CERVICAL INSEMINATION IN SHEEP

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ABSTRACT

In this study, the effect of the insemination dose (250 or 500 x 10^6 fresh spermatozoa) on pregnancy rate at 35 days post-insemination (PI) in synchronized ewes was analyzed. The 30 days non-return rate was determined by the introduction of an intact ram harnessed with a crayon at 10 days PI. At 35 ± 2 days PI, the ewes were scanned for pregnancy by means of transrectal ultrasonography (7 MHz). Five months PI, the lambing rate and fecundity were recorded. No significant differences ($p<0.05$) were found in non-return rate, pregnancy or fecundity between 250 and 500 x 10^6 fresh spermatozoa per insemination dose. Increasing the number of spermatozoa from 250 x 10^6 to 500 x 10^6 per insemination dose did however improve the lambing rate ($p<0.1$). Pregnancy control by transrectal ultrasound (7 MHz) at 35 ± 2 days was at least as reliable as a teaser ram.

SAMENVATTING

In dit onderzoek werd het effect van de inseminatiedosis (250 of 500 10^6 verse spermacellen) op de drachtigheidresultaten (PR) op 35 dagen post inseminatie (PI) nagegaan bij gesynchroniseerde ooien. Het percentage niet-terugkomers werd bepaald door introductie van een intacte ram 10 dagen PI. Het drachtigheidpercentage werd op 35 ± 2 dagen bepaald door middel van transrectale echografie (7MHz). Vijf maanden PI werden het aflammerpercentage en de worpgrootte bepaald. Er kon geen significant verschil ($p<0.05$) gevonden worden wat betreft de terugkomers, de drachtigheidresultaten en de worpgrootte tussen 250 en 500 miljoen verse spermacellen per inseminatiedosis.

Een verhoging van de inseminatiedosis van 250 naar 500 miljoen spermacellen had een gunstige invloed op het aflammerpercentage. Drachtigheidcontrole met behulp van transrectale echografie (7 MHz) op 35 ± 2 dagen PI, bleek minstens zo betrouwbaar te zijn als een zoekram.

Keywords: Sheep - Insemination - Fresh semen - Dose

INTRODUCTION

In Belgium, artificial insemination (AI) has so far not become a common procedure for sheep reproduction. In Australia, the former Soviet Union and several Scandinavian countries, however, much AI is performed and it produces acceptable conception rates. Both fresh and frozen-thawed semen are deposited by means of laparoscopic insemination in the uterus at doses of 50 x 10^6 and even less.

Most of the insemination procedures in sheep are performed after estrus synchronization. Cyclic ewes are routinely synchronized by means of an intravaginal progestagen sponge followed by an injection of the pregnant mare serum, gonadotropin (PMSG). Within three days after the progesterone withdrawal, 85 – 90% of treated ewes display estrus (Greyling *et al.*, 1997; Romano *et al.*, 2000). Ovulation most often commences within 57 h of progesterone removal and is completed within 81 h (Walker *et al.*,1989).

In cattle, transcervical insemination is the routine insemination technique, producing good conception rates using low numbers (10 x 10^6) of frozen-thawed semen. Transcervical insemination is not common in

sheep, however, because penetration of the cervix is a major problem in sheep. The complex anatomy of the ovine cervix and the impossibility of getting hold of it transrectally reduce cervix penetration rates to about 50%, depending on the anatomical variation between ewes and the skill of the inseminator. Moreover, the performance of AI in small flocks involves extra costs for oestrus synchronization in order to minimize labor.

Despite the problems associated with ovine AI, the technique is worthwhile in European sheep breeding. Thanks to AI, a higher rate of genetic progress may be made in a flock. Sire rams can have more descendants in a shorter time interval, and they can be used in several flocks without spreading infectious diseases. Moreover, the date of lambing can be determined more accurately by the farmer.

A practical solution for AI in sheep can be obtained by using cervical insemination instead of transcervical insemination. With cervical insemination the semen is deposited close to or in the cervix without penetrating it. If cervical insemination is the technique of choice, then much fresh, good quality semen is required to obtain acceptable conception rates. Doses of 100-600 million fresh diluted spermatozoa are required in a volume of 0.1 to 0.4 ml to obtain good fertilization results after cervical insemination in synchronized ewes .

Early and accurate diagnosis of pregnancy is important for the evaluation of the insemination results and for effective livestock management. Lack of knowledge to differentiate pregnant from non-pregnant animals results in uneconomical feeding of non-pregnant animals.

The present study was designed to evaluate the effect of cervical insemination, both with a high (500 x 10^6) and a low (250 x 10^6) number of fresh diluted spermatozoa, on the 30 days non-return-rate (NRR), the pregnancy rate (PR) at 35 days post-insemination (as assessed by ultrasonography), the fecundity (Fec) and the lambing rate (LR). A second goal of this study was to determine whether transrectal ultrasonography is a valuable alternative for pregnancy diagnosis, compared to using a ram.

MATERIALS AND METHODS

Preparation of ewes

At the end of September 1999, 96 crossbred ewes (3/4 Texel and 1/4 Suffolk) which had lambed at least once were randomly selected to take part in the experiment. The ewes were kept on good pasture without any supplementary feeding. The body condition score of the ewes ranged from 2.5 to 3.5 on a scale from 0 (extremely thin) to 5 (extremely fat). They were in good condition, and weighed between 50 and 70 kg. For practical purposes, the ewes were randomly divided into 3 groups of 32 ewes. After a 12 day treatment with an intravaginal progestagen sponge (Veramix, Upjohn; 60 mg medroxyprogesterone acetate), a single dose of 500 IU PMSG (Folligon, Intervet) was administered intra-muscularly. Insemination was performed 55 ± 1 h after sponge withdrawal, without detection of oestrus. In each group of 32 ewes, 8 ewes were randomly inseminated per ram; half of the ewes received a dose of 250 x 10^6 and the other half a dose of 500 x 10^6 fresh spermatozoa. Ten days post-insemination (PI), an intact ram harnessed with a crayon was introduced into the flock to mark the ewes returning into oestrus. The unmarked ewes at 30 days PI were used for the calculation of the non-return rate (NRR).

Preparation of rams and semen

Semen was collected, by means of an artificial vagina, from 2 purebred Texel and 2 crossbred Texel-Swifter rams. Their semen quality had been evaluated 14 days before the insemination trials. Cut-off values for semen quality of the ejaculates are presented in Table 1. Per ram, two ejaculates were taken at an interval of 15 min and pooled. The ejaculate was protected from light and temperature shock.

Immediately after collection, each ejaculate was evaluated for volume and wave motion. The volume was determined in the collection tube, which was graduated in 0.1 ml divisions. The wave motion was assessed macroscopically by deposition of a drop of pure semen on a warm (37°C) glass slide. After the macroscopic assessment of the ejaculate, the concen-

Table 1. Cut off values for semen quality of the rams included in the experiment.

Parameter	Value
Motility	> 80%
% Alive	> 80%
Concentration	>1.5 10^9 sp/ml
Volume	>0.8 ml
No. of spermatozoa per ejaculate	>3.0 10^9

tration, individual motility and morphology were assessed microscopically. The concentration was determined in the following way: 50 µl of raw semen was diluted in 9.95 ml HCl, mixed and put in a Bürker chamber. For the assessment of the individual motility, one droplet of semen was deposited on a warm glass slide, diluted with one droplet of 0.9% saline solution and put on a warm stage (37°C) under the phase contrast light microscope. A second droplet was stained with eosin-nigrosin to evaluate the percentage living spermatozoa and the morphology.

Ejaculates which met the criteria were centrifuged during 10 min at 1160*g. Subsequently the seminal plasma was removed, and the spermatozoa were diluted with Triladyl diluent to obtain the required number of spermatozoa in a 0.25 ml insemination volume. The diluted samples were maintained at 22°C ± 2°C. The time interval between sperm collection and insemination was 3 ± 0.5 h.

Insemination and experimental design

Ninety-six ewes, randomized in 3 treatment groups, were inseminated by the same operator on Monday, Wednesday and Friday of the same week, at 55 ± 1 h after sponge removal. No attempt was made to detect behavioural oestrus, and all ewes which had received sponges were inseminated. In each group, 8 ewes were inseminated per ram, half with an insemination dose of 250 x 10^6 / 0.25 ml and half with a dose of 500 x 10^6 / 0.25 ml fresh spermatozoa. The ewes were brought together in a small pen and presented for insemination by an assistant. Hindquarters were not lifted during or after insemination to avoid extra labor and stress. A vaginal speculum was introduced into the vagina to locate the cervix by means of a light source fixed on the speculum and the semen was deposited close to the external cervical os. Before the semen were expelled out of the insemination pipette, the speculum was removed to avoid pneumovagina and subsequent discharge of semen. After insemination, the ewes remained on pasture without any change in nutrition.

Pregnancy control

Ten days after AI, an intact ram provided with a marker on his chest was introduced in each group. The non-return rate (NRR) was defined as the percentage of inseminated ewes that were not marked by the ram 10 to 30 days after insemination. At 35 ± 2 days post-insemination, transrectal ultrasonography by means of a 7 MHz probe was performed for pregnancy control. When a fluid-filled uterus was visible (Fig 1), the ewes were designated as being pregnant, without any distinction being made between single and multiple pregnancies. In this experiment, the pregnancy rate is the percentage of ewes inseminated that were detected as being pregnant. At parturition, the number of lambs born per ewe, which is defined as fecundity, was noted. The lambing rate (LR) was the number of ewes that lambed 145 ± 5 days PI, divided by the number of ewes inseminated, times 100. The NRR and the PR were compared to the LR.

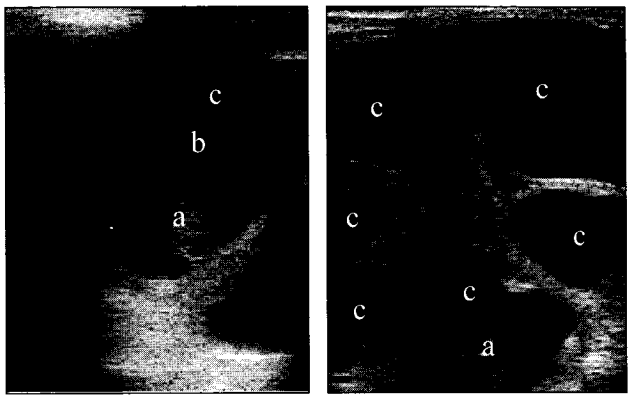

Fig 1. Pregnancy diagnosis in sheep by means of transrectal ultrasound at 35d PI.
a) embryo b) extra-embryonic membranes c) uterine lumen filled with fluid.

Statistical analyses

The effect of insemination dose (250 and 500 x 10^6 spermatozoa) and ram on the NRR, PR and LR was evaluated statistically using weighted logistic regression. The weighted Poisson (discrete) regression was applied to detect whether the fecundity was influenced by the ram or the insemination dose, and to verify whether there was a difference in fecundity between the groups of ewes. A 2*2 cross tabulation was used for the determination of the sensitivity, the specificity and the positive and negative predicting value of the NRR and the PR, compared to the LR.

RESULTS

The NRR, PR, LR and Fec are presented in Table 2. No sponges were lost during the synchronization treatment. The NRR, PR and LR were higher for the ewes inseminated with 500 x 10^6 than for ewes inseminated with 250 x 10^6 spermatozoa, but did not differ significantly ($p<0.05$). However, there was a tendency ($p = 0.096$) for the LR to be higher for ewes inseminated

Table 2. Mean values in percentage for non-return rate (NRR), pregnancy rate (PR, detected by ultrasound at 35 days ± 2d), lambing rate (LR) and fecundity (Fec) for the 250 10^6 and 500 10^6 insemination dose.

Insemination dose	Number of ewes	NRR (n)	PR (n)	LR (n)	Fec
250 10^6	48	58 **(28)**	65 **(31)**	40 **(19)**	1.94
500 10^6	48	71 **(34)**	69 **(33)**	56 **(27)**	1.62

Table 3. Difference in non-return rate (NRR), pregnancy rate (PR), lambing rate (LR) and fecundity (Fec) between the three groups of ewes.

	Number of ewes	NRR (n)	PR (n)	LR (n)	Fec
Group 1	32	65.5 **(21)**	69.0 **(22)**	37.5 **(12)**	1.5^a
Group 2	32	50.0 **(16)**	56.3 **(18)**	56.3 **(18)**	2.1^b
Group 3	32	71.9 **(23)**	75.0 **(24)**	50.0 **(16)**	1.4^a

Table 4. Sensitivity, specificity, positive and negative predicting value of the non-return rate (NRR) and pregnancy rate (PR).

	Sensitivity	Specificity	Positive predicting value	Negative predicting value
NRR	93.5	62.0	69.4	91.2
PR	95.7	60.0	68.8	93.8

with a dose of 500 x 10^6 (56%) than for ewes inseminated with a dose of 250 x 10^6 spermatozoa (40%).

The fecundity was higher for the low insemination dose (1.94), but the difference between the higher insemination dose (1.62) and the lower dose was not significant. In general, the NRR and PR were 13-25% higher than the LR (Table 2), indicating a substantial fetal loss. In the second group of ewes, the fecundity was significantly higher than in the first and third groups ($p<0.01$) (Table 3).

The sensitivity and negative predicting value are slightly higher for pregnancy detected by transrectal ultrasound than for the NRR (95.7% and 93.8% vs. 93.5% and 91.2%, respectively). However, the specificity and positive predicting value are slightly lower for the PR detected by ultrasound than for the NRR (60.0% and 68.8% vs. 62.0% and 69.4%, respectively) (Table 4).

DISCUSSION

This study was set up to evaluate the effects of two different insemination doses (250 and 500 x 10^6 fresh diluted ram spermatozoa) on pregnancy outcome in a flock of synchronized ewes, and to determine whether ultrasonography is a valuable alternative for pregnancy control, compared to using an intact ram.

Since PR and LR obtained after estrus synchronization are the same for cervical insemination with 500 x 10^6 fresh ram spermatozoa and for natural service (± 2.5 x 10^9 spermatozoa), it is not opportune to inseminate with a higher dose than 500 x 10^6 fresh spermatozoa.

The increase in the number of spermatozoa per insemination from 250 to 500 x 10^6 has a beneficial effect on the NRR, the PR and the LR, although not significantly. This may be explained by: 1) the number of

sheep in this experiment, which was not high enough to demonstrate a significant beneficial effect of doubling of the insemination dose; 2) the threshold level for optimal fertility, which was probably already reached with the dose of 250 x 10^6 fresh spermatozoa for cervical insemination in ewes. To know which of the two hypotheses is right, an experiment of greater size would have to be performed.

Greyling *et al.* (1997) conducted a similar experiment in a more fertile breed of sheep, using only 100 x 10^6 fresh spermatozoa per insemination. The NRR and LR he found (54.8% and 47.0%, respectively) were comparable to our results.

Ewes in this study were synchronized by administration of synthetic progestagens in sponges, which minimizes labor and enables planning of insemination and lambing dates. However, one major disadvantage of this kind of synchronization is the lowered fertility. This may be explained by the inhibitory effect of the progestagens on the movement, transport and survival of spermatozoa in the female reproductive tract, and by the altered timing of the peak in luteinizing hormone and the ovulation relative to estrus and insemination . Pregnancy rates after cervical insemination can be improved 1) by insemination after the onset of natural estrus, 2) by using only half the dose (30mg) of MAP for estrus synchronization during the mating season, and 3) by inseminating twice during the same estrus .

Pregnancy rates of up to 70% after only one insemination, even when frozen-thawed semen is being used, can be obtained if the cervical barrier is passed by. At present, the most popular technique for obtaining such results is laparoscopic intra-uterine insemination . While laparoscopic insemination is effective, its wider application is limited by its expense, the associated anesthesia, the required veterinary skills and the ethics of animal welfare, which do not allow surgical intervention for mere reproductive purposes. The semen can also be deposited into the uterus by means of transcervical insemination. One such transcervical technique is the Guelph system for transcervical AI (GST-AI), which involves traction on the cervix. With the GST-AI, conception rates similar to those realized by laparoscopic AI can be obtained, but only if the semen can adequately be deposited into the uterus. This occurs in 50% to 87% of the ewes . The administration of oxytocin helps to increase the penetration rate of the cervix. Sayre and Lewis (1996) found that IV administration of 200 - 400 USP units (~IU) of oxytocin causes dilatation of the cervix in 100% of the ewes; this dilatation occurs within 10 min and lasts for 7.4 hrs. Although the administration of oxytocin induces uterine spasms for a period of 30 min, the movement of sperm cells towards the region of fertilization is not affected. Out of the methods available for the diagnosis of pregnancy in sheep and goats , only a few are reliable and applicable under field conditions. Pregnancy in the ewe can be diagnosed by ultrasound with low accuracy from 17 days PI on . In our study, the pregnancy detection at 35 ± 2 days PI by linear array transrectal ultrasonography (7 MHz) seemed to be especially valuable as a negative control. In 94% of the cases the ewes were correctly diagnosed as not being pregnant. It was less reliable for use as a positive control. Only 69% of the ewes detected to be pregnant finally lambed. At day 45-50 PI, images of uterine fluid, placentomes and fetuses provide evidence of pregnancy with 90% accuracy , and at day 53-60 this accuracy even amounts to 100% . It is therefore apparent that day 45-50 is preferable to day 35 for making an accurate pregnancy diagnosis by ultrasonography. Ultrasonography can be used to screen a whole flock and is not time-consuming. It can be useful for separating ewes with singletons or multiple pregnancies. The main limiting factor for using this technique is the initial cost. Training and considerable experience are required to obtain good accuracy in terms of image interpretation in early pregnancy.

In our study, both the negative predicting value and the specificity of ultrasound for pregnancy control is higher than that of NRR, though not significantly higher. The sign of non-return to estrus due to pregnancy is not physically different from seasonal anoestrus. Therefore, neither the NRR following estrus induction and insemination at the end of the breeding season nor the NRR following estrus induction out of the breeding season is not reliable in sheep. Environmental factors also affect estrus exhibition, and it is difficult to differentiate between gestational anoestrus and anoestrus due to stress. Pregnancy detection using an intact ram is the most inexpensive method for pregnancy diagnosis: it is not time-consuming, and ewes returning into estrus can be mated by the ram.

The NRR and PR rates in our experiment were 10-25% higher than the lambing rates. This may be caused either by fetal loss and abortion, or by inaccurate ultrasound diagnoses at 35 ± 2 days PI. The earliest indications of pregnancy in the ewe are to be observed at 15 days PI using transrectal ultrasonography performed in dorsal recumbency. However, diagnostic accuracy is low until the 27th day of pregnancy. In our study, ewes were estimated to be pregnant if fluid was detected in the uterine lumen. This can contribute

to an over-estimation of the number of pregnant ewes, as ewes that had lost their embryo(s) before 35 days PI were also considered to be pregnant. Combined embryonic and fetal loss has been estimated to be 20% in ewes, with fetal death contributing less than 10% of this loss.

In our experiment, pregnancy control by transrectal ultrasound at 35d PI was at least as reliable as a teaser ram provided with a crayon, though it would be better if it were performed from 45d PI on to increase the accuracy.

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