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Current Topics in Artificial Insemination of Sheep

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Abstract

There have been developments in several aspects of artificial insemination (AI) in recent years, some of which have been directly responsible for proliferation of AI in the sheep-breeding industries of several countries. The most notable advances have probably been associated with the development of intrauterine insemination by laparoscopy. There is potential for refinement of some of the related techniques, particularly in the area of control of ovulation and definition of appropriate times and optimum doses of spermatozoa for insemination. It is unlikely that laparoscopic AI will be developed sufficiently that it will become readily affordable, and therefore widely practised, by commercial producers.

Unfortunately, there has been little progress in the past few years in improvement of the methods of cryopreservation of ram semen. There is considerable potential for AI to have a significant impact on the genetic improvement of sheep, though this has yet to be evaluated in practice. However, if the full potential of AI in sheep is to be realized, it will likely only happen when methods of freezing semen are improved sufficiently that cervical or even vaginal insemination can be widely used with frozen-thawed semen, or when practicable methods of deep cervical or intrauterine insemination through the cervix are developed.

Introduction

Artificial insemination (AI) of sheep is by no means a new technique. It has been practised for over 50 years, originally in the Soviet Union, and has been in routine, if sometimes limited, commercial use in other Eastern European countries, and in France, Ireland, Australia, New Zealand and many other countries for several decades. Until recently, however, inseminations were performed with fresh diluted semen, since the efficacy of using semen frozen, thawed and inseminated by the best available technology was low. The development of a method of intrauterine insemination with the aid of a laparoscope has now rendered the use of frozen semen in sheep a viable proposition. In the 1986–87 breeding season in Australia, more than 83 000 ewes were inseminated with frozen-thawed semen (Maxwell 1987); the number was negligible five years ago.

Intrauterine insemination by laparoscopy is probably one of the most important developments in AI of sheep in recent years. However, there have been several other developments. It is not the object of this article to analyse the development of semen preservation, as this has been reviewed by others (Salamon and Visser 1974*a*; Graham *et al.* 1978; Watson 1979; Maxwell 1984), but to summarize only the recent developments in AI technology and related topics and identify some deficiencies in knowledge of these subjects.

Preservation of Semen

The 1980's have seen little improvement in the methods of preservation of ram semen for AI. Semen is preserved for use in the short-term (chilled or liquid storage) or long-term (frozen storage) in much the same way as it has been for over a decade.

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obtained with cervical insemination (Salamon and Robinson 1962; Maxwell 1984). When intrauterine insemination was used, in this case by mid-ventral laparotomy, fertilizing capacity of liquid-stored semen was maintained for up to 10 days, though fertility declined after 6 days of storage (Salamon *et al.* 1979). There have been no trials reported on the use of liquid-stored semen inseminated into the uterus with the aid of a laparoscope, but obviously this insemination technique offers the potential to use semen which has been liquid-stored for longer periods than was hitherto possible.

Frozen Storage

There is potential for improvement in the techniques of cryopreservation of ram semen, as there is for semen of other species. Although good results have been obtained on occasions with cervical insemination of frozen-thawed semen (Salamon 1971; Visser and Salamon 1974; Colas 1975; Maxwell *et al.* 1980), these have been obtained only after stringent preselection of the semen and insemination of relatively high doses of spermatozoa. Conception rates of 10–40% are much more common and are unacceptable in commercial practice. The problem is associated with impaired transport of frozen-thawed spermatozoa through the cervix of the ewe (Mattner *et al.* 1969; Lightfoot and Salamon 1970*a*, 1970*b*), and possibly also with reduced retention in the female reproductive tract (Hawk 1983). The success of the laparoscopic method of intrauterine insemination of frozen-thawed semen has diverted attention from the deficiencies in the techniques of cryopreservation of ram semen. Laparoscopic insemination, though technically feasible, is not an economic proposition for many sheep breeders. Frozen semen would gain much more widespread use if acceptable fertility could be obtained with cervical or vaginal insemination; unfortunately, despite considerable research effort, the techniques for freezing ram semen have not yet been sufficiently refined to meet this requirement. Additionally, no practical technique of inseminating frozen-thawed semen into the uterus via the cervix has been developed.

There are, however, some constraints to the techniques of freezing ram semen which do not apply to other species, for example the bull. These are related to the insemination technique; in the cow, intrauterine insemination is a relatively simple procedure due to the open structure of the cow's cervix at oestrus. This permits insemination with relatively low numbers of frozen-thawed spermatozoa, and the bull's ejaculate can be diluted extensively before freezing. This ensures that the spermatozoa are preserved in a synthetic medium which is specifically designed with cryoprotective properties, with little contribution from the seminal plasma which is not a cryoprotective medium. The ram's ejaculate may not be extensively diluted if sufficient spermatozoa are to be contained in the small volume required for cervical insemination, unless the spermatozoa are to be reconcentrated upon thawing, an often cumbersome and damaging procedure. Laparoscopic insemination may now permit greater prefreezing dilution of semen, but if the long-term aim of successful cervical or vaginal insemination with frozen-thawed semen is to be achieved, it is likely that it will require a method of croypreservation with low prefreezing dilution rates.

There has been little development of the methods of freezing ram semen since that of the Tris-egg yolk-glucose pellet method (Salamon and Visser 1972; Visser and Salamon 1973). However, semen has also been successfully frozen in straws (Colas 1975). Both methods are described in detail by Evans and Maxwell (1987). Although there have been few direct comparisons of motility and fertility of semen frozen in pellet and straw form, the pellet method has hitherto been the most commonly used. Maxwell *et al.* (1980) reported that fertility tended to be higher when semen was frozen in pellets compared with straws. Hunton (1987) reported a consistent, but insignificant, advantage in terms of post-thaw motility for semen frozen in straws, but there was no difference in fertility of ewes following intrauterine insemination. The view held by most Australian workers appears to be that pellet freezing is superior to straw freezing, largely because of its simplicity and reliability. It is likely that variable results obtained with straw freezing can be attributed in part to variability in freezing rates due to inability to maintain a fixed cooling temperature in nitrogen vapour; otherwise, there is no

apparent reason why the two methods should differ. It is possible that attention will be turned more to the use of straws in future since it is becoming more common to sell semen from breeding centres. Consumers may demand the ready identification offered by straws, although, when pellet-frozen semen is properly handled and packaged, identification is not a problem. Nevertheless, it is possible that such a stimulus will lead to development of more reliable methods of freezing semen in straws.

There has not been any recent improvement in fertility of frozen semen attributable to the development of diluents. Most recent effort in this area seems to have come from the Soviet Union and Eastern Europe. There are several diluents recorded in the Soviet literature, but those most commonly used nowadays are saccharose- (sucrose-) based (Varnavskii and Varnavskaya 1978; Milovanov *et al.* 1985) and lactose-based media (Platov *et al.* 1983). The former diluent is combined with an antioxidant such as butylhydroxytoluene, 2,6-di-t-butyl-1,4-cresol, echinochrome A (sea urchin extract), or vitamin E (Milovanov and Sokolovskaya 1980; Grigoryan and Nazaryan 1981; Milovanov *et al.* 1981) with the intention of minimizing the oxidation of phospholipids and unsaturated fatty acids in the cell membrane.

At this stage, since intrauterine insemination with frozen semen is successful, we can conclude that cryopreservation techniques have been sufficiently refined to preserve the fertilizing ability of the spermatozoon. However, motility and viability of the spermatozoon are not preserved sufficiently to ensure passage through the entire female reproductive tract. It appears that research effort should be directed at finding new cryoprotective diluents, but directing attention particularly to the preservation of motility and viability of the spermatozoa.

Use of Antibiotics

Although semen of other species, particularly cattle, routinely contain antibiotics, use of these substances has been limited in preserved ram semen. Since intrauterine insemination is becoming more common, the question of sterility of semen has become more relevant. Although there is no documented evidence that intrauterine insemination causes infection of the uterus resulting in infertility, intrauterine infections, which could be attributable to contaminated semen, have occasionally been observed on flushing embryos 5–6 days after intrauterine insemination (G. Evans and N. W. Moore, unpublished observations.)

Several bacteriostats and bacteriocides have proven effective in controlling bacterial growth in bull semen *in vitro* (Zaugg and Almquist 1973; Almquist and Zaugg 1974; Berndtson and Foote 1976). Various concentrations of sodium penicillin and streptomycin sulfate have been tested for toxic effects on semen pellets frozen and thawed as described by Evans and Maxwell (1987); after incubation of thawed semen for 4 h at 37°C, there was no significant reduction in motility of spermatozoa in semen containing up to 1000 IU/ml of penicillin or 1 mg/ml of streptomycin in comparison with control semen (G. Evans, unpublished data). The effects of antibiotics have not been properly tested in fertility trials.

Use of Egg Yolk

Egg yolk has been used commonly in diluents for preserving semen of many species. Ram semen diluents are no exception. Egg yolk affords protection against cold shock during the cooling process, and concentrations of up to 12% (after dilution) have a beneficial effect on post-thaw motility of spermatozoa (Salamon and Visser 1972; Watson and Martin 1975). However, in diluents for freezing both ram semen and bull semen there is a suggestion that fertility may be impaired with egg yolk concentrations as low as 5–6% (Watson 1979). With the more widespread use of laparoscopic intrauterine insemination, it is perhaps of greater concern that foreign protein is injected through the uterine wall into the lumen of the uterus, potentially causing an immune reaction in the recipient. Evidence in the literature that cows may produce an immunological response to insemination with egg yolk-containing diluents is equivocal (see Watson 1979). The potential for this to occur in sheep is somewhat greater since the laparoscopic insemination technique not only results in desposition of diluted semen into the uterine lumen, but does so via a small puncture thereby allowing direct contact of

blood and diluent. There are no data to suggest that intrauterine deposition of semen results in permanent infertility, but this is a possibility and there is a need for properly controlled experiments in this area. Since semen of rams (Watson and Martin 1975) and goat bucks (Ritar and Salamon 1982) can be successfully frozen in diluents containing relatively low concentrations of egg yolk, there may be some benefit in using such diluents for freezing semen for use by intrauterine insemination. The use of antioxidants in the freezing diluent has also been reported to allow a reduction in the concentration of egg yolk (Milovanov and Sokolovskaya 1980).

Semen Assessment

There is obviously a need for further improvement of semen freezing methods, but progress in this area is always hampered by the lack of reliable laboratory methods of predicting the fertility of semen in the field. The only conclusive test of fertility is an insemination trial, which is both time-consuming and expensive. There has been little progress in development of laboratory diagnostic tests in recent years (Watson 1979; Salamon 1987). Standard visual motility assessment does not necessarily correlate with fertility (Eppleston *et al.* 1986). The technique of *in vitro* fertilization of ova harvested from heterologous laboratory species, particularly hamsters, has been adopted for use in human fertility studies (Yanagimachi *et al.* 1976; Yanagimachi 1984). The test has been used for laboratory evaluation of ram spermatozoa (Pavlok and Flechon 1985), though no attempt was made to relate the results to fertility of the semen *in vitro*. The authors concluded that the test was unlikely to be a useful diagnostic technique.

It is likely that development of a simple and reliable standard *in vitro* test for semen quality would be widely adopted in the industry, and would be particularly useful for standardizing semen for sale through breeding centres, but at present the prospects of this happening appear remote.

Insemination

The most widely used insemination technique for sheep is deposition of semen into the cervix, usually with the aid of a speculum using the 'over the rail' technique (Evans and Maxwell 1987). The cervix acts not only as a reservoir but as a barrier for spermatozoa and, in general, the deeper into the cervix is the deposition of semen, the higher the conception rate (Salamon 1976; Milovanov and Sokolovskaya 1980). The barrier is particularly effective for frozen-thawed spermatozoa which apparently have reduced viability in the female reproductive tract (Hawk 1983). Best results have been obtained with intrauterine insemination, either via the cervix or by direct injection into the uterine lumen by laparotomy or laparoscopy. Recently, the technique of vaginal insemination has attracted interest for use with fresh semen. The various methods differ in their complexity, cost, and effectiveness.

Cervical Insemination

Cervical insemination of fresh semen probably provides the most economical effective use of semen, but is not generally effective enough for use with frozen-thawed semen. The cervix of the ewe cannot be readily penetrated by conventional inseminating pipettes, though there have been attempts to inseminate ewes into the uterus via the cervix with the aid of special inseminating devices (Andersen *et al.* 1973; Fukui and Roberts 1976, 1978; Stoyanov 1980). These efforts have proved effective in the respect that the conception rate of ewes inseminated was high. However, the tortuous nature of the cervical lumen prevents this type of insemination in a large proportion of ewes. The method is therefore impracticable on a field scale. If a practical method were devised whereby the cervix could be easily penetrated from a vaginal approach, it would, in the foreseeable future, become the insemination method of choice for most purposes, but recent research in this area has been minimal.

Vaginal Insemination

This technique was originally developed in the U.S.S.R. as a more simple approach to artificial insemination than other methods (Lopyrin 1971). The method, often termed in Australia the 'shot in the dark' or S.I.D. method, quite simply involves depositing fresh diluted semen deep into the vagina of the ewe without attempting to locate the cervix. Although there have been some favourable reports on the effectiveness of the technique (Fairnie and Wales 1982; Tervit *et al.* 1984; Maxwell and Hewitt 1986), there is evidence to suggest that conception rates are lower than for cervical insemination, even with relatively large doses of semen (Kerton *et al.* 1984; Rival *et al.* 1984). The method is ineffective for frozen-thawed spermatozoa (Tervit *et al.* 1984; Maxwell and Hewitt 1986).

Intrauterine Insemination

As mentioned previously, intrauterine AI has found favour for use with frozen semen, since the cervical barrier is effectively circumvented, and conception rates are comparable with those of natural service or other forms of insemination with fresh semen (Maxwell *et al.* 1984*b*; Maxwell and Hewitt 1986; Maxwell 1986*a*). Until recently, the technique was limited to research projects, since it required a surgical approach (laparotomy) which was time-consuming and impractical on a large scale. There is also evidence that intrauterine AI via laparotomy results in abnormally high rates of embryo mortality, possibly due to uterine trauma occurring during surgery (Mattner *et al.* 1969; Lightfoot and Salamon 1970*b*; Salamon *et al.* 1979). The technique of intrauterine insemination with the aid of a laparoscope, first reported by Killeen and Caffery (1982) and described in detail by Evans and Maxwell (1987), has made the use of frozen-thawed semen effective and practicable. It is much more economical in terms of use of semen than other methods of AI (Maxwell 1984).

Since its inception, there has been considerable research effort put into development and refinement of laparoscopic AI with particular regard to the appropriate time of insemination and dose of inseminate. These two factors are inextricably linked as there is evidence to suggest that relatively low doses of semen are effective if they are inseminated at a precise time in relation to ovulation whereas larger doses are required at other times. This, in turn, is probably related to the viability of the spermatozoa.

Time of Insemination

Laparoscopic AI is almost always used in conjunction with synchronization of oestrus and possibly also with stimulation of ovulation. Since only small numbers of animals are usually inseminated, at least in relation to vaginal or cervical AI programs, synchronization is an essential component of a laparoscopic AI program so that all females can be inseminated over a short period.

There has been some research into determination of the optimum time of insemination in relation to the synchronization method and/or the time of ovulation, but data in this area are still very limited. Evans *et al.* (1986), using superovulated ewes, found that the time of insemination was much more critical for frozen-thawed semen than for fresh semen. Maxwell (1986*a*) determined that the optimum time for intrauterine insemination of ewes treated with a progestagen sponge and 400 i.u. pregnant mare serum gonadotrophin (PMSG) was 60–72 h after sponge withdrawal; the median time of ovulation was 56–60 h, indicating that lambing rates were higher when insemination took place shortly after ovulation. This observation is curious in view of the fact that, in naturally mated ewes, spermatozoa are normally present in the female tract before ovulation (Hunter *et al.* 1980). Maxwell *et al.* (1984*b*), however, observed an increase in embryo mortality when intrauterine insemination took place relatively early in relation to ovulation. Some workers have obtained satisfactory fertility when insemination took place at an earlier stage in relation to the sychronization treatment (Killeen *et al.* 1982; Davis *et al.* 1984; McKelvey *et al.* 1985). Many practitioners, who for convenience often inseminate 50–56 h after withdrawal of progestagen pessaries, complain of variable lambing results under apparently similar conditions. This may be related to variation in the time of the preovulatory LH surge and ovulation.

It has been reported that oestrus and ovulation is highly predictable within a homogeneous group of oestrus-synchronized ewes (Robinson and Smith 1967; Evans and Robinson, 1980; Robinson *et al.* 1987), with a close relationship between the onset of oestrus and the LH surge (Evans and Robinson 1980; Pearce and Robinson 1985; Robinson *et al.* 1987). On other hand it has been reported that the onset of oestrus can be highly variable (Maxwell 1986*a*), though the time of ovulation may nevertheless be predictable within each group. Smith *et al.* (1986) found the time of ovulation to be quite variable within a group of synchronized ewes of the same breed.

It is not suprising that some variation is experienced in the time of ovulation in ewes, given the number of factors which are known to influence the onset of oestrus and ovulation (see following tabulation):

Factor[A]	Reference
Breed	Reviewed by Bindon (1984)
Season	Evans and Robinson (1980)
Synchronization method	Robinson and Smith (1967); Gordon (1983)
Gonadotrophin type	Walker *et al.* (1986)
Gonadotrophin dose (and breed of sheep)	Bindon *et al.* (1986)
Gonadotrophin dose (and season)	Evans and Robinson (1980)
Association with males	Lindsay *et al.* (1975); Pearce and Oldham (1984); Maxwell (1986*a*)
Age of ewe	Reviewed by Scaramuzzi and Radford (1983) and Cahill (1984)
Body condition and nutritional status of ewe	Reviewed by Scaramuzzi and Radford (1983) and Cahill (1984)

[A]There is little direct evidence of the influence of these factors on the time of ovulation in ewes. However since it is known that these factors affect the time of onset of oestrus, that oestrus onset and the time of the LH surge are closely related, and that the interval from the LH surge to ovulation is reported to be constant (Cumming *et al.* 1971; Lindsay *et al.* 1975), it is presumed that these factors do, in fact, influence the time of ovulation.

In addition, there is some inherent biological variation in the time it takes ovarian follicles to develop to the stage at which they produce sufficient oestrogen, occasionally termed the threshold level, to trigger the ovulatory LH surge. What is, perhaps, surprising is the relatively high degree of precision of the timing of these events, and, of course, in sheep the follicular phase is so short in relation to that of many other species that fixed-time inseminations are frequently successful. Nevertheless, insufficient knowledge of the exact time of ovulation in particular circumstances may mitigate against successful fixed-time inseminations, particularly when frozen-thawed semen is used. Thus, if ewes are inseminated at a fixed time in relation to synchronization method and not in relation to ovulation one can expect variable lambing results, bearing in mind that the retention and viability of frozen-thawed spermatozoa in the female reproductive tract is less than for fresh spermatozoa (Hawk 1983) and that fertilization with aged semen may result in increased embryo mortality (Maxwell *et al.* 1984*b*).

Though it is apparent that we have insufficient knowledge of the time of ovulation under different circumstances, it may be possible to increase the synchrony of ovulation in commercial ewes for AI in much the same way that ovulation has been synchronized in superovulated ewes and cows. This involves the use of gonadotrophin releasing hormone (GnRH) or one of its synthetic analogues given at about the expected time of onset of oestrus or just before the expected time of the endogenous LH surge (or just before the anticipated 'threshold' level of follicular oestrogen is reached) (Nancarrow *et al.* 1984; Walker *et al.* 1986). At this stage the follicle and its oocyte are responsive to the endogenous LH surge, whether it is triggered

by endogenous oestrogen feedback mechanisms or by exogenous administration of GnRH. This treatment synchronizes the LH surge, and presumably ovulation, amongst all animals and removes some of the inherent biological variability.

The first reports of the use of GnRH in artificially inseminated ewes did not demonstrate an increase in the number of pregnant ewes over control ewes (Fukui *et al.* 1985; Smith *et al.* 1986), but nevertheless did considerably improve the synchrony of ovulation (Smith *et al.* 1986). Further refinement of the technique may produce positive results and, despite the added cost and effort, it may eventually prove to be worthwhile to use GnRH analogues in conjunction with intrauterine insemination of frozen-thawed semen.

Dose of semen

One of the great advantages of laparoscopic intrauterine insemination is that it permits the use of much lower doses of spermatozoa than are required for natural mating or other methods of insemination, thereby increasing the mating potential of superior sires (Maxwell 1984; Clarke 1986). Laparoscopic intrauterine insemination has been used for both fresh (Killeen and Caffery 1982; Rival *et al.* 1984; Tervit *et al.* 1984) and frozen-thawed semen (Killeen *et al.* 1982; Rival *et al.* 1984; Tervit *et al.* 1984; Maxwell 1984, 1986*a*, 1986*b*). Since acceptable fertility can be obtained with fresh semen using other methods of insemination, it is likely that laparoscopic insemination will be used mostly for frozen-thawed semen, unless the availability of fresh semen of a particular ram is severely limited. In both cases, it is of interest to determine the minimum effective or critical dose of spermatozoa for maximum fertility or most economical use of semen. Although Evans and Maxwell (1987) indicate that the minimum effective dose for intrauterine insemination of both fresh and frozen semen is 20 million motile spermatozoa, it should be pointed out that this has not been thoroughly investigated under various conditions, particularly with regard to the time of insemination. Doses larger than this have usually produced good results, but there have been reports of acceptable fertility with lower doses (Table 1). Again this variability may be related to the relative time of insemination and ovulation, though the time of ovulation is rarely documented. There may also be unknown ewe factors which influence fertility.

Table 1. Pregnancy rates after laparoscopic intrauterine insemination of ewes with different numbers of spermatozoa

10^{-6} × No. of spermatozoa[A]	Type of semen	Pregnancy rate (%)	Time of determination	Reference
50	Fresh	52	Non-return	Rival *et al.* (1984)
40*	Fresh	67	Lambing	Salamon *et al.* (1985)
30	Fresh	83	Lambing	Tervit *et al.* (1984)
12·5	Fresh	70	70 days	Davis *et al.* (1984)
>600	Frozen	65	50 days	Maxwell and Hewitt (1986)
120	Frozen	69	Non-return	Killeen *et al.* (1982)
>100	Frozen	56	50 days	Maxwell and Hewitt (1986)
100	Frozen	54	Late pregnant	Maxwell *et al.* (1984)
60	Frozen	38	Lambing	Tervit *et al.* (1984)
50	Frozen	60	Non-return	Rival *et al.* (1984)
10*	Frozen	58	Lambing	Salamon *et al.* (1985)
5*	Frozen	50	50 days	Eppleston *et al.* (1986)
0·5*	Frozen	29	Lambing	Maxwell (1986*b*)
25*	Frozen	56	Lambing	Maxwell (1986*b*)

[A]Numbers of spermatozoa are either *total* numbers or unspecified unless denoted by an asterisk, in which case the number is the number of *motile* spermatozoa.

Site of insemination

Maxwell (1986*b*) found that insemination into the lumen in the middle of the uterine horn was superior to that at the tip or bottom of the horn. Since the middle of the horn is the part most readily visualized during insemination, it is the site of choice for deposition of semen.

Evans *et al.* (1984) found that when spermatozoa were deposited in only one uterine horn, fertilization rates were as good in the contralateral horn as in the inseminated horn, indicating that spermatozoa are quite capable of travelling from one horn to the other. Other workers, however, have found a distinct advantage for inseminating into both horns rather than one horn (Killeen *et al.* 1982; Maxwell 1986*b*). The reasons for this are unclear, but it may be that insemination into both horns simply increases the probability of depositing some spermatozoa into the reproductive tract.

Other factors influencing fertility

As mentioned above, results of laparoscopic AI with frozen–thawed semen are often quite variable. It has long been known that there is marked individual variation in the ability of animal semen to withstand the freezing and thawing processes. There are recent reports that frozen–thawed semen of different rams results in different fertility after intrauterine insemination (Maxwell 1986*b*; Hunton *et al.* 1986; Hunton 1987). This, apparently, cannot be detected by conventional motility tests (Eppleston *et al.* 1986). It is unclear at this stage whether or not the reduced fertility of thawed semen of some rams can be overcome by increasing the inseminating dose.

Insemination of superovulated ewes

Although cervical insemination or natural mating of superovulated ewes has occasionally resulted in high fertility (Armstrong and Evans 1984; Evans and Armstrong 1984), it is generally accepted that intrauterine insemination with a large number of fresh spermatozoa is required if satisfactory fertilization is to be reliably obtained (Trounson and Moore 1974). The requirement to bypass the cervix is due to the disruption of the normal sperm transport mechanisms by superovulation treatments and possibly also to reduced retention of spermatozoa in the female tract (Hawk 1983; Evans and Armstrong 1984). Insemination with the aid of a laparoscope has again been used to facilitate this procedure. However, the appropriate dose of spermatozoa and time of insemination have not yet been fully resolved. High oocyte fertilization rates have been obtained with relatively high doses (in the order of 60 million–100 million) of motile spermatozoa (Killeen *et al.* 1982; Armstrong and Evans 1984; Evans *et al.* 1986). Lower doses, in the order of 1 million–10 million spermatozoa, seem to be less effective (Evans *et al.* 1984; Walker *et al.* 1984). Fertilization rates in superovulated ewes are higher when insemination takes place before rather than after ovulation (Armstrong and Evans 1984; Evans *et al.* 1984, 1986). The time of insemination of progestagen sponge-treated superovulated ewes appears to be optimal at 44–48 h after sponge withdrawal (Killeen *et al.* 1982; Armstrong and Evans 1984; Evans *et al.* 1984, 1985; Hunton *et al.* 1986). These studies also showed that fertilization rates are lower for frozen–thawed than fresh semen, and that the timing of insemination is less flexible for frozen–thawed semen. Since it has been reported that the time of ovulation in superovulated ewes can be quite variable, particularly in the non-breeding season (Maxwell *et al.* 1986*b*; Walker *et al.* 1986), it appears that more precise synchronization of ovulation with GnRH may be of benefit in terms of fertilization rates, particularly when frozen–thawed semen is used (Nancarrow *et al.* 1984; Maxwell 1986*a*; Walker *et al.* 1986).

Use of related technology

'Sexing' of semen to predetermine the sex of offspring has long been a goal of research workers, but good results have not been obtained consistently in farm animals. The technology has recently been applied to sheep. White *et al.* (1984), using a separation technique based on layering semen on protein-enriched columns (Ericsson *et al.* 1973), produced 63% females in the predicted X-enriched fraction and 75% males in the Y-enriched fraction, but the total number of lambs was small ($n = 46$). A further study involving more animals produced 58% males in the Y-fraction, but no difference in the X-fraction (Evans *et al.* 1987). The technique is not yet powerful or reliable enough for commercial use. If it does become viable, the technique will almost certainly require the use of intrauterine insemination since, at present,

the yield of the sexed semen is only 5–20% of the initial ejaculate, which necessitates collection and processing of large amounts of semen in advance of insemination.

Control of Oestrus and Ovulation

In many AI programs teaser rams are used to detect oestrus in naturally cycling females. On the other hand, for added convenience, many practitioners prefer to use some form of control of oestrus and ovulation (reviewed recently by Gordon 1983), and development of this technology has received a further boost with the development of AI technology. Laparoscopic insemination with frozen-thawed semen on a commercial scale virtually necessitates synchronization of oestrus and possibly also stimulation of ovulation. It would be inappropriate to discuss artificial insemination without mention of recent developments in the techniques for control of oestrus and ovulation.

Methods of synchronization of oestrus and stimulation of ovulation are often used together but should be regarded as separate entities. In most circumstances, the simplest and most effective method of oestrus synchronization at present is that of the intravaginal pessary (Robinson 1965), often used in conjuntion with PMSG to provide greater synchrony of oestrus and ovulation (Evans and Robinson 1980). However, there are several other methods for control of oestrus and ovulation. Methods for synchronization of oestrus include not only the intravaginal pessary but also progestagen implants, prostaglandin injection and the 'ram effect'. Ovulation can be stimulated either without or in conjunction with synchronization, depending on the method of choice; methods include administration of various gonadotrophins, immunization against ovarian steroids (Fecundin), the 'ram effect' or nutritional 'flushing'. As most of these methods, and the use of GnRH described above, have been well described elsewhere (Maxwell 1984; Scaramuzzi and Martin 1984; Evans and Maxwell 1987) this article will deal only with recent developments.

One recent development is that of the controlled internal drug release (CIDR) dispenser (Welch 1983). The device, which utilizes the intravaginal route for administration of progesterone (Robinson 1965), is reputed to have the advantage over conventional progestagen sponges in that its withdrawal is not associated with an unpleasant vaginal discharge. It is not yet licensed for use in many areas, but drug registration trials are in progress. Initial reports on its use in sheep showed there were no differences between the CIDR and the progestagen sponge in terms of fertility to natural or artificial insemination, or in time to onset of oestrus (Boland *et al.* 1983; Maxwell and Barnes 1986). However, Clarke *et al.* (1986) claim that the onset of oestrus is earlier with the use of CIDRs compared with sponges, and that they provide more precise control of oestrus without the use of PMSG. If this is borne out by more extensive trials, and the cost is comparable to the sponge, the CIDR will likely be adopted by practitioners of AI. It is not known whether the CIDR induces the adverse effects on sperm transport associated with synthetic progestagen sponges (Quinlivan and Robinson 1969; Hawk and Conley 1975). Before adoption of the CIDR for commercial use, it may be necessary to reappraise the timing and doses of insemination for all methods of AI.

The ram effect (Pearce and Oldham 1984) has received some considerable attention of late. It can be used for stimulating oestrus and ovulation in anoestrous ewes and has had a profound influence on AI practices in some areas, especially in Western Australia (Maxwell 1984). Teaser rams are also used for stimulation of oestrus and ovulation in ewes in which oestrus is synchronized by pharmacological means and in which timed inseminations are performed. This technique is used commonly on the basis of sound evidence of the stimulatory effect of rams on ewes, including stimulation of ovulation rate (Pearce and Oldham 1984), and the fact that higher ovulation rates result in higher lambing rates (Kelly 1984; Cameron *et al.* 1985). However, there is little experimental evidence to suggest that fertility of artificially inseminated ewes is improved by joining the ewes with teaser rams around the time of insemination. The study of Restall (1961) indicated that post-insemination teasing may have a beneficial effect on fertility, but the results did not reach significance. Clearly there is a need for more definitive research in this area.

One of the recent developments of potential use for increasing the ovulation rate of ewes at a natural as well as a synchronized oestrus is immunization against inhibin (O'Shea *et al.* 1984). Increasing the ovulation rate in this way may result in increased conception rates to AI without the adverse effects of increased embryo mortality associated with the use of Fecundin (Scaramuzzi and Martin, 1984). The technique is still under investigation and there is no commercially available product for immunization against inhibin at this time.

Genetic Impact of Artificial Insemination in Sheep

The final aspect which may be considered is the genetic impact of AI in sheep breeding programs. The fact that this is discussed as the last topic is no reflection on its importance. Indeed, the genetic consequences of implementation of an AI program should be the first consideration for the sheep breeder.

Although the genetic impact of AI programs has been well documented in cattle (Inskeep and Peters 1981; Van Vleck 1981), there has not been much research done on the genetic impact of AI programs in sheep (Inskeep and Peters 1981; Nicholas 1985). The problem has been addressed recently from a theoretical point of view (Maxwell 1984; Clarke *et al.* 1986; Maxwell and Ponzoni 1987), but there is a dearth of information on the actual effect of AI on production characteristics such as carcass quality or wool or milk production. Nevertheless it appears that AI of sheep can potentially have a significant impact on sheep production.

The advantage of AI over natural breeding is that superior males can be used more widely, both in terms of the number of matings possible within a given flock and over a greater geographical area. This has a number of potential genetic advantages. The selection intensity of sires can be increased with a resultant increase in production, provided there are no deleterious effects of inbreeding (Clarke *et al.* 1986). This may occur in small flocks, but can be overcome in large flocks or in group breeding schemes. Cooperative and group breeding schemes can operate more efficiently with AI, particularly when semen is collected at a centre and stored for distribution at the appropriate time (Clarke *et al.* 1986). Clarke *et al.* (1986) predict that, with collection and frozen storage of semen throughout much of the year, the genetic progress with AI used with the current rates of success would be 48% greater than that with natural mating. With progeny testing and sire referencing schemes, facilitated by AI, the potential increase is even greater (Maxwell 1984; Clarke *et al.* 1986; Maxwell and Ponzoni 1987).

There have been group breeding schemes in operation in Australia for several years. These schemes use a nucleus flock at the tip of a pyramid structure, and genes are usually disseminated down the pyramid, ultimately reaching the commercial flocks. The advantage of AI is that the genetic lag can be greatly reduced by allowing much more intensive and widespread use of rams (Nicholas 1985; Clarke *et al.* 1986).

A further genetic advantage of AI is that scarce resources, such as a superior breed or strain, can be spread much further than with natural mating. In addition, genetic material from rare breeds, or from breeds with rapidly changing genotypes, can be preserved for future use. Semen of rams can be frozen-stored for prolonged periods without loss of fertility, at least up to 16 years (Salamon and Visser 1974*b*; Salamon *et al.* 1985).

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