

DEVELOPMENT OF transcervical AI IN SHEEP

PROJECT REPORT TO HCC

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Introduction

While conception rates following cervical insemination of synchronised ewes with fresh semen are generally around 60-70%, conception rates with frozen-thawed semen are unacceptably low (<20% generally). These poor conception rates following cervical insemination with frozen-thawed semen appear to stem from two inter-related factors which are additive in their negative impacts: (a) a reduction in the viability and motility of sperm directly attributable to the freezing process *per se*, and (b) reduced sperm transport through the cervix arising from the need to synchronise ewes for AI.

A major challenge for the UK sheep industry is to improve carcass quality of the slaughter generation so that far more of the lambs meet the target market quality specifications. This is especially important in Wales, where the predominant breeds are hill types with their inherently small carcass weights and poor carcass quality attributes. In an effort to overcome these limitations it is necessary for the industry to implement breeding schemes where the emphasis is directed towards the genetic improvement of carcass quality. The advantage of properly structured breeding schemes is that the gains achieved are cumulative and permanent.

The greatest rates of genetic improvement can be achieved by the introduction of Sire Reference Schemes. In essence, Sire Reference Schemes provide a means of genetically linking together a number of different flocks into a single larger population through the use of common reference sires (on a proportion of the ewes in each flock). Such a system provides the statistical basis on which across-flock assessments of genetic merit can be determined. This facilitates the identification of the best genotypes, allowing increased selection pressure to be applied especially through the male line. This, in turn, promotes faster rates of genetic gain compared to those achievable through within-flock selection. A number of such schemes are the mainstay of the breed improvement work within the Welsh Sheep Strategy.

The development of sire referencing has been made possible only by the introduction of intrauterine insemination by laparoscopy to bypass the problems of sperm transport through the cervix, especially when using frozen-thawed semen. This approach results in conception rates with frozen-thawed semen similar to those achieved with cervical insemination of fresh semen. Being able to use frozen-thawed semen is important for a number of reasons: (a) guaranteeing a supply of semen for fixed-time insemination, no matter the geographical location; (b) allowing the insemination of large numbers of sheep at a similar time across a range of flocks in different locations; (c) reducing the costs of semen supply by facilitating the collection of semen during 'off peak' times for use during the intensive AI season.

However, intrauterine insemination by laparoscopy adds substantially to the costs of AI, since it is a full veterinary procedure. This compromises widespread uptake of breed improvement when farmers are required to cover the costs themselves. Far better would be a trans-cervical approach that allows intrauterine insemination, but which could be carried out by trained technicians.

While a number of previous attempts to develop trans-cervical insemination have been reported, most notably from Australia and Canada, these have met with inherent technical difficulties and welfare concerns that have precluded their widespread application. However, one notable result from all of these studies has been that, in those animals where the cervix has been successfully penetrated and semen has been deposited into the uterine lumen, conception rates using frozen-thawed semen have been akin to those using the laparoscopic technique. The major challenge, therefore, is to overcome these technical difficulties and develop a welfare-friendly, trans-cervical approach.

Recent fundamental research on the physiology of the sheep cervix, conducted at the Royal Veterinary College, has provided an insight into ways in which the cervix can be relaxed sufficiently to predictably allow an insemination catheter to be passed through the cervix, and thereby allow semen to be deposited directly in the uterine lumen without recourse to the surgical intervention of laparoscopy.

The procedure developed is both repeatable and appears to cause minimal welfare problems to the animal. However, to date this has only been undertaken in a 'laboratory type' environment, and no attempt has yet been made to evaluate conception rates following the use of this new approach.

The current study was therefore undertaken as a preliminary investigation of the fertility aspects of this new trans-cervical technique, before attempting to develop/refine the procedure further, and before entering into a full fertility trial.

Materials and methods

Animals and treatments

The research was conducted using the flock of hill ewes at Tan-y-Graig Farm. This flock comprised draft ewes of the Welsh Mountain, Hardy Speckled Faced and Beulah breeds. The ewes were randomly allocated by breed and condition score to four artificial insemination groups, with approximately 80 ewes per treatment group:

- (i) Cervical insemination with fresh semen (single AI);
- (ii) Cervical insemination with frozen-thawed semen (double AI);
- (iii) Intrauterine insemination with frozen-thawed semen (single AI);
- (iv) Trans-cervical insemination with frozen-thawed semen (single AI).

Oestrus was synchronised with progestagen sponges left *in situ* for 13 days, with an injection of 250 i.u. PMSG given at sponge withdrawal. AI was conducted at approximately 54 h after sponge withdrawal for all groups, with the second insemination given approximately 58 h after sponge removal for ewes in Group (ii). Cervical ripening/relaxation for ewes in Group (iv) was achieved by hormonal treatment given 24h prior to AI (subject to patent protection). Bluefaced Leicester rams were introduced into the flock 7 days after AI to cover returns to service. Using rams of two different breeds, i.e. Inverdale Texel for AI and Bluefaced Leicester to cover returns to service allowed us to unequivocally determine whether the lambs were born as a result of AI or not.

Semen collection, processing and insemination

Semen was collected from the same two Inverdale Texel rams for all treatment groups.

For the frozen semen, the semen was collected, evaluated, processed and frozen in 0.25ml straws by Innovis according to their standard protocols. Essentially, only ejaculates with >80% progressive motility and $>2500 \times 10^6$ motile sperm/ml were used, and were diluted to achieve around 80 – 100×10^6 motile sperm per straw. In addition, all ejaculates were checked to ensure that they had a minimum of 40% motility after freezing and thawing before they were accepted for use. The straws from each ejaculate from the individual rams were pooled prior to use. For the intrauterine and trans-cervical insemination groups (Groups (iii) and (iv)) a single straw was used to inseminate each ewe, whereas for cervical insemination of frozen-thawed semen (Group (ii)) a single straw was used for each of the two inseminations.

A standard, commercially available stainless steel sheep insemination pipette, designed for use with semen in straws, was used for cervical and trans-cervical insemination of ewes with frozen-thawed semen. The straws were thawed immediately prior to use by placing them in a water bath at 37°C for around 10 sec. They were then dried, one end was cut off and the straw loaded into the AI pipette. Depending on the treatment group, the semen was then expelled from the pipette by depressing the plunger and deposited either into the posterior os of the cervix (Group (ii)) or directly into the body of the uterus just anterior to the cervix (Group (iv)).

For laparoscopic intrauterine insemination of frozen-thawed semen (Group (iii)), specially designed perspex pipettes with a 21G needle fitted in the end and coupled to a 1ml syringe with rubber tubing were used. Following thawing, the semen from an individual straw was expelled into a test tube suspended in the water bath and aspirated in equal volumes into two AI pipettes, with one being used to deposit semen into the lumen of each uterine horn.

The same two rams were also used to supply fresh semen for ewes in Group (i). Ejaculates (3 per ram) were collected, checked for progressive motility, pooled and diluted with UHT skimmed milk to achieve a final concentration of 200×10^6 motile sperm/0.25ml. A conventional perspex insemination

pipette, coupled to a 1ml syringe with rubber tubing, was used to facilitate deposition of 0.25 diluted semen into the posterior os of the cervix.

Data collection

All ewes were individually tagged to facilitate identification, both at the time of AI and at lambing. Details of cervical penetration for individual ewes in Group (iv) were noted at the time of insemination. Details of the date of lambing, number of lambs born and breed of lamb (inverdale Texel or Bluefaced Leicester sired) were recorded for individual ewes over the lambing period. Pregnancy rates to AI were determined by using the combined information on date of lambing relative to date of AI and the breed of lamb produced.

Results

The overall results from the different insemination groups are presented in Table 1. The cervical ripening procedure used for Group (iv) was highly effective, facilitating an overall cervical penetration rate of 87.5%.

Table 1. Conception rates to AI and mean litter size per ewe lambing for the four different methods of artificial insemination

Treatment group	Number ewes with complete records	Number sperm inseminated/ewe*	Pregnancy rate to AI	Litter size/ewe lambing to AI
Cervical fresh	82	200x10 ⁶	44/82 (53.7%)	1.5
Double cervical frozen	81	160x10 ⁶	2/81 (2.5%)	1.5
Intrauterine frozen	79	80x10 ⁶	51/79 (64.5%)	1.7
Trans-cervical frozen	82	80x10 ⁶	1/82 (1.2%)	1.0

* For frozen semen groups it is the original number of motile sperm in the straw(s) prior to freezing

While acceptable pregnancy rates to AI were achieved with both the cervical fresh and intrauterine frozen insemination groups, the pregnancy rates for both the cervical frozen and trans-cervical frozen groups were extremely poor.

Discussion

The high cervical penetration rate for the trans-cervical insemination group clearly indicates that the cervical relaxation procedure used was highly effective. Without this treatment it is almost impossible to pass an insemination pipette through the cervix of the ewe because of its very tortuous structure. It was notable that the length of the currently available insemination pipette was too short to easily traverse the cervix in a number of ewes. It is quite possible, therefore, that with a longer pipette the penetration rate could well be higher than that achieved in the current study.

Bearing in mind the relatively small group sizes involved, the pregnancy rates following cervical insemination of fresh semen and intrauterine insemination of frozen semen were similar, and very much in line with expectations. Both are routine procedures, and the relative number of motile sperm inseminated/ewe conformed to recommendations for each procedure. These two groups were included in the study as positive controls for the trans-cervical insemination treatment.

A poor conception rate following cervical insemination of frozen semen was also expected, and this group was included as a negative control for the trans-cervical insemination group. Past research has clearly demonstrated that conception rates, even with the double insemination procedure used, are variable but low. The rate achieved in this study is at the very low end of expectations.

Low conception rates following cervical insemination of frozen semen in synchronised ewes is attributable to the combined influences of (a) poor sperm transport through the cervix, and (b) lower

than optimum numbers of motile sperm in the inseminate. The optimum motile sperm number required to maximise pregnancy rates following cervical insemination is around 200×10^6 in synchronised ewes, though this is reduced to around 80×10^6 for ewes inseminated at a natural oestrus. This difference is a measure of the impact of the impairment of sperm transport through the cervix arising from the synchronisation treatment, and these optimum sperm numbers in a maximum volume of 0.25ml can be readily achieved when extending fresh semen with appropriate diluents. However, the dilution rates required with freezing diluents to optimise post-thaw motility mean that it is impossible to achieve these optimum motile sperm numbers for synchronised ewes, even with a double insemination. This problem is exemplified by the total number of sperm inseminated with Group (ii) ewes in the current study. Even though the pre-freezing motile sperm number inseminated was close to the optimum at 160×10^6 (achieved with the double insemination procedure), the poor post-thaw motility of around 40% reduced the total number of motile sperm inseminated to around 65×10^6 per ewe.

Transport of sperm through the reproductive tract of the ewe to the site of fertilisation, once within the uterine lumen, is largely passive, in direct contrast the very active sperm motility required to traverse the cervix. Since the intrauterine insemination technique bypasses the problems of poor sperm transport through the cervix and their exacerbation due to synchronisation, it is able to result in acceptable conception rates even with relatively low motile sperm numbers per ewe that are inevitable when using frozen-thawed semen. This is exemplified by the results of the present study when comparing the conception rates to AI for Groups (ii) and (iii). Even though the intrauterine insemination technique used only half the sperm numbers compared with cervical insemination of frozen-thawed semen, the pregnancy rates achieved were substantially higher.

The pregnancy rates achieved following trans-cervical insemination were especially disappointing, particularly given the very high cervical penetration rates achieved. It is important to recognise that ewes inseminated by the trans-cervical route received the same total number of motile sperm as those inseminated by the intrauterine route and from the same semen pool. In both cases the semen was also deposited within the uterine lumen. The poor conception rates achieved cannot therefore be attributed to a deficiency in motile sperm numbers in those ewes inseminated via the trans-cervical route. One possibility is that it was due to some change in the uterine environment induced by the cervical relaxation treatment that compromised either sperm transport within the uterus to the site of fertilisation or of the ability of the endometrium to promote implantation of the embryo. Alternatively, it is possible that the physical process of passing the insemination pipette through the cervix was in some way antagonistic to sperm transport, fertilisation or implantation. However, previous studies had clearly shown that there was no evidence of trauma to the cervix when using the same cervical relaxation and trans-cervical insemination techniques. Whatever the reason, it is clear that the effects were transient because the vast majority of ewes returning to service conceived and carried their pregnancy to term. Further basic studies are therefore required to better understand the poor pregnancy rates achieved so that refinements can be made which might result in high pregnancy rates following the trans-cervical insemination procedure.