

Approaches Towards Efficient Use of Boar Semen in the Pig Industry

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Towards an efficient use of liquid, cryopreserved and sexed semen in the pig industry

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Abstract

The current cervical artificial insemination (CAI) procedure, involving deposition of excessive sperm numbers, is uneconomical for pig industry. The most obvious alternative requires uterine deposition in combination with fixed-time AI, which would reduce the number of sperm required per pregnant sow, thus allowing the best use of valuable boars and, ultimately, the commercial integration of frozen-thawed and sexed sperm. This review depicts possible best ways to implement an efficient use of liquid-stored, frozen-thawed and sexed sperm by the pig industry.

Introduction

Artificial insemination is extensively used by swine producers worldwide, playing a pivotal role on the improvement of global swine production. The current procedure, cervical artificial insemination (CAI) implies delivery of large sperm numbers (>2-3 billion spermatozoa) into the cervix two or three times per oestrus. Although the fertility outcomes parallel those of natural mating when using fresh or

liquid-stored semen, the large number of sperm required per pregnant sow (>7 billion) presses the collection frequency for valuable boars. In addition, it restricts the commercial use of frozen-thawed (FT) sperm, and limits the application of sexed sperm, ultimately limiting the economical profitability of valuable sires. Moreover, despite of the high number of sperm delivered via CAI, most of them do not participate in fertilization (Roca et al., 2006), since they are rapidly evacuated from the uterus by backflow during or immediately after AI (30-40% of the total number of spermatozoa deposited into the cervical canal), trapped and die in the cervical folds (5-10%) and phagocytized in the uterus (up to 60%).

Swine production has become highly competitive, and producers are forced to incorporate breeding methods of higher efficiency and profitability, so that productivity increases alongside with cost-reduction. In this context, decreasing the number of sperm required per pregnant sow should be useful. This review summarizes possible best paths for achieving this goal, still warranting high fertility.

Key approaches

The primary goal of any AI strategy must be ensure a sufficient sperm number in the oviduct at the time of ovulation for optimal fertilization, irrespectively of the sperm numbers delivered and sperm treatment used. Depositing the spermatozoa deeper into the reproductive tract would allow overcoming some of the weakness of CAI. Consequently, fewer sperm per AI-dose would be required to maintain fertility. Insemination procedures allowing semen delivery into the uterus body (intra-uterine insemination, IUI) and the proximal uterine horn (deep uterine insemination, DUI) are now commercially available for pigs (Roca et al. 2006). In addition, laparoscopy is

showed as an effective and efficient procedure for delivering very low numbers of expensive or very valuable sperm of limited availability into the utero-tubal junction or the mid-oviduct (Vazquez et al. 2008).

Although uterine insemination procedures can help to achieve a more efficient use of sperm, special emphasis should be placed on how far we can go in reducing sperm numbers per AI-dose. This reduction should never compromise that an optimal competent sperm population reach the oviduct at the time of ovulation. If an insufficient functional sperm population colonizes the oviductal reservoir, partial or no fertilization will happen and consequently the results will be reduced farrowing rates and/or poorer litter sizes. In addition, it is important to bear in mind that as the numbers of sperm per AI-dose is reduced, the influence of other variables in the fertility outcome increase. Variables such as actual functional sperm rate, boar fertility, sow management and timing of sperm delivery should be carefully considered. The relevance of some of these variables will be discussed in other lectures of this Boar Semen Preservation Conference.

The timing of sperm delivery relative to ovulation is always one of the most important variables affecting the overall success of AI, but it becomes more important as the number of sperm delivered is reduced (García et al., 2007b; Pelland et al., 2008). In addition, the optimal timing of AI is narrowed as delivered spermatozoa grow old or weaker. Therefore, establishment of a window for optimal timing of AI is the utmost objective. As ovulation usually occurs during the final third part of behavioral oestrus (Soede and Kemp, 1997), accurate detection of the onset of oestrus as well as monitoring its duration, are pre-requisites to properly forecast ovulation under field conditions. If oestrus is sub-optimally detected, ovulation can be synchronized using

exogenous pharmaceutical treatment. It allows greater accuracy in predicting the timing of ovulation, which also facilitates single fixed-time AI, and, finally, greater reduction of the numbers of sperm per litter. Use of Equine Chorionic Gonadotropin (eCG), administered 24 h after weaning or of Altrenogest treatment, in combination with human CG (hCG), porcine Luteinizing Hormone (p-LH) or Gonadotropin-Releasing Hormone (GnRH) agonist, 56-80 h later, is the most effective currently used treatment set-up. For more details of these and other effective hormonal treatments see the recent review of Brüssow et al. (2009).

Liquid stored semen

Although breeding sows via CAI with only 1 billion sperm per AI-dose can result in acceptable farrowing rate and litter size when inseminated close to ovulation time (Pelland et al., 2008), a minimum of 2.5 billion liquid-stored sperm per CAI-dose is recommended to achieve systematic high fertility (Reicks and Levis, 2008; Alm et al., 2006). This sperm number is far too excessive for an efficient use of valuable sires. Field IUI-trials have showed that 1 billion spermatozoa are enough to mimic outcomes achieved by CAI with 3 billion sperm (Watson and Behan, 2002; Rozeboom et al., 2004). Moreover, the most recent IUI-trial, conducted on Danish commercial farms and involving a total of 9,197 sows, showed high fertility outcome by using only 750 million spermatozoa, when deposited at a correct interval before ovulation (Olesen and Hansen, 2009). A greater lowering of the sperm number affects the reproductive outcomes of inseminated sows, particularly litter size (Rozeboom et al., 2004), this could be related with partial fertilizations. Although IUI for liquid stored semen is currently used by practitioners in several European regions, the sperm numbers per AI-

dose is not yet standardized. It appears that the threshold dose to achieve systematic high fertility success is at least 1 billion sperm (Roca et al., 2006). However, when farm conditions or sperm quality are sub-optimal, it would seem prudent to use sperm numbers up to 1.5 billion per IUI-dose (Roberts and Bilkei, 2005). If a stronger reduction in sperm number per AI-dose is needed (more profitable use of valuable ejaculates, for instance), then the DUI technique should be used. Although some unilateral fertilization occur, negatively affecting litter size, acceptable fertility outcomes are possible, after delivering in the depth of an uterine horn as few sperm as 150 million (Martinez et al., 2002). Partial fertilizations and consequently small litter sizes are avoided by increasing the number of liquid-stored sperm per DUI-dose to 600 million (Martinez et al., 2006).

Fixed-time AI, single or double, is not usual when liquid-stored semen is used. Currently, sows are bred every 24 h from the start of oestrus until oestrus is no longer detected, issuing >2.5 average number of AI per oestrus (Rozeboom et al., 2004). If two or even just one AI at fixed-time is to be tested, concerns rise. The optimal time for AI is in the interval from 28 h before to 4 h after ovulation (Nissen et al., 1997). However, highest fertility is seen when liquid-stored semen is placed in the reproductive tract within 12-24 h before ovulation, regardless of the sperm number delivered (Steeverink et al., 1997).

Frozen-thawed sperm

Ideally, frozen-thawed (FT) sperm should be usable for AI given their additional advantages respect to liquid-stored semen (Bailey et al., 2007). Despite this, they are yet scarcely used by the swine industry, being its only use transferring valuable genetic

material (Tribout et al., 2010). Its low fertility success compared to liquid-stored semen remains the burden of FT-semen when attempting integration in commercial swine AI programs. Field CAI-trials (Table 1) with fertility success similar to those with liquid-stored semen; e.g. with farrowing rates over 80% and more than 10 piglets born per litter (Eriksson et al. 2002; J. Roca, unpublished observations), have clearly rebutted this myth. These optimal fertility results were achieved in sows CAI-inseminated 2-3 times per oestrus with 5-6 billion total sperm per AI-dose (\approx 2-3 billion motile spermatozoa per dose). The biological reason of this large number of sperm per AI-dose would be related to weakness of FT-sperm, which are especially vulnerable during their transit through the long pig female reproductive tract. In fact, the number of functionally FT-spermatozoa available in the oviduct is usually 10-fold lower compared to fresh semen, even delivering double FT-sperm number (Wabersky et al., 1994). In comparison to liquid-stored semen, 4-6 times more FT-sperm are required per oestrus, implying few AI-doses prepared per ejaculate, a non-economical situation. Attempts have been made to reduce the number of FT-sperm required for a successful CAI insemination, with encouraging results. Recently, Thilmant (2009) reported farrowing rates over 90% with more than 12 piglets born per litter in sows CAI-inseminated with 2.5 billion motile spermatozoa per AI-dose. In despite of these promising fertility results, the number of motile spermatozoa is still too large, considering that the percentage of motile spermatozoa after thawing barely reaches over 60% (Rath et al., 2009), being close to previous trials (see Table 1). Therefore, the immediate use of FT-spermatozoa on commercial AI requires the drastic reduction of total sperm numbers to that needed to produce optimum litter size. Uterine AI techniques are an alternative to CAI for lowering sperm numbers per AI. To the

authors' knowledge, few field trials have been published evaluating the potential effectiveness of IUI for FT-sperm, and fertility outcomes are not too encouraging (Abad et al., 2007; Casas et al., 2009). For instance, in a recent on farm study conducted in Belgium, IUI-doses of 1 billion motile sperm resulted in 60% of farrowing rate compared with 91.7% when CAI-inseminated with 3.6 billion motile sperm (Thilmant, 2009). In contrast, DUI offers optimistic possibilities to an effective and efficient use of FT-sperm, achieving farrowing rates over 80% when inseminating as few total sperm as 1 billion (Bolarin et al., 2006; Bathgate et al., 2008).

Frozen-thawed boar sperm have a shortened fertile lifespan (< 8 h) once delivered in the female reproductive tract (Waberski et al., 1994; Wongtawan et al., 2006). Therefore, it is a common practice to inseminate sows three or, even, four times per estrous with the aim that one of them will fall inside the narrow window for optimal fertility. However, efficient semen usage call for breeding protocols with only double or even single fixed-time AI (Spencer et al., 2010). For them, sperm should be delivered within a short period of time before expecting ovulation time, which requires accurate knowledge of when ovulation occurs, being hormonal treatment advised to achieve it (Roca et al., 2003). In this context, a single DUI with 1 billion FT-spermatozoa (\approx 500-650 million live spermatozoa per dose) can achieve high fertility outcomes (farrowing rates over 80% with more than 10 piglets born per litter), comparable to liquid semen, if deposited close to the beginning of the ovulation (Bolarin et al., 2006; 2009). However, it is important to notice that a second fixed-time AI or doubling sperm numbers in single fixed-time AI partially compensates some variations in the expected time of ovulation (Bolarin et al., 2006; Spencer et al., 2010).

Sex-sorted spermatozoa

The advantages of using sex-sorted spermatozoa in AI-programs open a new dimension to pig industry. Sexed sperm has in itself the potential to substantially speed-up the rate of swine genetic progress when implemented as part of an AI program (Rath and Johnson, 2008). The still low speed of sorting (Sharpe and Evans, 2009) together with the short lifespan of liquid-stored- (Parrilla et al., 2005; Spinaci et al., 2010) and FT- (Bathgate, 2008) sorted sperm are obstacles to overcome towards a field application of sex-sorted spermatozoa. Deep uterine insemination has been postulated as the most suitable AI technique for producing litter size from bulk-sorted (Vazquez et al., 2003) and sex-sorted non-frozen sperm (Rath et al., 2003; Grossfeld et al., 2005), using as few as 50 million sperm. However, this is still a too large a number of sperm per DUI-dose (considering the current outcomes and losses of the sorting procedure) which, together with the low fertility rates so far achieved (up to 60%), limits the current possibilities for DUI as an efficient insemination tool for on-farm application of sexed sperm. Nevertheless, DUI will be attractive in the future as increasing sorting rates and extending lifespan of sorted sperm. Nowadays, the laparoscopy insemination technique, despite its high cost and skill requirements, offers to the pig industry a possibility for an efficient application of sexed sperm. The suitability of the laparoscopic technique to inseminate pig females was initially evaluated using fresh semen (Fantinati et al., 2005) and then successfully extrapolated to sex-sorted sperm (Garcia et al., 2007a). The procedure involving the direct delivery of spermatozoa into the utero-tubal junction and/or into the oviduct can be speedily performed (between 9 and 21 min, with an average of 12.6 ± 0.6 min in 27 sows; I. Parrilla, personal communication). Careful laparoscopic manipulation of the

reproductive tract did not have negative consequences to the future reproductive performance of the sows (Parrilla et al., 2010). Preliminary results (87 sows) of current experiments in our laboratory, showed that fertility rates over 70% and litter size as large as 10 piglets born, can be obtained with a single laparoscopic insemination delivering at optimal timing between 1 and 3 million liquid-stored sexed sperm per sow.

Similar to FT-sperm, the fertility outcomes of sexed sperm depend highly on the timing relative to ovulation (Vazquez et al., 2008). Although the lifespan of liquid-stored and FT-sexed sperm are still not well defined, it is apparently very short, considering that the highest fertility is achieved only when sperm is laparoscopically delivered shortly before ovulation. Therefore, hormonal treatments for accurate prediction of ovulation are required, and high eCG dosage (1500 IU) is advised to avoid possible small litter size related to recurrent unilateral or partial fertilizations (Garcia et al., 2007a). Sperm delivery at ovulation time or later, results in fertility loss related to a high incidence of polyspermy (Vazquez et al., 2006).

Conclusions and remarks

Uterine insemination procedures have shown to be beneficial for the efficient use of low numbers of liquid stored and frozen-thawed spermatozoa, achieving optimal farrowing rates and litter sizes using as few as ≈ 1 billion sperm per AI-dose. Laparoscopic insemination has enabled satisfactory fertility outcomes after insemination with 1-3 million liquid-stored sexed sperm. However, fertility success depends on proper timing of semen deposition relative to ovulation rather than on the site and number of sperm deposited. Therefore, convenient and economical protocols

to synchronize ovulation are needed for a profitable use of boar spermatozoa, particularly to frozen-thawed and sexed sperm, where single fixed-time AI should be required for efficiency.

The number of sperm required per litter size can define the cost-effectiveness of any insemination strategy. However, it should be more properly defined at farrowing, in terms of fecundity index, and at weaning where the average piglets weaning weight could be a better estimation, particularly when the ultimate goal of AI is to increase the impact of individual valuable boars on genetic progress.

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