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The Ovariectomized Ewe: Its Contribution to Controlled Breeding

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Abstract

The ovariectomized ewe has been used to establish principles and procedures which have proved invaluable in controlled breeding in entire animals. Bioassays in the ovariectomized ewe, using the end-point of oestrous behaviour, have been used to identify potent and rapidly metabolized progestagens which were subsequently used to control the time of oestrus and ovulation in cyclic ewes effectively, and to induce oestrus and ovulation in anoestrous ewes. Steroid hormone treatment of the ovariectomized ewe has been used to study relationships between the ovary and the pituitary — hypothalamic axis, to examine transport of embryos within the female tract and to establish the steroid hormone requirements of early pregnancy.

Introduction

The title of this paper may seem strange, as it could be said that ovariectomy would control breeding activity permanently and that would, of course, be the end of it. However, studies using ovariectomized ewes have, in many instances, provided knowledge essential for the development of procedures that have been successfully used in controlled breeding in sheep and the principles established for sheep have been extended to other species, notably cattle and goats.

The early work on the ovariectomized ewe carried out in the 1950's and 1960's is notable for three reasons. First, it showed that a farm animal could be used in types of experiments which had been previously restricted to laboratory rodents. Second, it showed that a behavioural activity, namely oestrus, could be used as a reliable and precise end-point in the bioassay of steroid hormones, and third, T. J. Robinson was responsible either directly, or indirectly through postgraduate students, for much of the work.

Relationship between Oestrogen and Progesterone in Oestrus in the Ewe

Prior to 1950 the roles played by oestrogen and progesterone in oestrous behaviour in rodents had been well established. In the ovariectomized rat (Boling and Blandau 1939) and mouse (Ring 1944) oestrus could be reliably induced by oestrogen, but only when a small dose of progesterone was given shortly after the oestrogen. However, the requirements for oestrus in the ewe turned out to be quite different from those for the rodents. Oestrus in ovariectomized ewes could be induced by a single injection of oestradiol but the ewes became refractory to repeated treatments. Sensitivity to oestradiol and reliability in response were restored when a period of treatment with progesterone *preceded* oestradiol (Robinson 1954*a*, 1954*b*). The realization that oestrus in the ewe was a function of oestrogen secreted after a period of progesterone provided support for the observation (Dauzier and Wintenberger 1952; Dutt 1952; Robinson 1952) that ovulation induced in the anoestrous ewe by pregnant mare serum gonadotrophin (PMSG) was accompanied by oestrus only if the PMSG was preceded by progesterone. The need for progesterone prior to oestrogen also provided the explanation for the observation made 20 years previously (Grant 1934) that the first ovulation of the breeding season was usually not accompanied by oestrus ('silent heat').

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A series of studies (Robinson 1955; Robinson *et al.* 1956; Moore and Robinson 1957) established the time–dose relationship for oestradiol and progesterone for the induction of oestrus in crossbred (Border Leicester × Merino) ovariectomized ewes. Progesterone (6 mg/day) given over 6 consecutive days was required to condition the ewes to respond to oestradiol and some 90% of ewes given 20 μg oestradiol benzoate 2 days after the last injection of progesterone were detected in oestrus. If given concurrently with oestradiol progesterone inhibited the capacity of oestradiol to induce oestrus, while if more than 2 days elapsed between the final injection of progesterone and the injection of oestradiol responses declined, indicating that the ewes remained primed by progesterone for only a limited period.

Contol of Oestrus and Ovulation

Of major importance in the development of effective methods for the control of oestrus and ovulation was the clear demonstration that information obtained from the ovariectomized ewe could be directly applied to the entire ewe (see Robinson 1967). The requirements of progesterone and oestrogen for oestrus in the ovariectomized ewe are remarkably similar to those for the inhibition and subsequent release of oestrus and ovulation in the entire cyclic ewe, and for the induction of oestrus and ovulation in the anoestrous ewe. In the entire ewe the oestrogen is, of course, of follicular origin, a consequence in the cyclic ewe of the endogenous release of gonadotrophins and in the anoestrous ewe of exogenous gonadotrophins, usually PMSG, given near the end of, or shortly after, a period of progestagen treatment. In the ovariectomized ewe progesterone primes a neural centre, as yet unidentified (Rice *et al.* 1984), to respond to oestrogen, whilst in the entire cyclic ewe the major action of progesterone is probably one of a negative feedback effect on the hypothalamus reducing the pulse frequency of release of releasing hormone (GnRH) with consequent inhibition of the luteinizing hormone (LH) surge associated with ovulation (Scaramuzzi *et al.* 1971). Progesterone may also reduce tonic LH secretion during the luteal phase of the oestrous cycle (Karsh *et al.* 1977).

To be of practical value procedures for the control and subsequent release of oestrus and ovulation in the cyclic ewe must provide high circulatory levels of progestagen, but at the same time they must involve infrequent treatment and limited handling of animals. Daily, or once every second day, injections of progesterone although effective in controlling the time of oestrus and ovulation, held little promise and attempts to use long-acting forms of progesterone (emulsions and suspensions) met with disaster (Robinson 1960). The administration of orally active progestagens, e.g. medroxyprogesterone acetate (MAP; Provera, Upjohn) did not provide an answer to the problem (Southcott *et al.* 1962; Lindsay *et al.* 1967). Few of the animals treated with the long-acting forms of progesterone showed oestrus after treatment, and of those which did show oestrus, few conceived. Following Provera the incidence of oestrus was acceptable but conception rates, particularly to artificial insemination, were low. It is now clear that the form of the progesterone and the method of administration of the Provera could not result in a rapid decline in the circulatory levels of progestagen at the end of treatment. Solution of the problem seemed to depend upon, first, the identification of potent progestagens which were not long-acting, and second, on the development of a method of administration which involved limited handling of animals, provided high circulatory levels of the progestagen shortly after the start of treatment, and gave a rapid decline in levels at the end of treatment. Promising progestagens were identified by the use of an assay procedure developed for use in the ovariectomized ewe (Shelton 1964, 1965; Shelton *et al.* 1967). In the assay the unknown progestagen given over 12 days at two daily dose levels was tested against progesterone and three graded doses of oestradiol were given 2 or 4 days after the final injection of progesterone or unknown progestagen. The end-point of the assay was oestrus. The search was for progestagens which would at a low dose provide a result, in terms of the nature of the dose–response characteristics, indistinguishable from that of progesterone. If the duration of activity of the progestagen was too long (or the doses chosen were excessively high) then the oestrous response was low with no difference between responses when 2 and 4 days elapsed between the end of progestagen treatment and the injection of oestradiol. If the progestagen was short-acting then with one or other of the two doses the responses closely resembled those following progesterone. In this way several promising progestagens, e.g. Cronolone (G. D. Searle) and MAP were identified. They were short-acting and some 20–25 times more potent than progesterone. The ability of these progestagens to control oestrus and ovulation in the cyclic ewe was correctly predicted from the results achieved in the ovariectomized ewe assays (Shelton and Robinson 1967). However, these progestagens, like progesterone, are lowly soluble. The problem of low solubility was solved by the use of a polyurethane sponge which provided a matrix on which the progestagen crystallized out from an ethanol solution, thus providing a large surface area. The impregnated sponges also solved the problem of frequent handling of animals. The sponges were effective when used subcutaneously or intravaginally (Robinson 1964), but for ease of insertion and removal the intravaginal site was preferred. Progesterone is effective when administered by intravaginal sponge (Robinson and Moore 1967) and recently a silastic device (Constant Internal Drug Release, CIDR; AHI Plastic Moulding Co., Hamilton, New Zealand) containing 9% progesterone has proved effective when used intravaginally for the control of oestrus and ovulation.

Induction of Oestrus and Ovulation during Anoestrus

Knowledge gained from work with the ovariectomized ewe has been equally applicable to the anoestrous ewe for the induction of oestrus and ovulation as it has been for the control of oestrus and ovulation in the cyclic ewe (Moore and Holst 1967; Robinson and Smith 1967), the only difference between the cyclic and anoestrous ewe being the need for gonadotrophin in the anoestrous ewe. The need for gonadotrophin in the anoestrous ewe, or more specifically the failure in most instances of full follicular development, oestrus and ovulation following the end of progestagen treatment of the anoestrous ewe, provided suggestive evidence of a difference between the anoestrous and cyclic ewe in the responsiveness of her pituitary–hypothalamic axis to steroid hormones.

In the ewe a dramatic rise of up to 10-fold in peripheral plasma levels of LH follows ovariectomy (Scaramuzzi *et al.* 1970). There is an increase in both the amplitude and frequency of LH pulses (Martin 1984). Physiological doses of oestradiol (25–50 μg oestradiol benzoate) given to the ovariectomized ewe are followed initially by a rapid decline in circulatory concentrations of LH and then a marked rise (Scaramuzzi *et al.* 1971), the rise being similar in nature to the preovulatory surge of LH seen in the entire ewe. The magnitude of the negative feedback effect of oestradiol on the tonic secretion of LH has been shown to vary with season (Legan *et al.* 1977), being greatest in the spring and early summer (anoestrous season) and least in the late summer and early autumn (breeding season). These authors suggested that seasonal breeding might be a resultant of this change in sensitivity of the pituitary–hypothalamic axis to oestradiol. The observations of Raeside and McDonald (1959) and Reardon and Robinson (1961) that the oestrous response of the progesterone-primed ovariectomized ewe to oestradiol varied during the year, being lowest in the winter and spring and the more recent finding of Evans and Robinson (1980) that the oestradiol production of ovarian follicles of Border Leicester × Merino ewes was lower in the spring than in the autumn might suggest an involvement of both production and sensitivity to oestradiol in seasonality of breeding. It has been suggested (Kennaway *et al.* 1982) that the influence of the pineal gland, through its production of melatonin, on seasonality of breeding might be mediated through an influence on the sensitivity to oestradiol. The results of the work of Bittman and Karsh (1984) in which they examined LH concentrations in the peripheral plasma of pinealectomized and ovariectomized ewes infused with melatonin support the suggestion of Kennaway *et al.* (1982) and they also provide a further example of the value of the ovariectomized ewe.

Changes in production and/or sensitivity to oestradiol may well provide an explanation for the dramatic change in the oestrous and ovulatory response of Cormo ewes to a follicle stimulating hormone (FSH) preparation (FSH-P, Burns-Biotec., Omaha, U.S.A.) seen in this laboratory in 1982 (R. J. Bilton and N. W. Moore, unpublished data). During May and June the oestrous and ovulatory responses were satisfactory. However, during July and August an increasing number of ewes failed to show oestrus, the ovulatory response declined and many of the ewes which failed to show oestrus failed to ovulate even though the dose of FSH had been increased. When a small dose of PMSG (around 500 i.u.) was given with the FSH, on the assumption that the PMSG might because of its steroidogenic activity (Jabbour *et al.* 1986) increase oestradiol production, there was a dramatic improvement in both the oestrous and ovulatory responses and the 'FSH–PMSG cocktail' (Ryan *et al.* 1984) has now been widely used in this and other laboratories as an effective preparation for the superovulation of ewes.

Effect of Rams on Breeding Activity in Ewes

It has been known for some 40 years that the unaccustomed presence of rams can induce ovulation and hasten the onset of breeding activity in anovular ewes (Underwood *et al.* 1944). Within minutes of their contact with rams the frequency of LH pulses in ewes increases (Martin *et al.* 1980) culminating in a preovulatory surge of LH occurring within 48 h of the introduction of rams (Pearce and Oldham 1984). The induced ovulation is not accompanied by oestrus (Schinckel 1954) and recently Oldham and Martin (1978) found that corpora lutea formed after ovulation often regressed prematurely 5–6 days after ovulation. Regression is generally followed by a further ovulation, usually without oestrus, but the resulting corpora lutea persist for the normal period. Similar problems of failure of oestrus and of abnormal luteal function follow the induction of ovulation in the anovular ewe by PMSG or GnRH (Haresign *et al.* 1975).

A single injection of 20 mg progesterone given just before the introduction of rams will ensure that corpora lutea do not regress prematurely, but the ewes still do not show oestrus (Pearce and Oldham 1984) and the same may be true of anovular ewes given PMSG or GnRH after a single injection of progesterone. Pretreatment with progestagens for several days is required if the ewes are to show oestrus as well as have a normal luteal phase following the introduction of rams (Cognie *et al.* 1980), injection of PMSG (Robinson 1952) or GnRH (McLeod *et al.* 1982). Results from studies in ovariectomized ewes (Robinson *et al.* 1956) would suggest, and observations on the behaviour of anovular ewes following the introduction of rams (Reeve and Chamley 1982) has confirmed, that a minimum period of 6 days pretreatment with progestagen is required for the ewes to show oestrus.

Hormone Requirements during Early Pregnancy

The ultimate in controlled breeding would be to mate at a predetermined hour of the day, get all ewes pregnant and for all to bear and rear multiples. A variety of techniques which will increase the incidence of multiple births is available (Scaramuzzi and Martin 1984). The time of mating can be controlled although perhaps not with the degree of precision desired but there remains the problem of some 20–30% of ewes failing to become pregnant following mating at one oestrous period. In the ewes which do not establish a successful pregnancy, failure due to death of embryos is more common than is failure due to lack of fertilization (Edey 1969) and most prenatal death occurs within the first 3 weeks after mating (Robinson 1951; Moore *et al.* 1959). Hence, the interest in early pregnancy particularly in ovarian secretory activity and the influence of ovarian steroid hormones on the uterus. The ovariectomized ewe has been used to establish the steroid hormone requirements of early pregnancy and to examine the roles played by progesterone and oestradiol in metabolic changes within the uterus and in the secretory activity, particularly protein secretion by the uterus (Miller and Moore 1976, 1983; Murphy *et al.* 1977).

Studies on ewes ovariectomized a few days after mating to fertile rams (Foote *et al.* 1957; Bindon 1971) or ovariectomized at the time of embryo transfer 3–4 days after mating to sterile rams (Moore and Rowson 1959) clearly established the need for progesterone secreted after ovulation, but a role for oestradiol secreted during the luteal phase of the oestrous cycle (Cox *et al.* 1971) could not be demonstrated. It has been suggested (Holst and Braden 1972) that luteal phase oestradiol is involved in the transport of embryos within the oviduct, but extensive studies in ovariectomized ewes have been unable to implicate oestradiol in either the retention or transport of embryos in the oviduct (Ryan 1985).

Results of a series of studies involving the transfer of embryos to ewes ovariectomized some time before transfer (Miller and Moore 1976; Miller *et al.* 1977; Moore *et al.* 1983) suggest, and one experiment in which embryos were transferred to anoestrous ewes following the induction of ovulation by PMSG (Moore and Miller 1984) confirmed, that a period under the influence of progesterone prior to oestrus is required for the subsequent survival and normal development of embryos. The minimum duration of this period under progesterone appears to be about 4 days (Moore 1985).

The benefits that could be achieved from control of the time of mating would be enhanced if the incidence of multiple births and conception rates to mating at the controlled mating could be increased. Preliminary studies in this laboratory (N.W. Moore, unpublished data) suggest that both may be possible. Merino ewes treated with intravaginal progestagen sponges to control the time of mating and given a small dose of PMSG (400–500 i.u.) sufficient to result in an average of 1·7 lambs per ewes lambing gave a conception rate to natural service at the controlled mating of 84% (273 of 325 ewes lambed). Untreated ewes of the same origin and mated at the same time gave conception rates to mating at one oestrous period of 71% (89 of 125 ewes lambed) with an incidence of twins of only 13%. There are numerous explanations to account for the high conception rate in ewes bearing multiples (e.g. improved fertilization, increased ovarian secretory function, extra mass of embryos in the uterus) but there is little evidence to support any of these suggestions. However, information from this laboratory (N. W. Moore, unpublished data) on the pregnancy rate following the transfer of one or more embryos to the uteri of entire and ovariectomized Merino ewes might provide some leads (Table 1). Embryos were transferred to the uteri 3 or 4 days after oestrus which in the ovariectomized ewes had been induced by oestradiol given after daily treatment with progesterone for 12 days. After oestrus the ovariectomized ewes were placed on a regimen of progesterone injections known to support the survival and normal development of embryos (Miller and Moore 1976). In the entire ewes the increase in pregnancy rate with increase in number of embryos transferred may have been simply due to lack of viability of some embryos so that the more transferred then the greater was the chance of each ewe receiving a viable embryo. This may be true only in part. No doubt some embryos are not viable because of genetic aberrations (Murray *et al.* 1986), but the lack of a difference in pregnancy rate between the ovariectomized ewes which received one and two embryos (Table 1) would suggest that the incidence of non-viable embryos was low. The improved pregnancy rate observed in the entire ewes which received two and three embryos appears to be due to maternal factors as well as to the increased chances of receiving viable embryos.

Table 1. Proportion of entire and ovariectomized Merino ewes pregnant following the transfer of one, or more, embryos

Proportion with one, or more, normal embryos 17–25 days after transfer. Embryos transferred 3 or 4 days after oestrus (induced in ovariectomized ewes)

No. of embryos transferred	Proportion pregnant	
	Entire	Ovariectomized
1	121/195 (62%)	36/49 (73%)
2	96/129 (74%)	66/87 (76%)
3	85/109 (78%)	—

In the entire ewe there is some suggestion that exogenous progesterone given during the second week after mating increases pregnancy rate, but perhaps only under the unusual conditions of a dramatic improvement in levels of feeding or following synchronized mating (Parr *et al.* 1986). In addition there is evidence of an effect of dose of progesterone on survival and size of embryo in ovariectomized ewes (Foote *et al.* 1957; Bindon 1971; Parr *et al.* 1982). There is need for further studies aimed at investigating the relationships between circulatory levels of steroid hormones and survival and development of embryos, and again, the ovariectomized ewe could be used to advantage.

There is no doubt that studies involving the ovariectomized ewe have made and will continue to make major contributions to our knowledge of reproductive biology as well as to the exploitation of that knowledge. There is also little doubt that much of the credit for the use of the ovariectomized ewe is due to T. J. Robinson.

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