inseminated twice with extended chilled single-sire semen that was concurrently assessed at the Charles Sturt University Andrology Laboratory (CSUAL), Australia. Boars represented eight genetically different sire lines and sows represented 11 dam lines. Mean parity of the sows was 3 (range 1-10). Artificial insemination (AI) was performed twice during standing estrus as per industry guidelines. The first insemination was performed 12 h after sow was observed to respond to back pressure test plus boar contact and subsequent inseminations at 12 h intervals with both inseminations involving semen from the same boar to ensure known paternity. Sows were inseminated with semen stored no longer than 5 days after collection. The type and composition of the semen extender was not divulged by PIC. The determination of which boar was used to inseminate a specific female was based on genetic breeding objectives. All inseminations were artificial; no natural matings took place and each AI dose was 85 ml containing approximately 3 billion sperm (mean 5 ± 1.5 billion sperm/dose; CoV =3.4). Pregnancy was determined using Agroscan™RealTime β-mode ultrasound 24-28 days after insemination. Non-pregnant pigs were re-checked 2 days later by a different technician. Approval was obtained from the Charles Sturt University Animal Care and Ethics Committee for all experimental procedures involving boar sperm.

Semen

Semen was collected from Large White and Duroc boars (n = 87) by the gloved hand technique (King and MacPherson, 1973) and processed per stud routine protocols. All semen samples were obtained from the same PIC boar stud and analysed on the same day or the day after collection. A chilled aliquot of 25 ml extended semen from each boar was sent to CSUAL within 24 h of collection. Incoming semen was checked for temperature and pH and placed on a slide warmer at 37°C until the semen had reached 37ºC before assessment. Sperm movement was analysed with a computer-assisted sperm analyser (CASA) Integrated Visual Optical System, Version 12.4, Hamilton Thorne, USA for motility (total, progressive and rapid - also known as 'local' motility), velocity (straight line or VSL, average or VAP and curvilinear, VCL), beat cross frequency ('head wobble' or BCF) as well as concentration. CASA assessments were done using 4-well Leja slides (Minitube Australia). CASA measurements were done using 45 frames at 60Hz frames/sec. Cell detection (minimum 200) used a minimum contrast of 46 and minimum cell size of 7 pixels.

Aliquots of semen were placed into vials of isotonic formal buffered saline (Hancock, 1957) for sperm morphology evaluation. This was conducted by observing 100 sperm using wet mounts under oil immersion at 1000X magnification with a differential interference contrast (DIC) microscope. Sperm morphology assessment categories were abnormal heads, acrosomes, midpieces and tails as well as the presence of retained cytoplasmic droplets (proximal and distal) and percent intact acrosomes (PIA), based on the presence or absence of a discernible apical ridge (Saacke and White, 1972).

Semen smears were prepared and stained with eosin-nigrosin (Lane Manufacturing, USA) for membrane integrity or 'live/dead' estimation (Gadea *et al*., 1998) Sperm were also stained with Diff-Quick® (Provet, Australia) for assessment of sperm DNA/chromatin status.

Insemination seasons were nominated as summer (Nov-Jan), autumn (Feb-April), winter (May-July) and spring (Aug-Oct). Sows were inseminated by industry-trained inseminators while in standing heat as detected by boar contact and back pressure test.

Female return type

Return data on inseminated females were obtained from a comprehensive proprietary database accessed courtesy of the collaborating farm. Inputs were derived from regular observations of inseminated females (approx 3 x day) by trained personnel. Heat detection was done at 18-23 days post-farrowing or post-insemination using visual clues such as standing reflex in response to back pressure, reddening and enlargement of vulva, vaginal discharge, interest in mature boars walking past the sows every day and increased vocalizations.

Inseminated females returning to estrus were classified into the following categories depending on how many days after insemination they were first detected as being in estrus (adapted from Koketsu *et al*., 1997):

1. Early (0-18 days).
2. Regular (normal; 19-23 days).
3. Early irregular (24-35 days).
4. Late regular (36-45 days).
5. Late irregular (>46 days).

Analyses

Data from female breeding records and sperm tests were collected between January, 2009 and November, 2010. The analyses had two objectives; firstly to establish relationships between return type and pig genetic lines and management factors, and secondly, to identify semen and sperm traits that were related to return type after accounting for dam- and sire-line genetics and management factors. The variables of

interest included continuous and count variables. The analysis methods used for these types of data were linear mixed models and generalised linear models, respectively.

For statistical analyses of the count variables, an ordinal logistic regression model was used to establish relationships between pig genetics (i.e. dam and sire lines), management factors such as inseminator effect and return type. This analysis was conducted using statistical computing software (R Development Core Team, 2010). The main effects in the model were sire line, dam line, parity and insemination season.

The second objective was pursued using linear mixed model methods in ASReml-R (Butler *et al*., 2007). The fixed terms included in the model were dam line, sire line, parity and insemination season. Also included were all the two-way interactions of these fixed terms. The random terms included inseminator, the individual boars and dams. Covariates, including semen and sperm traits, were added to the model. F-tests were used to determine significance between sow age, parity and dam line. Results are presented as predicted means (± SEP) with significance starting at $P < 0.05$.

Results

Of the 1205 females which were inseminated with semen concurrently examined at the CSUAL, 74.2% (894) farrowed, 0.2% (3) aborted and 25.6% (308) returned to estrus following insemination. The types of returns and days to outcomes are summarised in Table 1, as well as the predicted probability for each of the return type categories. In total, 0.8% (10) of inseminated females were observed to return within 0-18 days (Early), 7.2% (87) between 19 and 23 days (Regular), 6.7% (81) between 24 and 35 days (Early irregular), 2.7% (32) between 36 and 45 days (Late regular) and 8.1% (98) after 46 days (Late).

Of the 308 sows which returned to estrus following insemination, the largest group was the late return category (31.8%) followed by early regular (28.2%) and early irregular (26.3%). A combination of the 3 categories of irregular returns (i.e. early, early irregular and late) showed that 61.8% of returns to estrus were irregular; i.e. at times which were considered to be incompatible with normal estrus cycle length(s).

Relationship between return type and environmental factors

Neither insemination season nor inseminator significantly influenced estrus return type. Sow records were obtained during all 4 seasons. Inseminators were all trained to be competent and were supervised by a senior qualified technician during the insemination procedures.

Table 1. Types of returns to estrus and pregnancy outcomes; incidences and predicted probabilities.

Return type	Days to outcome	% of females inseminated (n)	% of female returns	Predicted probability
Early	0-18	10 (0.8)	3.2	0.1352
Early regular	19-23	87 (7.2)	28.2	0.5967
Early irregular	24-35	81 (6.7)	26.3	0.1715
Late regular	36-45	32 (2.7)	10.4	0.0359
Late	>46	98 (8.1)	31.8	0.0605
Aborted		3 (0.2)	na	na
Farrowed		894 (74.2)	na	na
Total returns		308 (25.6)	na	na
Total sows		1205	na	na

na = not applicable.

Relationship between return type and sow factors

Parity ranged from 1 to 10 and inseminated female age ranged from 221 to 1617 days. The age for females returning to estrus varied significantly for dam lines and parity (both $P < 0.001$; Table 2). Return type was significantly linked with parity and female age (both $P < 0.01$). The intermediate parities (2, 3 and 4) showed least variation in effects of parity on return type (Table 2). There was a significant correlation between parity and return type ($P < 0.01$, $r^2 = 0.02$). Only one sire line out of 8 genetic lines influenced return type ($P = 0.05$, $r^2 = 0.009$). Dam-line influenced parity ($P < 0.001$), but not return type.

Table 2. Correlations for parity, sow age and return type#.

Parity	Sow age (days)	Type of return
1 *vs*. 3*	≤300 *vs*. 500**	Early *vs*. regular**
1 *vs*. 4**	≤300 *vs*. 700**	Early *vs*. early irregular**
1 *vs*. 5**	≤300 *vs*. 900**	Early *vs*. late regular**
1 *vs*. 6**	≤300 *vs*. 1100*	Early *vs*. late**
2 *vs*. 3*	≤300 *vs*. 1300**	Regular *vs*. early irregular**
2 *vs*. 4*	≤300 *vs*. 1500**	Regular *vs*. late regular**
2 *vs*. 5*	≤500 *vs*. 700**	Regular *vs*. late**
2 *vs*. 6**	≤500 *vs*. 900**	Early irregular *vs*. late regular**
	≤500 *vs*. 1100**	Early irregular *vs*. late** Late regular *vs*. late**

*P < 0.05; **P < 0.01. #other parities and ages were not significantly linked with return type.

Relationship between parity and sperm factors

Table 4 summarizes the correlations between sow parity and sperm factors. Returns are mainly influenced by progressive motility, rapid motility, VAP and VSL in early parity sows (1-5). In contrast, sows of higher parity order (e.g. 7 and 8) appear to return to estrus mainly due to abnormal sperm tails (Table 4).

Relationship between return type and sperm traits

A summary of the mean values and range for sperm traits is shown in Table 3. Large ranges were observed for sperm motility assessments and a percentage of morphologically normal sperm as well as for individual categories such as abnormal midpieces, tails, retained distal droplets and membrane integrity (or "live dead"). Of the sperm traits assessed, the significant associations with sow return-type were sperm acrosome morphological abnormalities, retained cytoplasmic droplets, bacteria, average velocity (VAP) and curvilinear velocity (VCL) and percent intact acrosomes (Tables 5 and 6).

Table 6 shows predicted return types for those sperm traits which significantly affected return type; i.e. percent intact acrosomes (PIA) and abnormal acrosome morphology (mean values 85.8 ± 2.9 and 0.5 ± 0.2% respectively). For PIA, most of the effect was on late (>46 day) returns which differed (P < 0.05) from irregular (36-45 days) returns. Similarly for abnormal acrosome morphology in which late (>46 day) returns differed (P < 0.05), although in this case from regular (19-23 days) returns.

Table 3. Insemination mean values and ranges.

Covariate	Mean	Range
Normal sperm morphology (%)	73.20	(19 - 94)
PIA (%)	87.90	(54 - 99)
Abnormal Head (%)	7.30	(0 - 33)
Abnormal acrosome (%)	0.5	(0 - 12)
Abnormal midpiece (%)	8.30	(0 - 49)
Abnormal tails (%)	2.10	(0 - 33)
Proximal droplet (%)	2.50	(0 - 19)
Distal droplet (%)	4.50	(0 - 44)
Detached heads (%)	1.30	(0 - 9)
Intact membranes (%)	77.10	(42 - 97)
Total motile (%)	67.90	(1 - 97.2)
Progressive motile (%)	38.10	(0 - 74.2)
Rapid motile (%)	54.20	(0 - 92.8)
VAP (μm/sec)	82.50	(32.6 - 125.2)
VCL (μm/sec)	162.00	(70.9 - 249.8)
VSL (μm/sec)	50.90	(23.1 - 95.6)
BCF (Hz)	33.20	(6 - 41.1)
pH	7.50	(6.7 - 8.1)
Temperature (°C)	19.20	(10.8 - 24)
Concentration (million/ml)	53.40	(15.8 - 121.9)
DQ %	86.50	(71 - 97)

PIA = percent intact acrosomes. Intact membranes = "live/dead" determined with eosin-nigrosin stain. VAP = average path velocity. VCL = curvilinear velocity. VSL = straight line velocity. BCF = beat cross frequency. DQ = DiffQuik percent normal.

Table 4. Correlations between parity and sperm factors.

Progressive motility	Rapid (local) motility	VAP (average path velocity)	VSL (straight line velocity)	Abnormal sperm tails
2 *vs*. 3** r^2 = 0.06751	2 *vs*. 3** r^2=0.07297	1 *vs*. 5* r^2=0.0786	1 *vs*. 3* r^2=.0.04942	1 vs. 3* r^2=.0.05108
3 *vs*. 4** r^2 = 0.1458	2 *vs*. 5* r^2=.0.04324	2 *vs*. 3* r^2=.0.04383	1 *vs*. 5** r^2=.0.1191	1 *vs*. 7* r^2=.0.08759
4 *vs*. 5** r^2 = 0.1734	3 *vs*. 4** r^2=.0.1682	2 *vs*. 5* r^2=.0.07334	1 *vs*. 6* r^2=.0.07165	2 *vs*. 7** r^2=.0.1138
	4 *vs*. 5** r^2=.0.2025	3 *vs*. 4** r^2=.0.1209	2 vs. 5** r^2=.0.07318	3 *vs*. 7** r^2=.0.1985
		4 vs. 5** r^2=.0.2595	3 *vs*. 4* r^2=.0.1068	3 *vs*. 8* r^2=.0.1047
			4 *vs*. 5** r^2=.0.2630	5 *vs*. 7* r^2=.0.1839
			4 *vs*. 6* r^2=.0.1623	

1 = parity 1, 2 = parity 2 etc; *P < 0.05; **P < 0.01.

Table 5. Correlations between return type and sperm factors.

VAP	VCL	Normal morphology	Proximal droplets	Distal droplets	Acrosomes	Bacteria
1 *vs*. 3** r^2 = 0.08737	1 *vs*. 3 ** r^2 = 0.1639	2 *vs*. 3* r^2 = 0.04101	2 *vs*. 3* r^2 = 0.03775	2 *vs*. 3** r^2 = 0.05542	3 *vs*. 4* r^2 = 0.06128	2 *vs*. 5** r^2 = 0.08259
3 *vs*. 4* r^2 = 0.04063	1 *vs*. 4* r^2 = 0.1636	2 *vs*. 4** r^2 = 0.1140	2 *vs*. 4* r^2 = 0.66901	2 *vs*. 4* r^2 = 0.04948	3 *vs*. 5** r^2 = 0.06071	
3 *vs*. 5* r^2 = 0.03440	3 *vs*. 5* r^2 = 0.04460	2 *vs*. 5** r^2 = 0.09018	2 *vs*. 5** r^2 = 0.08517	2 *vs*. 5** r^2 = 0.06066		

1 = early return; 2 = regular return; 3 = early irregular return; 4 = late irregular return; 5 = late return; *P < 0.05, **P < 0.01.

Table 6. Predicted return type values from linear mixed model.

Return time	PIA	Abnormal acrosomes %
0-18 days	88.5 ± 3.6^{ab}	1.15 ± 0.38^{ab}
19-23 days	91.5 ± 2.7^{ab}	0.53 ± 0.16^{a}
24-35 days	88.8 ± 2.6^{ab}	0.70 ± 0.17^{ab}
36-45 days	85.8 ± 2.9^{a}	1.11 ± 0.24^{ab}
>46 days	92.1 ± 2.4^{b}	1.22 ± 0.16^{b}

PIA = percent intact acrosomes. Different superscripts within a column indicate significant differences; $^{a,b}P < 0.05$.

Discussion

Inseminated females which return to estrus at regular intervals coinciding with the normal length of the estrus cycle in pigs have probably either failed to conceive or undergone early pregnancy loss prior to implantation (Koketsu *et al*., 1997). Those returning at irregular intervals may be considered to have either undergone EPL (i.e. following uterine attachment and before fetal calcification) or been inseminated at a time which did not coincide with true estrus.

The categorisation of the type of return to estrus exhibited by inseminated females allows interpretations of possible causes as follows:

1. Early (0-18 days). Conception failure (e.g. fertilization failure).
2. Regular (normal; 19-23 days). Conception failure or EPL.
3. Early irregular (24-35 days). EPL.
4. Late regular (36-45 days). Returns to estrus after gestational day 35 often represent abortion. However, it is also possible that these sows had an earlier, missed, regular return to estrus which could have been due to either conception failure or embryonic loss.
5. Late (>46 days). Late returns can represent abortion (although these cases would have been deleted from the dataset if recorded by personnel). However, in group housing such as in the present case, such evidence might be lacking due to cannibalization of the aborted fetuses and fetal membranes. Alternatively, this category could also include missed earlier returns.

Although dam line was not implicated in return type, it is perhaps not surprising that both sire line and parity had a significant effect on this trait. Relatively little is known about genetic influences on returns to estrus even though commercial pig operations employ a number of different genetic lines to improve production. Reproductive traits enhanced by using specific genetic lines include total number of piglets born, piglet mortality and weaning-to-estrus interval (Bergsma *et al*., 2008). The weaning-to-estrus interval is an important factor in the timing of insemination during fixed-time

insemination. However, unfortunately the weaning-to-estrus interval for the sows in the present study was not known. Other traits which have been improved by crossing specific dam lines or sire lines include lifetime prolificacy (Serenius and Stalder, 2004; Serenius *et al*., 2008), back fat thickness (Bereskin, 1984) and average daily gain (Kaplon *et al*., 1991).

As in the present study, parity (range 1-10, mean = 3) influenced sow returns in a study by Koketsu *et al*. (1997) in which parities at both ends of the spectrum (parity 1 and parity 6 plus) differed ($P < 0.05$ or $P < 0.01$) from intermediate parities (parities 2 to 4). The farrowing rate was only 74% for this cohort of sows which is lower than expected; for example, Dutch pig farms use A.I. almost exclusively and achieve a mean farrowing rate of 86% (Broekhuijse *et al*., 2012). The lower farrowing rate could be due to a number of reasons beyond the scope of this paper, such as aggression associated with group housing of sows, sow age, and parity, as well as human error in detecting estrus or pregnancy.

Of those sperm traits examined by CASA in this study, the only ones linked with female return type were VAP and VCL. Although sperm motility is important for male fertility, its effects in this study were probably diminished by a skewed distribution of both motility and sperm concentration results towards the upper (or optimal) end of the spectrum. As sperm motility is regarded as a compensable sperm defect, i.e. one in which adverse results can be mitigated by increased sperm numbers in the insemination dose, these factors probably reduced the chance of sperm motility findings being significantly associated with sow return types. Another reason could be the relatively small number of inseminated sows in this study. Broekhuijse *et al*. (2012) used a dataset of >100,000 Dutch boar ejaculates used for 165,000 sow inseminations resulting in ~1 million piglets of known paternity whose sires had their semen tested before AI. These authors found that only 6% of total variation in fertility was due to boar and semen factors while motility did not have a significant effect on boar fertility unlike genetic sire line (Broekhuijse *et al*., 2012).

The large range of values for semen parameters such as proximal droplets, midpiece and head defects in Table 3 can be attributed to the sperm donors being of variable age including young recently post-pubertal boars whose sperm is not of optimum quality yet. Another reason for a higher proportion of sperm morphological defects was heat stress of boars during the summer, which had an adverse effect on sperm quality. Likewise, the large range in motility and velocity parameters is most likely due to semen being too chilled during transport to the laboratory in the winter or becoming too hot in transit in the summer. However, most samples arrived at or near 17°C displaying acceptable sperm parameter values.

The large variation in sperm concentration is due to the boar stud providing the lab with a small aliquot (~25 ml) of semen which does not always reflect the concentration within the whole AI dose. Sperm concentration within an AI dose or ejaculate is a crucial factor in fertility, especially regarding the determination of litter size in pigs (Xu *et al*., 1998).

Similar considerations are probably involved in the failure to detect a significant relationship between sperm DNA/chromatin status, as determined by Diff-Quik staining, and female return types. Although abnormal sperm DNA/chromatin is associated with EPL in humans and in livestock species (Lewis and Aitken, 2005; Chenoweth, 2007), the fact that a significant relationship was not obtained with female returns in this study is probably due to the relatively high levels of "normal" sperm observed in the study population.

Sperm morphology in general can affect return rates in female pigs as was the case in this study and the work of others. In one study (Alm *et al*., 2006), the proportion of morphologically normal spermatozoa was significantly correlated with non-return rate using either 2 or 3 billion spermatozoa per dose. Likewise, litter size was linked with sperm morphology when using AI doses of 2 billion sperm (Xu *et al*., 1998). However, competent, detailed sperm morphology assessment is not routinely practised in pig production units as it is time-consuming and requires trained observers.

The presence of an intact, normal acrosome is essential for successful sperm binding to the zona pellucida (ZP; Waberski *et al*., 2006). The sequence of events leading to fertilization of the ovum once the capacitated sperm reaches the ZP is complex. Briefly, it involves acrosome exocytosis followed by penetration of the extracellular matrix of the oocyte (i.e. the ZP) and then binding and fusion with the oolemma (Gadella and Evans, 2011). Therefore, fertilization is not possible when sperm acrosomes are lacking or defective. In turn, fertilization failure should result in the inseminated female returning to estrus on a regular schedule. Thus, it was not surprising that the predicted return type most adversely affected by morphologically abnormal acrosomes was the late regular category of 36-45 days, assuming that earlier regular returns (19-23 days) were probably not observed.

Problems associated with the acrosome may be attributed to a number of causes, including structural deformities that occur during spermiogenesis, due to either stress or genetic influences (Meyer and Barth, 2001). More commonly, however, defects occur due to sperm ageing or following sperm death (Saacke and Marshall, 1968) and include a lack or partial lack of the acrosome as well as disturbances of the adjacent membranes. Likewise, retained cytoplasmic droplets surrounded by the plasma membrane are normally shed under the influence of D-fructose before ejaculation (Harayama *et al*., 1996). Failure of the droplets to be shed from the sperm midpiece indicates a defect of testicular origin which has adverse effects on

conception rate and embryonic viability (Kuster *et al*., 2004). Thus, the presence of retained cytoplasmic droplets in boar ejaculates can negatively affect return to estrus rates as determined in the present study.

In this study, the relative importance of normal acrosomes when compared with the other semen traits regarding returns to estrus may be viewed in light of a study by Saacke and White (1972). Working with Holstein bulls in an AI Centre, these authors found that PIA was more related to 60-90 day NR rates than was sperm motility. An important caveat for this study was that the bulls were pre-selected for AI purposes and in a controlled environment in which a number of other variables associated with semen quality were probably minimised. A similar context could be suggested for the present study whereby the boars had been carefully selected and closely monitored, thus skewing semen quality traits towards the favourable end of the spectrum. However, as acrosome status is not usually monitored in production units due to the constraints described above, this particular aspect was probably neglected. These results indicate that the development of an accurate, rapid and economic method of assessing acrosomal form and function in boar sperm could be useful in predicting breeding outcomes.

In conclusion, the assessment of boar sperm morphology, particularly of the acrosomal region, is useful in predicting returns to estrus following insemination, particularly those associated with fertilization failure.

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