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Field fertility of frozen boar semen: A retrospective report comprising over 2600 AI services spanning a four year period

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ABSTRACT

Worldwide, greater than 90% of sows are inseminated with fresh semen. Less than 1% is inseminated using frozen semen. Albeit, frozen semen is an effective technology for the transfer of genes between breeding pyramids and also to reliably provide semen for planned matings. Little information exists on the long term use of frozen boar semen in commercial pork production operations. The objective in the present study was to assess application of frozen semen throughout a 4 year period comprising more than 2600 AI services. The frozen semen sourced from a boar stud in Manitoba, Canada. All artificial insemination (AI) occurred on a single 1800 sow farm in Indiana, USA. The sperm-rich fraction was collected and only those collections having ≥80% motility and ≤15% abnormalities were further processed. Semen was prepared for cryopreservation using Androhep® CryoGuard™, packaged in 0.5 mL French straws (average 500 million total sperm per straw) and frozen using a programmable freezer (IceCube™). For each frozen ejaculate, a post-thaw quality check was performed. Ninety eight percent of the ejaculates that were frozen showed at least a 50% post-thaw motility and were approved for shipment. For AI, eight straws were thawed (to achieve at least 2.0×10^9 motile sperm) and diluted with 60 mL of extender pre-warmed to 26 °C. Within 2–5 min of thawing, the sows or gilts were inseminated via intra-cervical deposition using a standard AI pipette. Sows and gilts were inseminated three times PM/AM/PM and AM/PM/AM, respectively. Of 2696 recorded services, 2122 (78.7%) of the females farrowed. The mean (±SD) total number piglets born were 12.5 (±3.9). A progressive improvement of fertility over time was observed mainly due to adaptive procedures associated with an introduced technology. In summary, acceptable fertility is possible with frozen semen and has merit for application as a reproductive management tool.

1. Introduction

Since the observation by Lasley and Bogart (1944) that ejaculated boar sperm are sensitive to cool temperatures (10 min at 0 °C), much progress has been made relative to cryopreservation of sperm for use in artificial insemination (AI). Pigs were first successfully produced from frozen semen over 40 years ago (Polge et al., 1970) albeit using surgical insemination into the oviduct. And a year later there were three reports demonstrating the fertility of frozen boar semen when inseminated via the cervix (Crabo and Einarrsson, 1971; Graham et al., 1971; Pursel and Johnson, 1971). Nevertheless, it is well recognized that the fertility of frozen boar semen is not comparable to the fertility of fresh boar semen (Johnson, 1985).

Wagner and Thieber (2000) reported in 2000 that, worldwide, greater than 90% of sows were artificially inseminated with fresh extended semen and less than 1%

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of the sows were inseminated using frozen semen. Irrespective of the lesser fertility in females where frozen boar semen was used, there are several reasons why it is as an effective technology (Bailey et al., 2008) including: transfer of genes between breeding pyramids; available semen-on a daily basis-for planned inseminations; export of germplasm; protecting the health status of a herd by controlling transmission of particular pathogens; gene cryobanking to retrieve a desired genotype not maintained at a particular nucleus herd; genetic linking of nucleus herd for companies operating internationally and contingency in case of disaster events.

Despite these important applications of frozen boar semen, a variety of reasons exist for the limited use of frozen semen in commercial pork production operations including: variability in resistance to cryopreservation; large number of sperm required per AI dose; and lesser fertility achieved (Johnson, 1985; Johnson et al., 2000; Reed, 1985; Watson, 2000). And it is generally agreed that the shortened lifespan of frozen-thawed boar sperm in the uterus is the major reason for its limited commercial application (Pursel et al., 1978). Nevertheless, as advances are made to boar sperm cryopreservation (for reviews see Rath et al., 2009; Roca et al., 2006) there is more emphasis on the importance of proper placement of semen in the sow's reproductive tract and proper timing of insemination relative to ovulation.

Even though excellent fertility is achieved in commercial pork production operations using fresh-cooled boar semen (Johnson et al., 2000; Weitze, 2000) there will always be an interest in utilizing frozen boar semen in commercial pork production. As research continues to generate efficiencies through improvements in boar semen cryopreservation (Bailey et al., 2008; Rath et al., 2009; Carvajal et al., 2004; Saravia et al., 2005; Morrell et al., 2009); sperm concentration and semen deposition (Bathgate et al., 2008; Martinez et al., 2001, 2002; Wongtawan et al., 2006), insemination frequency or number, and timing relative to ovulation (Waberski et al., 1994; Bolarin et al., 2006; Spencer et al., 2010) the efficacy of frozen boar semen will continue to be evaluated in field tests enabling progress to be measured (Johnson, 1985; Hammitt and Martin, 1989; Didion and Schoenbeck, 1996; Almlid and Hofmo, 1996; Martin et al., 2000; Eriksson et al., 2002; Yamaguchi et al., 2009).

To that end this report presents retrospectively on the commercial application of frozen boar semen over a 4 year period (2007–2011) comprising over 2600 AI services. The data suggest that the fertility of frozen boar semen is comparable to fresh semen when control of the various factors influencing fertilization is carefully managed (Parrilla et al., 2009).

2. Materials and methods

2.1. Animals, ages and site locations

Fertility data were collected throughout a 4 year period (2nd quarter 2007 through 2nd quarter, 2010) on a single 1800-sow farm located in Indiana, USA. The frozen semen was sourced from a single, 100-boar stud located in Manitoba Canada. Boars were fed a standard boar ration and provided with water ad libitum. The genetic background of all boars to be used for frozen semen was pure line Yorkshire with an average age of 12–15 mo. All frozen semen was used as single-sire (i.e., no semen pooling) to mate pure line Yorkshire great-grandparent sows and gilts located in Indiana, USA to produce grandparent females. The average parity was 1.9 for sows inseminated with frozen semen. The genetic background of all boars used for fresh semen was Landrace with an average age of 12–18 mo. All fresh semen were pooled and used to inseminate Yorkshire sows and gilts to produce an F1 parent.

2.2. Collection, processing, cooling and cryopreservation of boar semen for frozen AI

The sperm-rich fraction of boar semen was collected by the gloved-hand method. The semen was directed through a filter into a plastic bag (US Bag™, Minitube, Verona, WI, USA) that lined a pre-warmed (37 °C) insulated semen collection cup (Minitube, Verona, WI, USA). The volume of semen was recorded and extended 1:1 with pre-warmed (37 °C) Androhep® ApX^2 (Minitube, Verona, WI, USA) semen extender and placed in a 17 °C cooling cabinet for a 12–15 h period. After cooling to 17 °C, a 4 mL aliquot was removed and placed in a glass cuvette and warmed for 20 min at 38 °C. Sperm motility, morphology and concentration were examined within 12–15 h of semen collection using a computer-assisted semen analysis (CASA) system (SpermVision™ Minitube, Verona, WI, USA). Only those collections having ≥80% motility and ≤15% abnormal morphology were further processed. After CASA evaluation, the total number (semen volume multiplied by sperm concentration) and total motile sperm (semen volume multiplied by sperm concentration multiplied by motility) were calculated. The semen from each boar was transferred to a 225 mL graduated conical centrifuge tube. Semen was prepared for cryopreservation based on the protocol supplied with a commercially available boar semen freezing extender (Androhep® CryoGuard™; Minitube). The cooling extender containing 20% egg yolk was prepared as recommended then allowed to cool to 17 °C. The freezing extender contained 20% egg yolk, Equex STM Paste (NOVA Chemical Sales Inc., Scituate, MA, USA) and 6% glycerol (Sigma–Aldrich, St. Louis, MO, USA) and was cooled to 5 °C. The cooled semen (17 °C) was centrifuged for 20 min at 800 × *g*. After centrifugation, the supernatant was removed using a pipette and the total number of sperm remaining in the semen pellet was determined. The semen pellet was re-suspended using enough cooling extender so as to achieve 50% of the final freezing volume at a concentration of 2.0×10^9 motile sperm/mL. The extended semen was slow cooled (2.0 h) to 5 °C using a 250 mL beaker containing 150 mL water in which the semen was placed in and held for >30 min. The freezing extender (5 °C) was then added to provide the remaining 50% of the final freezing volume. Semen was packaged at 5 °C in 0.5 mL polyvinyl chloride straws (catalog # 13048, Minitube, Canada and catalog # 5707 IMV, France) at an average of 500 million total sperm per straw and placed in a programmable freezer (IceCube) previously set to 5 °C. Semen freezing was performed with