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Editorials

Foals of desired sex - and with minimal sperm numbers

Sex preselection is based on flow-cytometric measurement of sperm DNA content, which is slightly different for X- and Y-chromosome-bearing spermatozoa (Johnson and Welch 1999). The difference in X/Y DNA is 3.7% in the stallion and 3.8% in the bull, but only 2.8% in man. Spermatozoa are treated with a fluorescent dye (Hoechst 33342) that binds to DNA, but does not decrease the fertilising capacity of sperm. Because of the small difference in X/Y DNA, the variability in fluorescence caused by the different position of the sperm head to the laser beam during sorting would mask the X/Y difference. Therefore, only those spermatozoa that face the laser beam correctly oriented are sorted to X and Y. Today, maximally 11 x 10^6 X-bearing sperm can be produced in an hour with 85–90% purity. Sperm can be sorted more rapidly, if a lower purity is acceptable. Johnson and Welch (1999) have described the development and the present status of the sex preselection methods in cattle in a review article.

The low speed of sorting is a problem. The standard insemination dose of mares, 500 x 10^6 pms (progressively motile spermatozoa) (Pickett and Voss 1975) would take days to sort. Since the number of sex-sorted sperm is limited, special insemination techniques are needed. Oviductal insemination is possible (McCue *et al.* 2000), but not applicable in practice because it requires laparatomy. In the 2 papers by Lindsey *et al.* (2002a,b) in this issue (pp 121, 128), mares were inseminated into the tip of the uterine horn ipsilateral to the preovulatory follicle. Semen was deposited onto the uterotubal junction papilla using a videoendoscope. This technique was introduced earlier by Vazquez *et al.* (1998), Manning *et al.* (1998) and Morris *et al.* (2000). The latter group achieved pregnancy rates comparable to normal doses by inseminating sperm numbers of 10, 5 or 1 x 10^6. Even lower numbers, 0.5, 0.1 or 0.001 x 10^6 resulted in pregnancies, but at lower rates than normally. These results are amazing, e.g. the dose of 1 x 10^6 is only 0.2% of the recommended dose. Similarly, in the study of Lindsey *et al.* (2002b), a 50% pregnancy rate was obtained by inseminating 5 x 10^6 fresh spermatozoa in a volume of 100 µl via an endoscope.

The hysteroscopic insemination technique seems to yield good results with small sperm numbers. However, the instrument is probably too expensive and the method too time-consuming to become a commonly used method in studfarms. Buchanan *et al.* (2000) managed to get mares pregnant by deep intracornual insemination, whereas Lindsey *et al.* (2002b) did not. In this technique, semen is deposited at the tip of the uterine horn ipsilateral to the preovulatory follicle and the position of the catheter is confirmed by transrectal ultrasonography prior to insemination. There were differences between the studies, e.g. in the timing of insemination in relation to hCG or GnRH administration and in the insemination volume. Lindsey *et al.* (2002b) preferred the small volume of 100 µl to keep semen from spreading along endometrial folds. With the transrectal technique the positioning of sperm is presumably not as accurate as via endoscopy and, therefore, larger volumes up to 1 ml, as used by Buchanan *et al.* (2000) might be beneficial. Ultrasonography is not necessary in the deep intracornual insemination technique that Woods *et al.* (2000) applied. The uterotubal papilla is easily palpated transrectally. The pipette is passed through the cervix as far up the horn as possible. The arm is then removed from the vagina and inserted into the rectum to grasp the uterine horn and manipulate the pipette to the tip of the horn. It remains to be seen, if the simple method used by Woods *et al.* (2000) will result in satisfactory pregnancy rates when very small sperm numbers are used.

In the work of Lindsey *et al.* (2002a), the 37.5% pregnancy rate obtained by inseminating as little as 5 x 10^6 progressively motile frozen-thawed sperm is very interesting. Could this technique improve our results of frozen semen inseminations? Seminal plasma is totally removed during the semen freezing. It has been shown in swine that seminal plasma facilitates transport of frozen sperm into the oviducts (Einarsson and Viring 1996). Perhaps, in the absence of seminal plasma, the placement of sperm close to the uterotubal junction is important for their survival and entrance into the oviduct. Could this technique also provide a solution for subfertile stallions, which produce very small numbers of live spermatozoa? Unfortunately, the results of Woods *et al.* (2000) do not support this idea. The uterine horn insemination did not produce significantly better results than the uterine body insemination, when a fertile (63% pregnancy rate after horn inseminations and 56% after body inseminations) or a subfertile stallion (29% pregnancy rate for both methods) was used. However, it is worth noticing that only 25 x 10^6 spermatozoa were used. Although the minimum insemination dose probably varies between stallions, the commonly used doses of 500 x 10^6 pms fresh sperm (Pickett and Voss 1975) or 300 x 10^6 pms frozen sperm (Leipold *et al.* 1998) are perhaps not required for most stallions. The fact remains that we do not know for sure what the minimum dose is that would result in commercially acceptable pregnancy rates for fresh, cooled or frozen stallion semen. Basic studies with large numbers of mares and stallions are needed.

It is to be expected that the flow-cytometric sorting would decrease fertility of spermatozoa. They are exposed to many potentially harmful treatments during sorting; staining, ultraviolet light, pumping through fine tubing at a high pressure at a speed of 100 km/h and storage for several hours (Buchanan *et al.* 2000). In spite of that, Buchanan *et al.* (2000), who reported the first equine pregnancies with sexed spermatozoa using the ultrasound-guided technique, obtained satisfactory results. The pregnancy rates of 57% (25 x 10^6 pms, 1 ml) and 35% (5 x 10^6 pms, 1–0.2 ml) were obtained. Lindsey *et al.* (2002b) reported a 25% pregnancy rate by endoscopic insemination of 5 x 10^6 fresh sorted spermatozoa and Lindsey *et al.* (2002a) a 37.5% pregnancy rate with the same dose. With the small numbers of mares, the pregnancy rates were not significantly different from those obtained by fresh sorted sperm, although the figures seemed to be lower.

The first steps in preselection of sex in horses have been taken, but many problems have to be overcome before sexed sperm is available for horse breeders. All authors who inseminated mares with sexed semen reported pregnancy losses; 3/8 (Buchanan *et al.* 2000), 1/5 (Lindsey *et al.* 2002b) and 1/8 (Lindsey *et al.* 2002a). Since numbers of mares are small, it is difficult to draw conclusions, but the total loss rate (5/21 = 24%) seems to be somewhat higher than in a normal mare population. For use in practice, sperm has to be frozen after sorting. In cattle, sex-sorting and subsequent freezing has produced satisfactory pregnancy rates (Seidel *et al.* 1999). Lindsey *et al.* (2002a) reported a 13.3% pregnancy rate for frozen sorted stallion semen. In horses, both freezing and sorting have to be developed in order to achieve commercially acceptable results. Refinement of sorting techniques, resulting in increased speed of sorting, will probably take place in the near future. Although there are problems to solve, it is very likely that one day breeders will be able to order a filly or a colt in advance.

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References

Buchanan, B.R., Seidel Jr, G.E., McCue, P.M., Schenk, J.L., Herickhoff, L.A. and Squires, E.L. (2000) Insemination of mares with low numbers of either unsexed or sexed spermatozoa. *Theriogenol.* **53**, 1333-1344.

Einarsson, S. and Viring, S. (1996) Distribution of frozen-thawed spermatozoa in the reproductive tract of gilts at different time intervals after insemination. *J. Reprod. Fertil.* **32**, 117-120.

Johnson, L.A. and Welch, G.R. (1999) Sex preselection: high-speed flow cytometric sorting of X and Y sperm for maximum efficiency. *Theriogenol.* **52**, 1323-1341.

Leipold, S.D., Graham, J.K., Squires, E.L., McCue, P.M., Brinsko, S.P. and Vanderwall, D.K. (1998) Effect of spermatozoal concentration and number on fertility of frozen equine semen. *Theriogenol.* **49**, 1537-1543.

Lindsey, A.C., Schenk, J.L., Graham, J.K., Bruemmer, J.E. and Squires, E.L. (2002a) Hysteroscopic insemination of low numbers of flow sorted fresh and frozen/thawed stallion spermatozoa. *Equine vet. J.* **34**, 121-127.

Lindsey, A.C., Morris, L.H.A., Allen, W.R., Schenk, J.L., Squires, E.L. and Bruemmer, J.E. (2002b) Hysteroscopic insemination of mares with low numbers of nonsorted or flow sorted spermatozoa. *Equine vet. J.* **34**, 128-132.

Manning, S.T., Bowman, P.A., Fraser, L.M. and Card, C.E. (1998) Development of hysteroscopic insemination of the uterine tube in the mare. In: *Proceedings of the Annual Meeting of the Society for Theriogenology.* pp 84-85.

McCue, P.M., Fleury, J.J., Denniston, D.J., Graham, J.K. and Squires, E.L. (2000) Oviductal insemination of mares. *J. Reprod. Fertil., Suppl.* **56**, 499-502.

Morris, L.H., Hunter, R.H. and Allen, W.R. (2000) Hysteroscopic insemination of small numbers of spermatozoa at the uterotubal junction of preovulatory mares. *J. Reprod. Fertil.* **118**, 95-100.

Pickett, B.W. and Voss, J.L. (1975) The effect of semen extenders and sperm number on mare fertility. *J. Reprod. Fertil., Suppl.* **23**, 95-98.

Seidel, Jr, G.E., Schenk, J.L., Herickhoff, L.A., Doyle, S.P., Brink, Z., Green, R.D. and Cran, D.G. (1999) Insemination of heifers with sexed sperm. *Theriogenol.* **52**, 1407-1420.

Vazquez, J.J., Medina, V., Liu, I.K., Ball, B.A. and Scott, M.A. (1998) Nonsurgical uterotubal insemination in the mare. In: *Proceedings of the Annual Meeting of the Society for Theriogenology.* pp 82-83.

Woods, J., Rigby, S., Brinsko, S., Stephens, R., Varner, D. and Blanchard, T. (2000) Effect of intrauterine treatment with prostaglandin E_2 prior to insemination of mares in the uterine horn or body. *Theriogenol.* **53**, 1827-1836.