Animal Reproduction Science 151 (2014) 28–33

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Semen effects on insemination outcomes in sows

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ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form
19 September 2014
Accepted 26 September 2014
Available online 8 October 2014

Keywords:
Boar sperm
Sperm morphology
Litter size
Born alive

ABSTRACT

Sows ($n = 1205$) were artificially inseminated with semen from single sires ($n = 166$). Semen was previously analysed for sperm concentration, motility, velocity, morphology (using DIC microscopy) and membrane integrity, sperm clump score, temperature on arrival and pH. Percent normal sperm influenced both numbers of pigs born alive ($P < 0.01$) and litter size ($P < 0.05$) which, in turn, was also influenced by abnormal sperm head morphology ($P < 0.05$) and retained distal cytoplasmic droplets ($P < 0.01$). Percent stillbirths were influenced by sperm flagellar beat cross frequency ($P < 0.05$) and semen arrival temperature ($P < 0.05$).

1. Introduction

Artificial insemination (A.I.) is common practice in the global pig industry (Boe-Hansen et al., 2008) with reliance on extended, chilled semen (Vazquez et al., 2005), permitting more rapid genetic gain than that achievable *via* natural breeding (McLaren and Bovey, 1992). This places more emphasis on quantitative and qualitative aspects of boar semen, which can include variables such as sperm motility, morphology, concentration, chromatin condensation and membrane integrity. Such assessments, which can help to detect subfertile boars and/or ejaculates, are most effective when semen from a single sire rather than pooled semen is employed for insemination (Flowers, 2009) as the latter can mask individual problems (Safranski, 2008). When sperm numbers per inseminate are reduced to increase the number of A.I. doses per ejaculate, compensable morphological defects become more important as these can lead to reduced litter size (Saacke et al., 2000). Thus, failure to monitor boar semen quality can result in significant economic loss, especially when one problem boar is used to inseminate large numbers of females (Foxcroft et al., 2008).

Both quantitative and qualitative aspects of an ejaculate are influenced by factors such as collection frequency, age of the boar, breed and season (Wolf and Smital, 2009; Colenbrander and Kemp, 1990). Although relationships between semen or sperm variables and fertility of A.I. boars are not always clear (Xu et al., 1998), it is clear that competent screening of boar semen can reduce reproductive outcome variability. In this respect (McPherson et al., 2014), sperm morphology, motility, pH, membrane integrity and chromatin condensation were all associated with litter size, pigs born alive and stillbirths as well as sows returning to oestrus (conception failure and embryonic loss).

Despite this, it should be acknowledged that female management is also important in determining reproductive outcomes (Colenbrander et al., 2003). Maternal factors can influence the litter size, number of pigs born alive,

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http://dx.doi.org/10.1016/j.anireprosci.2014.09.021
0378-4320/

stillborn pigs, lactation performance and wean-to-service interval. These factors include breed (Spötter and Distl, 2006), age and bodyweight at first service (Tummaruk et al., 2007), parity (Clark and Leman, 1987), number of services per oestrus (Clark et al., 1988) and dam line (Roehe and Kennedy, 1993). In addition, environmental factors such as humidity and ambient temperature can also contribute to mating outcomes (Kabuga and Annor, 1991; Xue et al., 1994).

Improvements in the assessment of boar sperm morphology and motility have occurred with the employment of technologies such as high power differential interference contrast microscopy (DIC) and computer assisted sperm analysis, or CASA (Broekhuijse et al., 2011). Boar sperm motility assessments performed with the CASA system have shown relationships with fertility (Hirai et al., 2001) although this procedure has yet to adopt a definitive role in the laboratory prediction of fertility parameters (Amann and Waberski, 2014).

In the present study, it was hypothesised that defective boar sperm, as identified using a variety of conventional and newer techniques, would significantly influence mating outcomes such as litter size, pigs born alive and stillbirths.

2. Materials and methods

2.1. Animals

Sows and boars were housed indoors at a commercial Australian boar stud in New South Wales where breeding occurred along specific genetic lines to maximise selected genetic traits. Semen was collected by gloved hand method (King and Macpherson, 1973) from Large White and Duroc boars ($n = 166$) and chilled aliquots ($n = 284$) at 17 °C were transported on collection day to the Andrology Laboratory (AL) for analyses (see Section 2.3). Sows ($n = 1205$ of which 894 or 74% farrowed), representing eight sire lines and 11 dam lines, were twice inseminated within 4 days of the collection date with 85 ml of the assessed single sire semen with mating records being obtained 4 months later. Semen collections occurred at approximately weekly intervals over 22 months from January 2009 until end of October 2010. For some boars, multiple semen samples were tested (single, $n = 109$; double, $n = 36$; triple or more, $n = 21$), resulting in a total of 284 aliquots assessed.

2.2. Herd management and data recording

Barren sows were housed in group pens as per industry guidelines and exposed to a boar every day to determine oestrus status based on standing heat, reddening of the vulva and vocalisations with the boar. Sows ($n = 1205$ of which 894 or 74% farrowed), representing eight sire lines and 11 dam lines, were twice inseminated with single sire semen within 4 days of the collection date with 85 ml (~3 billion sperm/dose). An aliquot of the same single sire semen was assessed with mating records being obtained 4 months later. Sows farrowed in individual farrowing crates and data regarding litters were obtained as soon as practical either immediately after farrowing (in daytime) or at the start of the next working day for litters born overnight.

2.3. Semen handling and analyses

Upon arrival at the AL, semen temperature within its packaging was measured using a laser thermometer (Minitube, Australia) before being warmed to 37 °C in a water bath. After appropriate mixing, a small sample was placed within a vial containing buffered formal saline (BFS) fixative (Hancock, 1957).

2.3.1. Computer-assisted sperm analysis

Once warmed and mixed, all semen samples (20 μl for each) at 37 °C were analysed by Hamilton Thorne CASA Version 12.3 for sperm total motility, progressive motility, rapid motility, concentration, straight line velocity (VSL), curvilinear velocity (VCL), average velocity (VAP) as well as beat cross frequency (BCF), using Leja® four well 20 micron counting slides (Leja Products, The Netherlands) within 24 h of collection. The CASA variables for boar semen were set at an image capture of 45 frames using frames/s of 60 Hz. Minimum cell size was set at 7 pix. An arbitrary sperm clump score (0 = no clumps, 1 = one clump per view; 2 = several clumps per view; 3 = almost complete clumping) was assigned based on the CASA acquire screen view. For each Leja well, four fields were analysed and repeated four times, with a requirement that at least 250 individual sperm were assessed. In this trial, as progressive and rapid motility were highly and significantly correlated with total motility, only total motility was used in the statistical analyses.

2.3.2. Sperm morphology

A wet mount was made of the fixed semen and left to settle for >30 min before reading. Morphology readings were performed by counting 100 spermatozoa/slide with only one main defect assigned to each sperm. Microscopy was performed using a Nikon Differential Interference Contrast microscope (model) at 1000 magnification using oil immersion. The percentages were obtained for morphologically normal sperm, presence of proximal or distal cytoplasmic droplets, abnormal heads (deformed, diadem/crater defect, misshapen), midpieces or tails as well as abnormal acrosomes and detached or detached heads. A separate count of the fixed semen slide was then done specifically for a percentage of intact acrosomes (PIA). Acrosomes were considered to be intact if the entire acrosomal cap was present as viewed under oil immersion microscopy at 1000× magnification.

2.3.3. Other semen variables

Semen pH was measured using a digital pH metre. For membrane integrity, several drops of semen were mixed with one drop of eosin/nigrosin stain (Lane Manufacturing, Colorado) and then a smear was made with a spreader slide to create different bands of variable staining intensity. Stained compared with non-stained sperm heads were counted using brightfield microscopy at 400× magnification and are hereafter referred to as live/dead counts.