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INTRAUTERINE INSEMINATION, TRANSCERVICAL INSEMINATION AND EMBRYO TRANSFER FOR PRACTICAL BREED IMPROVEMENT IN SMALL RUMINANTS

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Abstract

The development of laparoscopic intrauterine insemination for sheep has been essential for the implementation of national Sire Reference Schemes in the UK. This technology has developed contemporaneously with ultrasonic scanning techniques for *in vivo* assessment of carcass characteristics in sheep, and with the application of BLUP (Best Linear Unbiased Prediction) techniques for the accurate estimation of genetic merit. Together these techniques offer the real possibility of accelerating the rate of improvement in genetic merit for economically important characteristics in the terminal sire breeds of sheep. However, laparoscopy is an invasive procedure and future progress relies on the development of alternative, non-invasive insemination procedures. Embryo transfer has the potential to accelerate response to selection by increasing selection intensities and reducing generation intervals in females. The response may be enhanced further by embryo splitting. However, in sheep, results remain unpredictable due to the great variation in superovulatory responses of donor animals; responses in goats are less variable. This variation in response has an effect on genetic gain and especially inbreeding.

ARTIFICIAL INSEMINATION IN SMALL RUMINANTS

Use in Genetic Improvement

The breeding technology of Artificial Insemination (AI), combined with the objective assessment of genetic merit on a national basis using Best Linear Unbiased Prediction (BLUP) techniques, has transformed the efficiency of dairy cattle breeding in many countries and is also beginning to have some effect in beef breeding schemes (Simm, 1993). A similar transformation of the sheep industry has also started in the UK through the adoption of Sire

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Reference Schemes by several of the terminal sire sheep breed societies (Lewis, Simm, 1995). Until very recently pedigree sheep breeders in the UK had taken a very traditional attitude to genetic improvement programmes, basing many of their decision-making processes on their visual assessment of an animal's conformation, and on fashionable breed characteristics. However during the past decade objective measurements of carcass characteristics have been made possible through the development of ultrasonic scanning of carcass characteristics have been made possible through the development of ultrasonic scanning for assessment of muscle and fat depths to predict body composition. Currently about 350 pedigree flocks in the UK utilise these techniques through the Signet Sheepbreeder Service which provides breeders with within-flock comparisons of genetic merit for carcass composition.

However in order to obtain across-flock comparisons of genetic merit it has been necessary to develop co-operative breeding schemes where various flocks in a programme can be linked by common sires through the use of AI-Sire Reference Schemes. The reference sires leave lambs in many flocks which are related to lambs born at around the same time in the same and other flocks.

Related animals have genes in common - the higher the relationship, the greater the proportion of genes in common, and the greater the resemblance between relatives in performance. When related animals occur in different management of contemporary groups, it is possible to simultaneously estimate breeding values and management effects using BLUP. The most sophisticated version of BLUP-so-called "animal model BLUP", uses information from all categories of relatives to make the best possible separation of genetics and management, and hence gives the most accurate Estimating Breeding Values (EBV's). When animals occur in different flocks in different years, EBV's can be estimated across flocks and years. This allows selection of animals across flocks and years and allows estimation of genetic trends across time.

The existing widespread use of AI in dairy cattle meant that BLUP techniques could be applied without any structural changes in the dairy industry. However for sheep the creation of links across flocks was a necessary prerequisite for across-flock genetic evaluations. There are now approximately 150 flocks comprising nine different breeds involved in Sire Reference Schemes in the UK. The three largest of these are the Suffolk, Texel and Charollais breeds (Guy, Croston, 1994), whose progeny account for 69% of the lambs slaughteres in the UK.

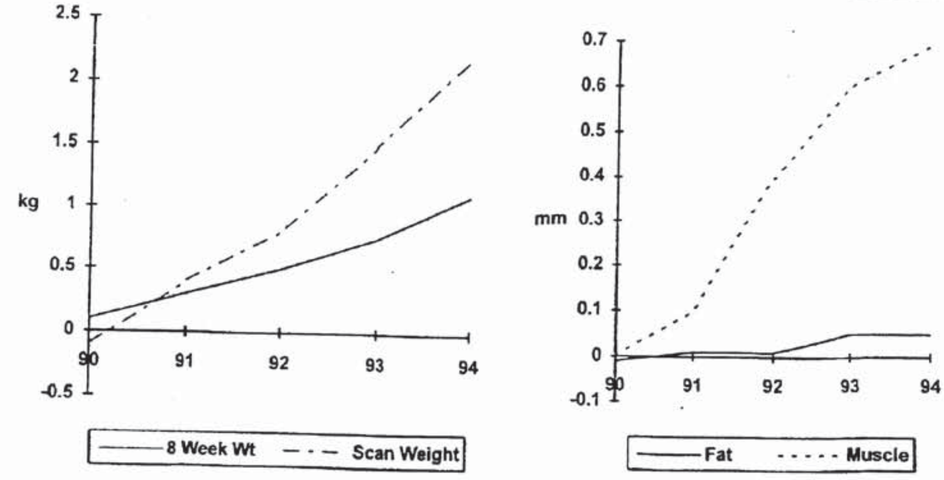
In order to develop effective Sire Reference Schemes on a national scale it has helped to utilise artificial insemination. The broad geographical distribution of members' farms throughout the UK means that the use of frozen semen in desirable. However fertility following artificial insemination with frozen-thawed semen in limited by the ability of spermatozoa to transit the cervix (Eppleston, 1992). Despite maintaining the ability to fertilise, most frozen-thawed spermatozoa are unable to colonise or traverse the length of the cervix and are rapidly lost from the female reproductive tract. Thus insemination of frozen-thawed semen into the cervix results in poor conception rates which are not commercially acceptable. The development of Sire Reference Schemes has therefore necessarily relied on the use of direct intrauterine insemination carried out by laparoscopy. This technique allows the operator to bypass the cervical barrier and to restore fertility to normal levels (Maxwell and Hewitt, 1986; McKelvey, 1994).

During the first five years of the Suffolk Sire Reference Scheme (the largest of these co-operative breeding schemes in the UK) conception rates have risen steadily from 53% to 71%. This progressive improvement has been largely due to greater attention to detail by farmers in programming their sheep and to increasing quality control in semen freezing techniques.

Basic records are collected for all progeny in the scheme which includes lamb birth weight, 8 week weight, 20 week weight and the depth of back fat and muscle measured by ultrasonic scanning at 20 weeks of age. From this data, and the relevant pedigree records, the EBV's for live weight, muscle depth and fat depth for every animal in the scheme can be derived. These are then used to calculate an index score for lean meat production which uses a relative weighting of +3 for lean kg and a weighting of -1 for fat kg (Simm, Dingwall, 1989). Ram selection for the following year is based on this lean index together with visual assessment of conformation and breed points by the breeders themselves.

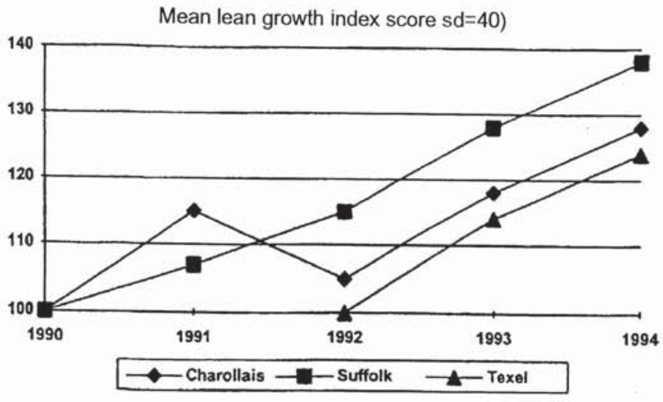
After several years of operation each scheme has been able to demonstrate a regular and significant yearly increase in the lean index and associated traits. The progress to date for scan weight and muscle depth for the Suffolk scheme is depicted in Figure 1, whilst Figure 2 depicts the progress in lean growth index score for the Suffolk, Texel and Charollais schemes to date.

As the number of co-operating members increases so the selection intensity for the desirable traits can be increased. It is expected that the number of members of the terminal Sire Reference Schemes will increase three fold over the next three years. This will in turn lead to a significant increase in the market share of rams sold from Sire Reference Scheme members' farms and thereby have a very real influence on the carcass characteristics of a significant proportion (10% in 1997 and 14,5% in 1998) of the lambs produced nationally each year.



Source: MLC and Sufolk Sire Reference Scheme Ltd.

Figure 1 - GENETIC TRENDS IN SUFFOLK SIRE REFERENCE SCHEME (1990-1994)



Source: MLC, Signet, Sufolk Sire Reference Scheme Ltd, Charollais Sire Referencing Ltd, Elite Texel Ltd.

Figure 2 - GENETIC TRENDS IN THREE LARGEST SIRE REFERENCE SCHEMES IN BRITAIN

Practicalities of AI in Sheep

As described above the development of Sire Reference Schemes has necessarily relied on the use of direct intrauterine insemination, carried out by laparoscopy, in order to achieve commercially acceptable pregnancy rates. However laparoscopic AI is an invasive procedure which, under UK legislation, may only be carried out by a veterinarys surgeon. It is therefore relatively expensive and requires to be serviced by highly trained professionals. Furthermore, despite the fact that recent experimental evidence demonstrates that penetration of the body wall by a laparoscope, under local anaesthesia, appears to impose little further stress on the animal over and above that attributable to restraint alone (Haresign et al, 1995), the public perception of the procedure has led to its use being questioned on welfare grounds. Specific guidelines have recently been proposed to restrict circumstances under which laparoscopis AI may be employed for insemination of sheep in the UK (Banner Committee Report, 1995).

The UK sheep breeding industry therefore faces a dilemma. It has, at its fingertips, the technology for objective assessment of carcass characteristics and for the collection and analysis of genetic data on a national basis. It now also has the goodwill and co-operaton of many breeders within the pedigree industry. What is not availably is a technique which is fully acceptable for the further development of a widespread AI service for frozen semen. The development of that service is crucial to the successful expansion of any future co-operative breed improvement programmes. There is therefore a need to develop alternative procedures by which viable frozen-thawed spermatozoa can be delivered to the sites of fertilisation without the need for laparoscopy.

There have been several reported attempts to traverse the ovine cervix with the aim of performing non-surgical intrauterine insemination (Salamon, Lightfoot, 1970; Fukui, Roberts, 1976; Olaffsen, 1979). These methods generally involved grasping the external cervical os, and sometimes stretching the cervix, whilst manipulating a pipette past the folds of the cervix. Whilst the fertility of ewes was high whenever the insemination catheter could be successfully passed through the cervix into the body of the uterus, the techniques are time consuming and by no means all cerivces could be penetrated. There was often no overall increase in fertility and in some ewes there was physical damage to the cervix (Fukui, Roberts, 1976). However, if an effective method for penetration of the cervix could be developed, fertility equivalent to that obtained after laparoscopy could be achieved without any increase in the dose of spermatozoa required; this has been demonstrated by Eppleston (1992) who showed that, irrespective of the sperm dose, insemination of frozen-thawed semen into the body of the uterus resulted in the same level of fertility as laparoscopic insemination directly into the horns of the uterus.

Considerable impetus was given to the search for a successful transcervical AI technique for sheep following publication of reports from the University of Guelph by Halbert et al, (1990 a and b) and Buckrell et al (1992). High rates of both intrauterine penetration via the cervix, and fertility following transcervical deposition of frozen-thawed semen, were reported. These reports led us to initiate a series of trials at our own unit in Edinburgh to investigate whether this technique resulted in similar pregnancy rates in UK breeds of sheep.

Our first experiment was carried out in the Autumn of 1992 using four hundred commercial ewes which were synchronised into oestrus and inseminated using a common pool of frozen-thawed Texel semen (Mylne et al, 1993). Despite using twice as many spermatozoa (80 x 10^6 motile spermatozoa) for the transcervical technique as for the laparoscopic procedure the pregnancy rate was only 18%. This was significantly less than that which was achieved using laparoscopic artificial insemination (57%) and was not in a range which would be acceptable to commercial breeders. (Table 1). These results are very similar to those which have been reported by Windsor et al (1994) for Merino ewes in Australia.

Table 1. - LAMBING RESULTS FOLLOWING INSEMINATION USING EITHER A LAPAROSCOPIC OR THE GUELPH TRANSCERVICAL (GST-AI) TECHNIQUE

Method	No. Inseminated	No. which aborted or died	No. inseminated (adjusted)	No. lambing to AI	% lambing to AI
Laparoscopis					
Greyface	101	12	89	55	62%
Blackface	99	15	84	44	52%
GST-AI					
Greyface	100	12	88	17	19%
Blackface	100	6	94	15	16%

(Source: McKelvey, 1994)

Of even greater concern than the low pregnancy rates was our finding that a number of ewes which were subjected to transcervical insemination suffered pathological lesions of the pelvic organs as a result of inadvertent penetration of the mucosa of the reproductive tract by the insemination pipette. From the two hundred ewes which underwent transcervical AI, forty-three barren ewes were identified and subsequently slaughtered. Of these forty-three, six ewes had pathological lesions involving the uterus and cervix. These varied from discrete peri-cervical abscessation (2 cases), severe peritonits with tracking to a cervical penetration lesion (1 case) to chronic metritis and pyometra (3 cases). Additionally, subsequent to transcervical AI, one ewe died from a severe peritonitis in which lesions were found tracking cranially from the cervix.

Subsequent trials using the Guelph transcervical technique have failed, in our hands, to produce pregnancy rates greater than 20% and, indeed, subsequent trials using dye markers rather than semen, with subsequent slaughter of ewes following AI, have also demonstrated that there is a significant danger of causing damage to the cervical cannal and peri-cervical organs using this technique. We have therefore concluded that, at the current stage of development, this technique not only results in unacceptably low pregnancy rates but is also potentially traumatic to the reproductive tract and therefore of detriment to animal welfare.

However trial work will continue in our laboratory with renewed emphasis being given to further studies on the physiology of the ovine cervix with a view to developing pharmacological regimes which will result in softening and dilatation of the cervical canal, thereby permitting the atraumatic passage of an insemination device through the cervical conal into the body of the cervix. These investigations will be based on the observation that, during naturally-occuring oestrus, there is some degree of cervical dilation as a result of peri-ovulatory endocrine changes. To understand the mechanisms controlling this dilation, and it further stimulation, studies will be made of the temporal expression of the cervical hormone receptors (oxytocin, relaxin, oestradiol, progesterone, PGF2α, PGE2, interleukin 1b and interleukin 8) that are likely, on the basis of studies on a wide range of species, to be involved in the softening of the cervical collagen fibres.

MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET) IN SMALL RUMINANTS

Use in Genetic Improvement

It is now over one hundred years since the first report of successful transfer of embryos from one female reproductive tract to another (Heape, 1891). However much of the embryo-related technology for farm animals has only been developed within the past 20 years (see review by Betteridge, 1981), and during the past decade the emphasis in both research and commerce has shifted from "conventional" embryo transfer (where fertilised ova are collected *in vivo* and transferred intact to recipient females), to manipulation of the embryo and of gametes themselves. Many of the embryo manipulative procedures will be reviewed in the following paper by Dr Wilmut. The purpose of this section of the present paper is to review the practical and commercial uses of MOET for genetic improvement programmes in small ruminants, as they exist at the present time.

The rate of genetic progress in an improvement programme depends on the accuracy of selection (which itself depends on the heritability of the trait under selection), the amount of genetic variation in the trait, the intensity of selection and the generation interval. Whilst the heritability and genetic variation are controlled by the inherent biology of the species, and are therefore largely outwith the control of the breeder, the selection intensity and generation interval can be manipulated for the female of the species, within biological limits, by using MOET.

Whilst embryo transfer technologies have found their greatest application in cattle there is little doubt that their greater use in small ruminants could significantly contribute to improved efficiency of production. MOET may be used to accelerate genetic improvement in existing breeds within a country or to introduce new livestock species, breeds or strains into those countries which need to diversify their traditional agricultural production systems. For example in Scotland there may be a role for substituting some traditional livestock with goats in order to produce cashmere for the textile industry (Russel, Bishop, 1990):

Land and Hill (1975) first drew attention to the potential benefits of using MOET in a 'structured' way in breed improvement. They assumed a closed nucleus herd of beef cattle, selected for increased growth of superior females, and reported expected rates of genetic change about double those possible in a conventional breeding scheme. Later, Smith (1986) reported similar results for the use of MOET in sheep selected for growth or carcass traits measured prior to reproductive age and a slightly lower advantage of around 60% for selection on wool characteristics measured at a later age. In both cases relatively high yields of embryos at a young age were assumed. As a result of this, and a number of other assumptions, these early studies are now believed to have overestimated responses to selection and underestimated rates of inbreeding. A recent re-evaluation of the use of MOET in beef cattle has shown that, at the same rate of inbreeding, increases of about 30% in genetic gain can be achieved in schemes using MOET compared to those using natural reproduction (Villaneuva, Simm and Woolliams, 1995). Considerable progress has recently been made in improving both the response to superovulation and the survival of embryos in UK breeds of sheep (Dingwall, et al, 1993; Haresign et al, 1994 a and b); furthermore embryo recovery techniques have been considerably improved thereby increasing the ability of operators to repeatedly recover embryos from the same animal (Mylne et al, 1992). Therefore similar additional genetic gains to those possible in cattle could probably be achieved in sheep MOET schemes which are designed to improve growth and carcass quality.

For goats, consideration of the theoretical benefits of MOET have, to date, centred largely around the improvement of fibre characteristics in Angora and Cashmere producing animals. Calculations indicate that in a conventional breeding programme using two hundred cashmere does, optimal improvements in fibre characteristics can be achieved when there are one hundred does in each of the age categories of two and three years, and these are mated to the top ten buck kids in any one year. However, using MOET, and assuming a yield of eight embryos per donor from a single flush, the two hundred doe herd can be replaced by 40 donors, who also carry their own kids, and 160 recipients. Assuming that the best ten buck kids are used in each year the rate of improvement of cashmere quality can be shown to be 27% greater than that which would be achieved using a conventional programme (Bishop and Wray, 1991). Similar calculations for improving mohair characteristics indicate an increase of 55% in the rate of genetic gain through the use of MOET over natural breeding methods (Wichkam, 1987).

Nucleus MOET schemes would also be of considerable value in genetic improvement programmes for dairy goats, especially in developing countries where progeny testing schemes might not be feasible. By centralising breeding activities into one nucleus MOET herd, and by using sibling evaluation of milk production, an entire national breed improvement programme could be undertaken on one site, thereby concentrating human and field resources and allowing the dissemination of improved animals through the distribution of bucks or semen to co-operating farmers (McGuirk, 1989).

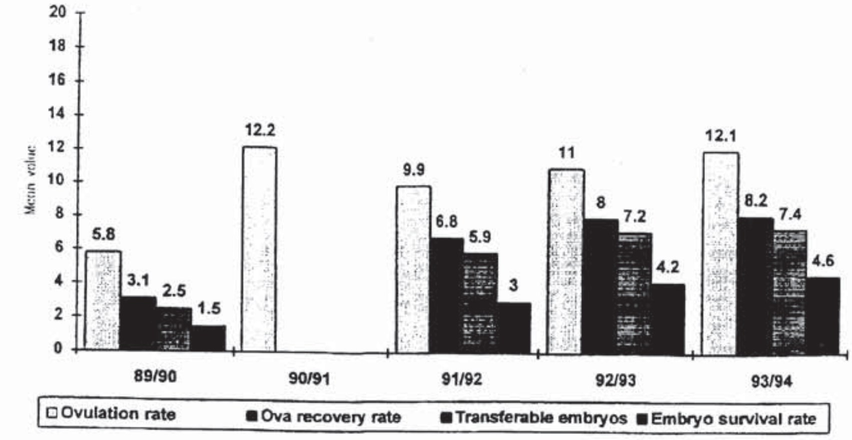
Practically however, for goats, MOET has primarily been used up until now for the international movement of germplasm because embryos are now universally regarded by national veterinary regulatory authorities as being the safest medium for the import of genetic material into any particular country (Singh, 1988).

PRACTICALITIES OF MOST IN SMALL RUMINANTS

Sheep

As has been mentioned above, the original studies on MOET suggested that in order to significantly increase the rate of genetic improvement of sheep, where carcass traits are the subject of selection, some ten progeny require to be produced from each donor ewe per year. With this target in mind we commenced, in 1989 in Edinburgh, to develop a MOET protocol for Suffolk sheep. Minimally invasive techniques for the recovery and transfer of embryos by laparoscopy had been available for some years (McKelvey et al, 1985 and 1986), so that the main impediment to achieving success was the low and variable superovulatory responses of donor ewes. The superovulatory regime used at that time, based on the use of porcine FSH, gave a mean response of 5.8 ovulations per donor ewe programmed with only 2.3 transferable embryos being produced. This resulted in a mean number of 1.3 lambs per donor ewe programmed (see Fig. 3) with only 11.4% of donors being represented by at least four progeny (Dingwall et al, 1993). Clearly these results were unacceptable if genetic progress were to be significantly accelerated.

Figures expresed on a per-ewe basis



Source: Dingwall et al, 1993.

Figure 3. SAC SUFOLK SELECTION EXPERIMENT - MOET RESULTS FOR 1989/90, 91/92, 92/93 AND 94/95 AND OVULATION DATA FOR 1990/91

In 1990, Ovagen (ICP Hamilton, New Zealand), a purified ovine FSH, became available in the UK. The advent of this product resulted in an increase in the mean superovulatory response of Suffolk ewes to 9.9 ovulations per donor programmed (Dingwall et al, 1993). This response was further ameliorated by the incorporation of a single dose of PMSG (400 units) on the first day of the superovulatory regime as originally suggested by Ryan et al (1984). The mean number of embryos recovered increased to 8 of which 7.2 were considered to be of transferable quality and this resulted in a mean number of progeny of 4.6 lambs per donor ewe programmed.

Although, in Suffolks, this is less than half way to the target of ten progeny per donor ewe suggested by Smith (1986), it may well be sufficient to significantly increase response (eg. see Villanueva et al, 1995). It may be that repeated flushing of donor ewes or splitting of embryos could help accelerate responses further (Matthews et al, 1994).

The responses to superovulation are generally more consistent for hill than for lowground breeds of sheep (see Haresign et al 1993, 1994 a and b). However the mean numbers of transferable embryos produced by these two classes of sheep are not significantly different. It appears, indeed, that the influence of breed, body condition and nutritional status on the number of embryos produced per ewe are each very limited (Fernie et al, 1993).

The commercial activity involved in sheep embryo transfer in the UK is much less than that for cattle. However at our own unit in Edinburgh, during 1994/95, we recovered 2248 embryos from 320 commercial sheep of which

1721 were viable. Of these 1288 were transferred fresh and 433 were frozen. Mean pregnancy rates for fresh transferred embryos are in the order of 65% and for frozen embryos the mean pregnancy rate is in the order of 50-55%.

Goats

Commercial embryo transfer in goats has been largely confined to several major programmes designed for the international movement of embryos; the level of activity has largely been dictated by changing requirements for new genetic resources. Examples are the movement of cashmere embryos from the UK to the Czech Republic (Riha et al, 1994) and the movement of Boer Goat embryos from South Africa to Canada in the past year.

Responses of goats to FSH are much more predictable and are generally better than those recorded for sheep; responses of dairy goats generally tend to be better than those of fibre producing or meat producing animals (Baril et al, 1989; Tervit et al, 1985; McKelvey 1992).

Embryo Bisection

In order to increase the response rate for the genetic improvement which can be achieved in a MOET programme it may be desirable to split embryos in order to increase the number of progeny which can be produced from a particular donor. Large scale commercial splitting programmes have been undertaken for goats (Udy, 1987) and for sheep (Vivanco et al, 1991). These have demonstrated that the ratio of offspring to embryos collected can exceed 100%. Our own experience indicates that, in sheep, the stage of embryo chosen for bisection is critical, with optimum results being achieved when early blastocysts are available for manipulation (Matthews et al, 1994).

Conclusions

This paper has shown that artificial breeding technologies such as AI and MOET have significant theoretical and practical benefits for small ruminant breed improvement programmes. They have already been successfully applied to economically significant schemes within the UK and overseas. Assuming that current research will yield more "welfare-friendly" techniques for AI and MOET such schemes will develop and expand over the coming years and will play an ever increasing role in maintaining and improving the competitiveness and efficiency of European small ruminant production systems.

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INTRAUTERINA INSEMINACIJA, TRANSCERVIKALNA INSEMINACIJA I PRENOŠENJE ZAMETKA U SVRHU IZVEDIVOG POBOLJŠANJA PASMINE MALIH PREŽIVAČA

Sažetak

Razvoj laparoskopske intrauterine inseminacija ovaca od najveće je važnosti za provođenje nacionalnih Programa oplodnje u Velikoj Britaniji. Ovaj se postupak razvio istovremeno s postupcima skeniranja ultrazvukom u svrhu *in vivo* procjene karakteristika trupa ovaca, a primjenom postupka BLUP-a (Best Linear Unbiased Prediction) u svrhu točne procjene genetskih odlika. Ovi postupci zajedno pružaju stvarnu mogućnost ubrzanja stope poboljšanja genetskih odlika za ekonomski važne karakteristike krajnjih pasmina za oplodnju ovaca. Međutim laparoskopija je invazivan postupak, pa se budući napredak oslanja na razvitak alternativnih, neinvazivnih postupaka inseminacije. Prenošenje zametka sadrži mogućnost brže reakcije na izbor (selekciju) i to većim intenzitetom izbora te smanjenjem generacijskog razmaka u ženka. Reakcija se može nadalje pojačati cijepanjem zametka. U ovaca se, međutim, rezultati ne mogu predvidjeti zbog velikog variranja superovularnih reagiranja životinja davalaca; reakcije u koza su manje promjenljive. Ovo variranje reakcija utječe na genetski probitak i to osobito u uzgoju u srodstvu.

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