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Effect of Different Sperm Numbers on Fertility after Artificial Insemination of Foxes

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Fougner, J. A. and M. Forsberg: Effect of different sperm numbers on fertility after artificial insemination of foxes. Acta vet. scand. 1987, 28, 000-000. A total of 325 blue for vixens were inseminated with fresh semen from 50 silver fox males. Each ejaculutate was divided into 4 portions and diluted so as to contain 100, 60, 40, and 20 million sperm/ml. Vixens in groups 1, 2, 3 and 4 had been randomly assigned to their group at the time of insemination. The animals were inseminated once with either 100, 60, 40, or 20 million sperm. Vixens in groups 5 and 6 were selected by the technician after detecting signs of estrus during a physical examination. Animals judged to be at their optimal time for conception were assigned to group 5 and inseminated once with 20 million sperm. Animals considered to be early in their heat were assigned to group 6 and inseminated twice within 24 to 36 h with 20 million sperm per insemination dose. All inseminations were performed within 3 h of semen collection. A 1-ml total volume of extended semen was used for intrauterine deposition. In the random group inseminated once with 20 million sperm (group 4), both pregnancy rate and litter size were lower compared to the other random groups (groups 1, 2, and 3), although the difference was not statistically significant. Among the vixens inseminated with 20 million sperm (group 4, 5, and 6) there was a significant difference in fertility between animals randomly selected and inseminaed once and those selected by the technician and inseminated twice (group 6). Our results suggest that for the crossbreeding of foxes 20 million sperm is the minimum insemination dose required for acceptable fertility with the present tecnique for sperm preservation and estrous determination.

Blue fox; crossbreeding; reproduction; semen preservation; silver fox.

Introduction

Breeding of foxes for production is of considerable economic importance in the Nordic countries, Canada, and the Soviet Union. Since the use of artificial insemination (AI) in foxes as a commercial breeding tool was introduced into Norway (1979), this technique has been adopted in other countries as well. In 1986 approximately 150.000 vixens were artificially inseminated with fresh semen in 4 of the Nordic countries. To improve the efficiency of AI using fresh semen, the number of sperm per insemination dose needs to be reduced. In this context »efficiency« includes both economic and genetic considerations. Earlier investigations have concluded that a minimum of 100-150 million sperm per insemination dose are necessary to acieve maximal numbers of fertilized ova when utilizing fresh semen (*Aamdal et al.* 1978).

If semen is collected by digital manipulation the total sperm number per ejaculate averages 650 million from blue fox (*Alopex lagopus*) (*Aamdal* 1972) and 550 million from silver fox (Vulpes vulpes), enough for 4-5 inse-

minations per ejaculate. A pilot study in 1985 (*Fougner & Forsberg,* unpublished results), in which blue fox vixens were inseminated with fresh silver fox semen, showed that good fertility could be achieved by inseminating twice within 24 to 36 h with 30 million sperm per insemination dose. Thus about 20 doses were obtained from each ejaculate, which was sufficient for inseminating 10 vixens. The present experiment was undertaken to more accurately determine the number of spermatozoa required to obtain a high degree of fertilization using fresh semen in the AI of foxes.

Materials and methods

This study was carried out as a field trial from March 25 to April 10, 1986 in Opdal, Norway. A total of 325 blue fox females from 7 farms owned by experienced fox farmers were inseminated with fresh silver fox semen.

Semen collection and treatment

Semen was collected from 50 silver fox males 2 or 3 times a week. The ejaculates were obtained by digital manipulation and fractionated as described in earlier experiments (*Aamdal* 1972, *Fougner* 1986). The sperm-rich fraction was initially diluted 1:3 in Varohm (Kiew) extender, which was composed of glucose (6.000 g), EDTA (0.370 g), sodium bicarbonate (0.120 g), sodium citrate $2H_20$ (0.375 g), neomycine sulphate (0.100 g), and aqua bidest (100 ml). Ph was adjusted to 6.8 ± 0.1 with sodium hydroxide.

The concentration of spermatozoa in the ejaculates was determined with a modified turbidimetric photometer (AB Leo Diagnostics, Helsingborg, Sweden). The instrument was calibrated against diluted semen of known concentration, as determined by direct haemocytometric counting in duplicate (28 ejaculates, coefficient of correlation = 0.97).

Sperm motility was estimated within 10 min of collection under a light microscope. Ejaculates with less than 75% motile spermatozoa were discarded. Ejaculates with more than 20% of their sperm abnormal were discarded even if motility exceeded 75%. Each ejaculate was divided into four portions that were diluted so as to contain 100, 60, 40, and 20 million sperm/ml. All inseminations were performed within 3 h of semen collection.

Experimental design

A summary of the grouping and utilization of males and vixens is presented in Table 1.

A strict split ejaculate trial could not be performed; still every ejaculate used in groups 4, 5 and 6 had a corresponding control in both group 1, 2, and 3. Ejaculates from 50 males were combined for use in the trial to reduce the potential for individual male effects on fertility.

Vixens in groups 1, 2, 3, and 4 were randomly assigned to a group at the time of insemination. Vixens in groups 5 and 6 were selected by the technician at the time of insemination after a physical examination for signs of estrus (vulvar swelling, cervical consistency). Animals judged to be at their optimal time for conception were assigned to group 5, and those considered to be early in their heat were assigned to group 6. Females in group 6 were reinseminated within 24 to 36 h.

Estrus detection and insemination

Estrus control was performed by the farmers. Evaluation of vulval swelling and the change in electrical resistance in the vaginal tract were criteria used to determine the optimal day for insemination in the individual vixen. Electrical resistance was measured with a modified ohmmeter (SiLi3 digital

heat detector, LIMA AS, Sandnes, Norway).

Intrauterine insemination was performed using the method described by *Fougner et al.* (1973). A 1-ml total volume of extended semen, was used for intrauterine deposition.

Statistical methods

Statistical analyses were performed to test the effect of different sperm numbers on fertility. Differences in fertility after insemiation were tested by Wilcoxon's rank sum test for unpaired measurements. Litter size was used as a fertility index for each vixen. Nonpregnant vixens were assigned a value of zero. This procedure allowed comparisons of pregnancy rates and litter sizes between groups. Statistical analyses were performed with procedures available in the Statistical Analysis System (*SAS Institute Inc.*).

Estimation of the results

Vixens aborting during pregnancy or killing their cubs at parturition were considered as pregnant in calculations of fertility. Litter sizes were calculated from observations made within 48 h of parturition. In the following text »fertility« refers to both pregnancy rate and litter size.

Results

The fertility of vixens inseminated with various amounts of sperm is summarized in Table 1.

Most striking was the high fertility in all groups, expressed in both pregnancy rate and litter size. In the random group inseminated with 20 million sperm (group 4), both pregnancy rate and litter size were low compared with other random groups inseminated with higher sperm numbers (groups 1, 2, and 3), although the difference was not statistically significant. Among the vixens inseminated with 20 million sperm (groups 4, 5, and 6) there was a significant difference ($p < 0.05$) in fertility only between those randomly selected and inseminated once (group 4) and those selected by the technician that were then inseminaed twice (group 6).

Discussion

In the fertility tests performed, all inseminations were carried out by the same technician. The trial was, to a large extent, of the split ejaculate type. Thus the results should be regarded as reliable representation of the relationship between the sperm numbers tested and fertility, although the number of vixens in the experimental groups was limited. It was evident that silver fox semen extended to 100, 60, 40, and 20 million sperm cells per millilitre yielded similar rates of fertility when inseminations were performed in randomly selected blue for vixens. The pregnancy rates and litter sizes in all experimental groups were equivalent to or exceeded results of routine AI work in Norway associated with the crossbreeding of silver- and blue

Table 1. Summary of the experimental design and results.

Group	No. males (silver fox)	No. vixens (blue fox)	Sperm *10 per AI dose	Pregnancy rate %	Litter size
1	31	49	100	85.7	8.2
2	34	74	60	81.1	8.3
3	36	88	40	85.2	8.1
4	18	31	20	74.6	7.8
5	22	35	20	88.6	8.6
6	29	48	20	83.3	8.9

fox. I 1986 litter sizes were 7.9 and 8.0 when inseminating once and twice respectively (data obtained from 11.228 and 8.614 animals in each group) (*Fougner,* unpublished results).

Earlier experiments indicated that 100-150 million sperm per insemination dose would be necessary to achieve optimal fertilization in the blue fox (*Aamdal et al.* 1978). In those experiments IVT (Illinois Variable Temperature) plus 10% egg yolk was utilized as semen diluent, while in the present investigation Varohm was used as extender for semen preservation. In addition also, storage temperatures differed betwen the two trials, 5°C was used in the former study while ambient temperature was used in our study. Moreover, since the time of those early studies, improved methods for estrus determination have been developed. (*Møller* 1980, *Møller & Fougner* 1981, *Fougner* 1983). Consequently, there were a number of factors that probably helped contribute to the differences observed in the results of the two investigations.

Our results show that using the present techniques for sperm preservation and estrous determination, 20 million sperm per insemination dose can give acceptable fertility when cross breeding foxes. Other investigations support this conclusion. Inseminations with 20 million sperm per dose have been used successfully for the pure breeding of red foxes in the Soviet Union. Pregnancy rates in those trials ranged between 80 and 86% (*Bautina et al.* 1983).

Our results also suggested that 20 million sperm per insemination dose might be the minimum dose required for acceptable fertilization. Further investigations are needed, however, to more firmly establish the optimal dose necessary for minimizing sperm use and maximizing fertility. This could be done by inseminating with sperm numbers below 20 millions. Below the minimum or threshold value there should be a high correlation between sperm number per insemination and fertility, but this correlation should decrease as the number of spermatozoa used for insemination aproaches the threshold value. At this point and above it there should not be any relationship between the number of spermatozoa inseminated and fertility rate (*Salisbury et al.* 1978).

Silver fox semen is usually of high quality. In this investigation all ejaculates had a motility equal to or exceeding 75% and total sperm abnormalities never exceeded 20%. We do not know whether these criteria for semen selection can be improved. It is conceivable, however, that the threshold value for sperm numbers per insemination can be further reduced by imposing more strict criteria for semen evaluation. We conclude with this word of caution: above the threshold value, increasing the number of sperm per insemination will not improve the low rate of fertility that results from using poor quality semen, nor does it compensate for reduced fertility caused by poor or unskilled technicians.

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Sammendrag

Spermietallets innvirkning på fruktbarheten ved kunstig sædoverføring på rev.

325 blårevtisper, fordelt på 6 forsøksgrupper, ble inseminert med fersk sæd fra 50 sølvrevhanner. Dyra i grupperne 1-4 ble inseminert med henholdsvis 100, 60, 40 og 20 millioner spermier. Dyra i gruppe 5 ble inseminert en gang med 20 millioner spermier etterat inseminøren kontrollerte rett inseminasjonstidspunkt ved palpasjon av cervix. Dyr i gruppe 6 ble inseminert 2 ganger med 24-36 timers mellomrom med 20 millioner spermier. Alle inseminasjoner ble utført innen 3 timer etter sæduttak. Inseminasjonsdosen var 1 ml og hvert ejakulat var mest mulig jevnt fodelt (split sample) mellom gruppene.

Resultatene viste at med nåværende teknikk for spermiehåndtering og brunskontroll, syns 20 millioner spermier pr. ml å være den minste inseminasjonsdose som gir akseptabel fruktbarhet ved artskrysning mellom blårevtispe og sølvrevhann.

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