Effect of vaginal and cervical deposition of semen on the fertility of sheep inseminated with frozen-thawed semen

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The effect of vaginal and cervical deposition of frozen-thawed semen on the fertility of sheep was tested in a field trial in which 543 Norwegian crossbred ewes aged between six months and five-and-a-half years from 10 farms were inseminated after natural oestrus. Cervical insemination with 200 x 10^6 spermatozoa resulted in 25-day non-return and lambing rates of 75·4 and 72·7 per cent, respectively, and vaginal insemination gave rates of 71·3 and 67·4 per cent; the cervical inseminations produced significantly higher lambing rates (P=0·04). There were significant differences between the lambing rates for different rams (P=0·006) and different farmers (P=0·003), and there was a significant interaction between farmer and deposition site (P=0·03). After vaginal insemination fertility was encouragingly high, but the results varied with the farmer, and different flock and management conditions.

THE breeding season of Norwegian crossbred ewes is in November and December. The ewes are seasonally polyoestrous with an oestrous cycle lasting 16 to 17 days. Oestrus lasts approximately two days and ovulation occurs in the second half of oestrus. Normally two to four eggs are ovulated 30 to 36 hours after the onset of oestrus. Ewes mature at approximately six months of age, and the majority are mated at first oestrus. The fertility of Norwegian ewes is very high, with natural mating being predominant. Because the average flock size is only approximately 52 adult animals, a cooperative scheme for breeding has been developed. During the mating season rams are moved between farms that belong to the same 'ram circle' or breeding group, according to their breeding requirements (Gjerdrem 1969, Andersen Berg 1999).

Cervical insemination with liquid semen was developed in Norway for routine use in artificial insemination (AI) in the 1960s (Aamdal and others 1966). At that time, AI was introduced as a supplement to natural mating and only a limited number of highly selected ewes were inseminated artificially. The small number of inseminations and the logistical problems involved with liquid semen favoured the use of frozen semen. As a result, cervical insemination with frozen semen was established in Norway in the late 1960s, and the procedure was improved in the 1970s (Aamdal and Andersen 1968a, b, Andersen and others 1973, Olafsson 1980). However, over the next few years, less than 1 per cent of ewes were inseminated artificially. The Norwegian animal health authorities have issued several restrictions on the movement of sheep between flocks during the past seven to eight years, to control the emergence of scrapie. Moving rams between farms is currently prohibited in some regions, reducing the effectiveness of the ram circles. As a result, the use of AI has increased rapidly from 2·3 per cent in 1999, to 3·5 per cent in 2001. The increase in AI during the late 1990s was mainly based on the use of liquid semen produced at local AI stations. Owing to the geographical spread of sheep holdings throughout Norway and the limited storage time for liquid semen (12 hours), it is considered more practical under most conditions to use frozen semen. Frozen semen started to be used more extensively in the breeding season of 2000.

The successful application of AI in sheep depends upon the availability of a cheap and effective insemination technique. To attain this goal, an AI technique has to be found that can be performed by farmers themselves. Vaginal insemination (Fairnie and Wales 1982) is simple because only an insemination pipette is required. A Norwegian study by Paulenz and others (2002) compared the vaginal and cervical deposition of liquid semen, and found no statistically significant difference between the fertility achieved by using the two deposition sites. However, several studies have revealed low fertility rates after the vaginal deposition of frozen-thawed semen (Tervit and others 1984, Maxwell and Hewitt 1986).

The aim of this field trial was to test the effect of the vaginal and cervical insemination of sheep with frozen-thawed semen during natural oestrus on their fertility, in terms of the percentage non-return rate 25 days after insemination and the lambing rate.

MATERIALS AND METHODS

Rams and semen processing

Semen from six Norwegian crossbred rams with proven fertility aged between one-and-a-half and two-and-a-half years was used for AI. The animals were owned by the Norwegian Association of Sheep and Goat Breeders (NSG) and housed at the NSG Semin AI station in south-east Norway, near Hamar, a region with an inland climate.

At the beginning of the breeding season (November to December) semen was collected three to four times weekly from each ram by using an artificial vagina, and the semen quality was assessed. To be accepted as a donor, each ram's ejaculates had to satisfy the following criteria: volume of at least 0·5 ml, macroscopically having good visual mass activity, with a sperm concentration of at least 3 x 10^9/ml, progressive sperm motility of at least 75 per cent, and at least 90 per cent of the sperm having normal morphology. The volume, mass activity, concentration and motility of the sperm were evaluated routinely, but the morphology was assessed only when it appeared to be abnormal when motility was being assessed. Sperm concentration was estimated with a spectrophotometer (IMV; Accucel) which was calibrated for ram semen. Sperm motility was assessed at x 200 magnification, and sperm morphology was assessed at x 400 magnification by phase-contrast microscopy.

Each ejaculate was collected in a prewarmed graduated glass vial, in which it remained while it was being processed. The ejaculates were placed in a water bath at 35°C immediately after collection, and the semen quality was assessed. Within 10 minutes after collection each ejaculate was diluted 1+4 to 1+6 with a milk-based extender (E1) at 35°C, which was prepared from non-fatty milk powder (11 per cent w/v) and distilled water, heated to 95°C for 10 minutes, and then

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TABLE 1: P values and chi-squared values for the effects of the age of the ewes, farmer, ram, deposition site and interaction between farmer and deposition site on the 25-day non-return rates and lambing rates of 543 ewes, aged between six months and five-and-a-half years, inseminated either in the vagina or cervix with 200 x 10^6 spermatozoa in frozen-thawed semen collected from six mature rams

Source of variation	Degrees of freedom	Chi-squared		P	
		Non-return rate	Lambing rate	Non-return rate	Lambing rate
Age of the ewes	5	12·3	10·0	0·032	0·076
Farmer	9	20·9	25·2	0·013	0·003
Ram	5	13·1	16·4	0·022	0·006
Deposition site	1	2·3	4·2	0·133	0·041
Farmer x deposition site	9	19·4	18·6	0·022	0·029

cooled to room temperature before egg yolk (5 per cent, v/v), penicillin and streptomycin were added. Each vial containing the extended semen was wrapped with paper to reduce the risk of inducing cold shock. The semen was then cooled to 5°C over approximately 30 minutes by placing it in a room at that temperature. The cooled semen was then diluted 1+1 with an extender (E2) at the same temperature. E2 was the same as E1 except that it also contained glycerol (14 per cent v/v), resulting in a final glycerol concentration of 7 per cent; the E2 was added stepwise over approximately 15 minutes. After this second dilution the semen was allowed to equilibrate for 60 to 90 minutes at 5°C. The semen was then concentrated by centrifugation at 1000 *g* for 10 minutes, and enough supernatant was removed to yield a final sperm concentration of approximately 1000 x 10^6/ml. Aliquots of 0·2 ml of the prepared semen were then placed in 0·25 ml plastic straws (Minitüb), to provide an insemination dose of approximately 200 x 10^6 spermatozoa. The straws were sealed with plastic balls and frozen in a programmable freezer (Digicool 5300; IMV) in which the temperature decreased from 5°C to –10°C at a rate of 5°C per minute, and then from –10°C to –130°C at 60°C per minute. The straws were then transferred to liquid nitrogen for storage. One straw from each batch from each ram was thawed, and only batches showing at least 50 per cent progressive motility were approved for use. The straws were thawed by immersing them in a water bath at 70°C for eight seconds.

Ewes and artificial insemination

During a period of approximately five weeks, from the beginning of November to the beginning of December 2001, a total of 543 Norwegian crossbred ewes were inseminated. They were between six months and five-and-a-half years old when they were inseminated and were housed at 10 different farms in the western part of Norway, in a region (Rogaland) with a coastal climate. The farmers inseminated the ewes themselves, after they had attended an AI training course. At least 50 ewes were inseminated in each flock.

The ewes were inseminated alternately into the cervix or the vagina with a dose of semen from the same ram, but the semen from the six rams was used at random; semen from a different ram was used for the next pair of inseminations, and so on. Only one straw was thawed at a time and used immediately for AI.

In general, the farmers checked the ewes for oestrus with a teaser ram twice a day, approximately every 12 hours, and the ewes were inseminated once, recommended to be between 12 and 24 hours after oestrus had been detected. For the cervical inseminations, the doses of semen were deposited to a depth of 5 to 12 mm into the cervical canal with an insemination pipette after the portio had been located by means of a speculum with a speculum light, as described by Andersen and others (1973). The insemination pipette was 4 mm in diameter and was equipped with an eccentric tip (Minitüb). The vaginal inseminations were made with an insemination pipette with a diameter of 5 mm and a rounded tip (Minitüb); without using a speculum, the lips of the vulva were parted and the semen was placed as deeply as possible into the vagina, as described by Fairnie and Wales (1982).

Fertility

To detect ewes returning to oestrus, all the ewes were checked by the farmers from day 12 to day 25 after insemination by using a teaser ram. Ewes not returning to oestrus were considered to be pregnant, and recorded as the percentage of ewes that had not returned to oestrus by 25 days after AI. The lambing rate (the percentage of ewes lambing) was recorded by the farmers on each farm during the following spring, and a written report was forwarded by each farmer.

Statistical analysis

The binomial responses not returned to oestrus (NR) and lambing rate were analysed by using a linear logistic model, the GENMOD procedure for categorical data (Statistical Analysis Systems statistical package, version 6.12; SAS Institute). The model chosen was:

log odds of NR and lambing rate = age of the ewes + farmer + ram + deposition site + interaction farmer x deposition site

where the NR and lambing rate for each ewe is either yes or no, the age of the ewe at parturition is one, two, three or more than three years, the farmer is one of 10, the ram is one of six, and the deposition site is either the vagina or cervix.

The parameters were fitted by the maximum likelihood principle, and effects were tested by the chi-squared test (Cox 1970, Agresti 1990). The significance of the main effects was confirmed by Fisher's exact tests for deposition site and sperm dose. Several alternative statistical models were tried, including combinations of main effects with interactions. The results of the chosen model were in accordance with simpler models, and the ones with interactions that were possible to run. Because the farmers were responsible not only for the flock management, for example, the oestrus check, but also performed the inseminations, the effect of the inseminator could not be included in addition to the farmer. To avoid overlapping, only the effect of the farmer was included in the statistical model. The level of significance was set at 0·05.

RESULTS

The levels of significance for the effects of the age of the ewes, the farmer, the ram, the deposition site and the interaction between the farmer and the deposition site are shown in Table 1.

There was a significant difference in the lambing rate, but not in the non-return rate, between the ewes inseminated into the vagina and those inseminated in the cervix. Both the farmer and the ram had a significant effect, and the age of the ewe had a significant effect on the non-return rates. There was a significant effect of the interaction between the farmer and the deposition site for both the non-return rate and the lambing rate. The results obtained by the individual farmers are shown in Fig 1. Only the results from farmer 1 showed a significant difference between the results of the vaginal and cervical inseminations.

The 25-day non-return rates and the lambing rates for vaginal or cervical insemination of the 543 ewes are shown in Table 2. The cervical inseminations resulted in not significantly higher 25-day non-return rates (75·4 v 71·3 per cent, P=0·13) but significantly higher lambing rates (72·7 v 67·4 per cent, P=0·04) than the vaginal inseminations. The difference between the overall 25-day non-return rate and the lambing rate was 3·3 per cent. Fourteen ewes (2·6 per cent) returned to oestrus later than 25 days and four (0·7 per cent) aborted.

TABLE 2: Twenty-five-day non-return rates and lambing rates after the cervical or vaginal insemination of 543 ewes, aged between six months and five-and-a-half years, inseminated either in the vagina or cervix with 200 x 10^6 spermatozoa in frozen-thawed semen collected from six mature rams

Site of insemination	Number of inseminations	Number (%) of Non-returns	Lambings
Vaginal	279	199 (71·3)	188 (67·4)
Cervical	264	199 (75·4)	192 (72·7)
Total	543	398 (73·3)	380 (70·0)

Each farm received 60 AI doses. As a result of withdrawals, for example, because of defective straws, on average 54·3 ewes were inseminated per farm, with a range from 48 to 60. The 25-day non-return rates varied between 60·4 and 86·1 per cent for the different farmers, and the lambing rate varied between 56·3 and 83·3 per cent. The average time from the detection of natural oestrus to insemination was approximately 14 hours, with a range from five to 28 hours. The average number of lambs per ewe was 1·50 for the 26 primiparous ewes, 2·16 for the 366 pluriparous ewes, and 2·15 overall.

The fertility of the different rams varied between 64·6 and 81·4 per cent for the 25-day non-return rate and between 58·5 and 78·3 per cent for the lambing rate. On average, 90·5 AI doses were used from each ram, with a range from 82 to 97.

DISCUSSION

Cervical deposition gave higher fertility results than vaginal deposition, with a significant difference in the overall lambing rate, but not in the non-return rate. There were also significant differences between the fertility of the six rams and between the results achieved by the 10 farmers. In addition, there was a significant interaction between the effects of the farmer and the deposition site.

The differences between the 10 farmers in their non-return rates and lambing rates are in accordance with the results of studies by Söderquist and others (1999) and Paulenz and others (2002). Although the farmers were confident with the AI techniques, because they had taken part in earlier studies with liquid semen, there was considerable variation between their results. Four of the 10 farmers had numerically better results from the ewes inseminated vaginally, and five farmers had the opposite result (Fig 1). However, the difference between the results of the vaginal and cervical inseminations was statistically significant for only one farmer; this result contributes to the statistically significant overall difference in the lambing rate observed between the two AI techniques. The differences between the results of the farmers would have been due not only to their level of technical skill but also to flock conditions, such as the detection of heat and signs of oestrus (Andersen Berg and Aamdal 1991), and possibly to other factors, such as feeding and animal handling. These factors are probably associated with different flock and management conditions, but further investigations would be required to determine their relative importance.

There were considerable differences in the non-return rates and lambing rates of the ewes inseminated with semen from the different rams. Such variations in the fertility of rams are well documented and have been reported after cervical inseminations with fresh semen (Salamon and Robinson 1962, Paulenz and others 2002). Sire effects after the use of frozen semen have been described by Colas (1979) Windsor (1997) and Söderquist and others (1999) after cervical insemination, and by Maxwell (1986), Eppleston and others (1986, 1991) and Eppleston and Maxwell (1995) after laparoscopic insemination. The rams used as donors were selected after an

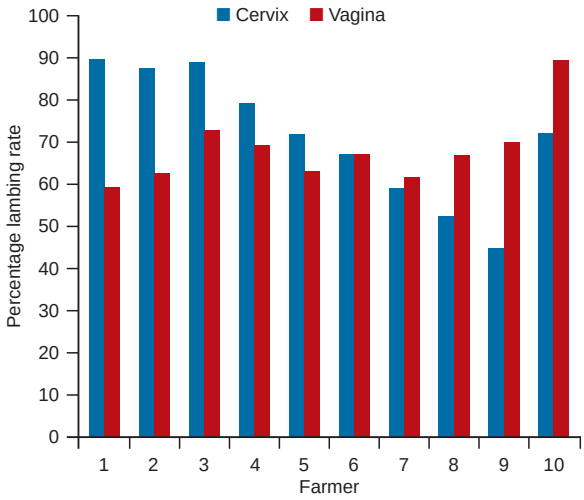

FIG 1: Percentage lambing rates achieved by 10 farmers after inseminating 543 ewes either into the vagina or the cervix with frozen-thawed semen collected from six mature rams. Only the results from farmer 1 showed a significant difference between the results of the vaginal and cervical inseminations

estimation of their breeding value based on the results of natural matings in the previous season. Though the breeding value includes fertility as an important factor, the variation observed between the six rams emphasises the need for improved methods for the selection of AI rams.

There have been few reports comparing cervical and vaginal inseminations with frozen semen. Tervit and others (1984) inseminated an unspecified number of Perendale ewes, after they had been synchronised hormonally twice, with a dose of 900 x 10^6 spermatozoa, resulting in a lambing rate of 17·0 per cent after cervical deposition and 22·7 per cent after vaginal deposition. Maxwell and Hewitt (1986) reported pregnancy rates of 18·4 per cent after the cervical insemination of 49 Merino ewes, and 17·6 per cent after the vaginal insemination of 51 ewes with a dose of 100 x 10^6 spermatozoa, after they had been synchronised hormonally. Despite the difference observed between the results of vaginal and cervical deposition of semen, the overall fertility results in the present study (73·3 per cent for the 25-day non-return rate and 70·0 per cent for the lambing rate) were much higher than in the earlier reports. However, the 25-day non-return rates observed in earlier field trials in the same region of Norway, in which 150 million spermatozoa were deposited intravaginally in liquid semen (Paulenz and others 2002), were in line with the present results. In Norway, the overall fertility results in 2001, available only as 25-day non-return rates (Colbjørnsen 2002), were almost identical when comparing the 14,472 AIs with liquid semen (150 x 10^6 spermatozoa; 69·6 per cent) and 16,143 AIs with frozen-thawed semen (200 x 10^6 spermatozoa; 69·8 per cent). However, it is not known how many of these inseminations were made into the cervix and how many were made into the vagina.

Possible factors that may have contributed to the higher fertility results achieved in this study than in earlier studies include the facts that the ewes were inseminated at natural oestrus and were of a fertile breed, the farmers had had experience of AI and the flocks were small. It has been reported that AI after hormonal synchronisation results in lower fertility than AI at natural oestrus (Hafez and Hafez 2000). The fertility of Norwegian crossbred ewes is very high compared with many other breeds. This is partly due to favourable environmental conditions and low milk yields, and partly to the fact that ewes that do not become pregnant after mating in, at most, the two following oestrus cycles are usually culled (Andersen Berg 1999). The farmers in this study must be considered as experienced inseminators, because they had all been using AI on a do-it-yourself basis for several years. The small flock size, between 70 and 120 ewes, makes it possible for the farmer to give each ewe individual attention, and this probably decreases the stress experienced by the ewes when

they are handled, for example, when inspecting them for oestrus and at AI.

In this field trial slightly higher fertility was achieved in the ewes inseminated into the cervix compared with those inseminated into the vagina, and there was a significant difference in the overall lambing rate. However, these results interacted with factors associated with the farmers, the different flocks and the management conditions, factors that need to be studied more comprehensively. Vaginal insemination is a simple, less costly and less time-consuming technique than cervical insemination, and may have advantages in terms of the animals' welfare at AI. As the aim is to establish a technique that could be applied on a large scale by all farmers, vaginal insemination must be considered as the method of choice for the AI of ewes with frozen-thawed semen in Norway.

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