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Sperm sorting and low-dose insemination in the pig – an update

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Introduction

During the last decade efforts have been made to develop techniques for pre-selection of the sex of the offspring. Such pre-selection may be desirable for different reasons; production of female hybrid pigs in multiplying herds or production male pigs for breeding reasons (e.g. AI boars or breeding boars). These are niche areas, and application of new techniques may therefore be done with less focus on efficiency and economy. However, pre-selection of the sex for use in commercial routine piglet (or slaughter pig) production due to banning of castration requires a different view on application of such a technique. It must be cost-effective and easy to implement in routine semen production. In addition, it must be possible to use sorted semen without having to use a sophisticated insemination technique.

This article will review the challenge concerning sperm cell sorting and low dose insemination for use in commercial pig production

Sex sorting of sperm cells

At present time, the only means of farrowing pre-selected piglets is by sorting sperm cells according to the Beltsville Sperm Sexing Technology (BSST) [1]. This method includes a flow cytometer and a cell sorter, and the sorting is based on differences in the DNA content between X and Y chromosome bearing sperm cells. In the pig this difference is 3.6 %; the X-bearing sperm cell ("female sperm") having the highest DNA content. This difference varies across species, and in chinchilla the difference is as high as 7.5 % while in humans only 2.8 % [2].

The sorting process includes dilution of the semen, staining the sperm cells, identifying X and Y chromosome bearing sperm cells, sorting X and Y chromosome bearing sperm cells and recovering and storage of the sorted sperm cells. There are several major challenges among these operations that have to be solved before the method can be used on a regular basis for commercial slaughter pig production.

In the flow cytometer, laser light is used to illuminate stained sperm cells as they pass through a laser beam, one at a time, in a fine fluid stream. A critical part of the process is to ensure a uniform staining intensity within the cell population. Variation in the staining intensity will reduce the efficiency in separating the cell populations significantly.

Light scattered by the cells and light emitted by the fluorescent dye are analysed by several detectors and processed by a computer. The X and Y bearing sperm cells are distinguished on the basis of the DNA content. Since the head of the sperm cell is paddle shaped, the cells must be oriented with the flat side against the laser beam. Two laser beams (0° and 90°) and a special needle are used so that when the fluid stream containing the sperm cells pass the laser beam, a high proportion of the cells will be facing the beam in the proper plane. The efficiency in distinguishing the two populations are depending on that a high proportion of the sperm cells are oriented in the proper way.

After passing the laser beam, the X and Y chromosome bearing sperm cells are given different charge and the cells are sorted as they pass between two continuously charged plates. The sperm cells are sorted in three populations; X chromosome bearing sperm cells, Y chromosome bearing sperm cells and waste (non-sorted sperm cells). The chal-

lenge in this operation is to reduce the waste, by enhancing proportion of sorted cells.

During sorting, the sperm cells are highly diluted. Once sorting is terminated, the sperm cells must be centrifuged (300 × g) to increase the density. The density will vary depending on the method selected for use of the sorted sperm cells. This process affects the viability of the sperm cells and it has been shown that the fertilising ability of sorted sperm cells is beginning to decrease at 5 hrs of storage [3]. It is therefore necessary to deposit sorted semen as close as possible to the ovulation time and the site of fertilisation. Premature capacitation of sorted sperm cells is one of the changes that shortens the viable lifespan for *in vivo* insemination.

Concerns have been raised whether exposure to the dye (Hoechst 33342) and the laser beam might damage the DNA. However, there seems not to be any higher proportion of abnormalities among offspring from sorted sperm cells compared to not sorted sperm cells. Furthermore, the mutagenic effect has been investigated without evidence of any negative effect on offsprings from stained sorted sperm cells [4].

State of the art from a practical point of view

To be used in commercial semen production, the speed of the sorting as well as the purity of the sorted sperm cells are crucial. The purity (efficiency of the sorting) will largely depend on the speed, making these two elements contradictory; the higher the speed, the lower the purity of sorted sperm cells. The present technology makes it possible to sort 15 million sperm cells per hour [1]. Although deep intrauterine insemination may reduce the number of sperm cells required for fertilisation significantly, the number is still too large to be considered for practical conditions.

Other methods for sex sorting of sperm cells have been discussed, and the focus has mostly been towards a possible difference in surface proteins between X and Y chromosome bearing sperm cells. If so, one could produce an antibody to attach to the X or Y chromosome bearing sperm cells and then use magnetic beads to separate the two populations. This method could provide a large scale separation process easily adaptable to AI centres. Approximately 1000 surface proteins have been mapped but no difference has been detected between proteins isolated from X versus Y chromosome bearing sperm cells [5,6].

Low dose insemination in the pig

Optimum fertilisation rate is depending on several factors; the interval between insemination and ovulation, the quality of the sperm cells, the life span of the sperm cells *in utero* and the site of deposition of the sperm cells. Traditional artificial insemination in the pig is carried out using 2.5 – 4 billion sperm cells deposited in the posterior part of *cervix uteri*. Only a very small proportion of the sperm cells reaches the site of fertilisation (the oviduct) due to a combination of loss through back flow and phagocytosis of sperm cells by polymorphonuclear leukocytes. It has, however, been shown that acceptable fertility can be achieved by deposition of only 10 million sperms at the uterotubal junction by using surgical insemination close to ovulation [7].

Surgical insemination can not be used in commercial farms, and during the last 5–7 years efforts have been made to develop new devices to be able to deposit the semen either in the uterine body or deep in the uterine horns. The main obstacles are the cervical folds in *cervix uteri* and the long uterine horns (up to 1.5 meters). However, two new procedures for deposition of the semen cranial for the cervix have been developed; post cervical insemination and deep intrauterine insemination.

Post cervical insemination

By post cervical insemination, the semen is deposited in the uterine body, just cranial for the cervix. Several different (commercial) catheters are available, and the aim is to reduce the number of sperm cells. The method may be used on a regular basis in commercial herds, and it has been shown that it is possible to reduce the number of sperm cells from 3 to 1 billion without compromising the fertility [8]. However, for use in combination with sorted semen this technique requires too many sperm cells.

Deep intrauterine insemination

Deep intrauterine insemination requires a long flexible catheter to pass the cervix and enter the uterine horns. It has been reported that it is possible to deposit semen close to the uterotubal junction with a 20-fold reduction of the number of sperm cells without affecting farrowing rate or litter size [9]. However, by reducing the number of sperm cells further it seems like the litter size is compromised although pregnancy and farrowing rates are not affected compared to traditional AI with ~3 billion sperm cells [10.11].

Conclusion

In conclusion: It is possible to sort sperm cells in X and Y chromosome bearing populations. The only method proven is the Beltsville Sperm Sexing Technology which uses a flow cytometer and a cell sorter. The sorting speed is at present approximately 15 million cells per hour.

To be able to use sex-sorted semen in commercial pig production the number of sperm cells per insemination must be reduced tremendously. None of the newly developed technologies makes it possible to reduce the number of

sperm cells inseminated to such an extent that sorted semen may be used.

The major challenge in sex sorting boar spermatozoa is to increase the speed of BSST-sorting or to develop new technologies. Furthermore, the damage caused by the sorting must be reduced, the storage time for sorted sperm cells must be increased and the waste (non-sorted population) must be reduced.

References

1. Johnson LA, Rath D, Vazquez JM, Maxwell WM, Dobrinsky JR: **Preselection of sex of offspring in swine production: current status of the process and its application.** *Theriogenology* 2005, **63:**615-624.
2. Johnson LA: **Sex preselection by flow cytomertric separation of X and Y chromosome bearing sperm based on DNA difference.** *A Review Reprod Fertil Dev* 1995, **7:**893-903.
3. Parrilla I, Vazquez JM, Cuello C, Gil MA, Caballero I, Almiñana C, Roca J, Martinez EA: **Influence of storage time on functional capacity of flow cytometrically sex-sorted boar spermatozoa.** *Theriogenology* 2005, **64:**86-98.
4. Parrilla I, Vazquez JM, Cuello C, Gil MA, Roca J, Di Berardino D, Martinez EA: **Hoechst 33342 stain and u.v. laser exposure do not induce genotoxic effects in flow-sorted boar spermatozoa.** *Reproduction* 2004, **128:**615-621.
5. Hendriksen PJ, Welsh GR, Grootegoed JA, Van der Lende T, Johnson LA: **Comparison of detergent-solubilized membrane and soluble proteins from flow cytometrically sorted X- and Y chromosome bearing porcine spermatozoa by high resolution 2-D electrophoresis.** *Mol Reprod Dev* 1996, **45:**342-350.
6. Hendriksen PJ: **Do X and Y spermatozoa differ in proteins?** *Theriogenology* 1999, **52:**1295-1307.
7. Krueger C, Rath D, Johnson LA: **Low dose insemination in synchronized gilts.** *Theriogenology* 1999, **52:**1363-1373.
8. Watson PF, Behan JR: **Intrauterine insemination of sows with reduced sperm numbers: results of a commercially based field trial.** *Theriogenology* 2002, **57:**1683-1693.
9. Martinez EA, Vazquez JM, Roca J, Lucas X, Gil MA, Parrilla I, Vazquez JL, Day BN: **Minimal number of spermatozoa required for normal fertility after deep intrauterine insemination in non-sedated sows.** *Reproduction* 2002, **123:**163-170.
10. Vazquez JM, Martinez EA, Parrilla I, Cuello C, Gil MA, Lucas X, Roca J, Vazquez JL, Didion BA, Day BN: **Deep intrauterine insemination in natural post-weaning oestrus sows.** In *Proceedings of the 6th Int Conf on Pig Reprod Columbia, MO, USA*; 2001.
11. Day BN, Mathias K, Didion BA, Martinez EA, Caamano JN: **Deep intrauterine insemination in sows: first field trial in USA commercial farm with a newly developed device [abstract].** *Theriogenology* 2003, **59:**213.