CONTROL OF REPRODUCTION IN SHEEP AND CATTLE

(Invited Paper)

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Summary

The paper is presented in three parts. It is not a review of the literature but a statement of the authors' views in the light of their experience, and backed by their recent experimental results.

Introduction. The physiological principles of control of the ovarian cycle are discussed, together with the practical problems associated with such control. The complexities of the problems directly and indirectly associated with controlled breeding are such as to render useless much of the purely empirical field testing which has characterised this area of study.

The ewe. The development of the spayed-ewe assay technique for the characterization of progestagens is described. This has led to the testing, in intravaginal sponges, of several progestagens for the control of the ovarian cycle in cyclic and anoestrous ewes. One steroid (SC-9880 —17α-acetoxy-9α-fluoro-11-β-hydroxypregn-4-ene-3,20-dione) has been extensively tested and the results are reported. The nature of the sub-fertility which accompanies artificial insemination at the first oestrus following sponge withdrawal is discussed. A major part of this problem is contributed by the enormous variation between rams and by an apparent necessity for greater than normal numbers of spermatozoa at first oestrus.

The cow. For many reasons, work an the cow lags behind that on the sheep. Hence emphasis is placed on physiological aspects of ovarian function together with some thoughts about what should be done to solve the problem of synchronization. It is concluded that although a partial solution of the problem may come from extrapolation of the work in the ewe, much more work is required in order to know the basic mechanisms involved in control of ovulation. A list of six areas for work is given. These include studies on ovarian morphlogy, steroid production, on endocrine and social factors involved in oestrous behaviour, on bovine gonadotrophins, on the use in the field of known and as yet unknown gonadotrophins, and finally an the use of long acting progestagen preparations for long-term suppression of ovarian function to prevent conception.

I. INTRODUCTION

Knowledge of the endocrine and other factors that influence ovarian function has many practical implications. In particular, acceptable and reliable techniques for suppressing ovulation or interfering with implantation in humans as a means of contraception, and for the induction and synchronization of oestrus and ovulation in domestic animals in order to breed at will and to reduce the costs of artificial insemination, are needed. In general, the empirical approach to the

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problem of human contraception has been moderately successful in that some hundreds of compounds, most of them steroid analogues of the natural oestrogens and progesterone, have been tested and some are available commercially and are being used satisfactorily. As might be expected, it has proved much easier to suppress ovarian function in order to interfere with human fertility than to control the ovary to provide normal fertility at a stimulated ovulation, as is required in the domestic animals.

The concept of control in sheep and cattle includes suppression, synchronization and, in the case of the anoestrous animal, stimulation of ovulation, with normal fertility at the synchronized oestrus. An understanding of the physiological mechanisms of successful synchronization in the cyclic animal might reasonably lead to a solution of related problems such as ovulation of a desired number of follicles (multiple ovulation), and the initiation of reproductive activity during anoestrus.

The physiological basis of synchronization during periods of normal cycles is that progesterone, which is produced by the corpus luteum, suppresses follicular development in the ewe and follicular maturation in the cow. The detailed relations between the daily production of progesterone throughout the cycle and follicular development are being investigated in a number of laboratories. If the progesterone content of the corpus luteum can be taken as a guide to secretion and physiological function, then a content of 100-200 μg progesterone is adequate and normal in the cow. The presence of a corpus luteum containing less than this amount has been correlated with inability to maintain pregnancy.

It has been inferred that there would be an optimum dose and method of administration of progesterone, or an analogue, for suppression in both the ewe and the cow. Inadequate doses will permit ovulation during treatment; excessive doses will delay the commencement of the oestrous cycle after cessation of treatment. There is a good deal of evidence to indicate that the optimum treatment may depend on the substance; the class of animal, including age, breed, body condition, whether lactating; season; and management factors such as the presence of a male or density of population.

In anoestrous animals, some form of stimulation of ovarian function is required. It is clear that progesterone, or an analogue, is needed prior to stimulation in order to prime the ewe. The position has not been clarified in the cow. Physiological problems of ovarian stimulation are so poorly understood that in most instances the use of a gonadotrophin is empirical. The most commonly used hormone is pregnant mare serum gonadotrophin (PMS), which has predominantly follicle-stimulating (FSH) activity. However, sheep pituitary FSH and horse pituitary FSH have been used. Difficulties associated with the use of the gonadotrophins are due to insufficient knowledge of their action, especially in relation to the cessation of the suppressive or priming treatment.

It has not generally been possible to indicate the reasons for poor fertility after satisfactory suppression and synchronization. Few studies have included adequate evaluation of semen quality, sperm numbers and fertilizing capacity, or time of ovulation and rate of ovum transport, fertilization, etc., so that in the event of failure there has been considerable difficulty in evaluating the effects of treatments on gamete transport, fertilization and nidation. The physiology of fallopian tube function is not understood in either the ewe or cow; nor are the hormonal factors associated with nidation.

The complexities of the problem of fertility after manipulation of ovarian function are so great that often it is as difficult to understand why a particular treatment gave satisfactory fertility, as it is to determine why another treatment gave poor results. Unhappily, too often all the good results are remembered along with only those poor ones for which an obvious explanation is forthcoming. Further, while not unique in this respect, this field is characterized by a remarkable proportion of field tests so lacking in perception or design as to be incapable of contributing anything either to our understanding of the basic principles involved or to an ultimate solution to the practical problems.

This paper is not intended as a review, several of which have appeared recently (Robinson 1959, 1960; Hansel 1961; Anderson, Schultz and Melampy 1964; Lamond 1964). Rather it is an assessment by the authors, each of whom has worked independently on different aspects of control of ovulation in sheep and cattle, of the present state of knowledge.

The body of the paper deals with recent advances in control of oestrus and ovulation in the ewe and the cow. Largely because of its size, ease of handling and relatively low cost, particularly in Australia, far greater progress has been possible with the ewe than with the cow. A great deal is known of endocrine interactions and the physiology of oestrus, largely as a result of detailed studies in the spayed animal. In the intact ewe, suppression, synchronization and fertility have been obtained with progestagens alone, provided dose is adequate. It is generally accepted now that the major problems associated with synchronization of ewes in the field are those of cost, ease of administration and safety, both for the margin in treatment and from the public health viewpoint. Some problems associated with fertilization remain to be solved, but these should not be too intractable.

The position is not so clear for the cow. The basic work on endocrine interactions and physiology of oestrus has not been done in the spayed animal In the intact cow, progesterone or progestagens alone continue to give variable results. There are factors associated with their use that are not fully understood.

The Section concerning the ewe deals first with the physiology of oestrus and the use of our knowledge of progestagen-oestrogen interactions for screening progestagens of unknown characteristics in the sheep, second with the development of techniques for the use of new and highly active progestagens, and third with the results obtained and experience gained in large scale field testing of these techniques.

This Section concerning the cow emphasises physiological aspects of ovarian function together with some thoughts about what should be done to solve the problem of synchronization.

II. THE EWE

Oestrus in the ewe is caused by a finely balanced interaction between progesterone and oestrogen. In the spayed ewe, progesterone conditions the animal to respond to injected oestrogen, provided the latter is given at an optimum time after cessation of progesterone treatment. If given too early, oestrus does not result-it is suppressed by the progesterone. If given too late, the effect of progesterone priming is lost and a high dose of oestrogen is required to induce oestrus (Robinson 1959).

This phenomenon has been used by Shelton (1965) as a bioassay technique for screening progestagens of unknown characteristics. By the use of a multidimensional model incorporating various doses of progestagen, time intervals between cessation of treatment and oestrogen, and doses of oestrogen, it is possible to assess the activity, relative to progesterone, of an unknown progestagen, and also its relative duration of activity. Using this technique, Shelton, Robinson and Holst (unpublished data) have characterized a number of progestagens, including the two commonly used orally (MAP-l 7α-acetoxy-6α-methylpregn-4-ene-3, 20-dione; and CAP- 17α-acetoxy-6-chloropregn-4, 6-diene-3, 20-dione). Of the compounds tested, one (SC-9880-l 7α-acetoxy-9α-fluoro- 11 β-hydroxypregn-4-ene-3, 20-dione) was found to have all the characteristics of progesterone but was 20 to 25 times as active. Its duration of activity was indistinguishable from progesterone. Another (SC-9022-l 7β-hydroxy-2 1 -methyl-2 1 -methylene-19-Nor-1 7α-pregn-4-en-3-one) had similar characteristics but was less active than SC-9880. Three others, SC-10363 (17α-acetoxy-6-methylpregna-4, 6-diene-3, 20-dione), MAP and CAP also were highly active—of the same order as SC-9880—but appeared longer acting. When used by injection in intact cyclic ewes, SC-10363 interfered with fertility more than did SC-9880 (Shelton and Robinson, unpublished data), so the latter was chosen as the steroid of choice for intensive study.

The next step was the development of an intravaginal sponge pessary impregnated with SC-9880 (Robinson 1965). Preliminary experiments showed this to be a highly effective technique for the synchronization of oestrus and, following treatment, fertility appeared normal. A series of experiments was then conducted in Merino ewes in the spring of 1964 at "Cocketgedong", Urana, N.S.W. Intravaginal sponges impregnated with various doses of several progestagens-progesterone, SC-9880, SC-10363, MAP, SC-9022 and Enovid—were compared for their ability to suppress ovarian function and then allow synchronized oestrus and ovulation after withdrawal. Of these, SC-9880, SC-9022 and MAP were highly effective in blocking and releasing oestrus. The time of release was earlier and more uniform with SC-9880 and SC-9022 than with MAP, so confirming the evidence obtained from the spayed ewe. Attention was focussed on SC-9880 and the minimum effective dose for use in a sponge was found to be 10 mg. A dose of 5 mg suppressed ovulation and oestrus, and released ovulation after sponge withdrawal, but oestrus was not observed in many ewes. Hence there was a high incidence of "silent heats" at this dose (Robinson, Moore, Holst and Smith, unpublished data).

A concurrent field test gave a 50% conception rate to artificial insemination using semen pooled from five rams and diluted with skim milk.

Concurrently, Moore and Holst (unpublished data) were studying the use of SC-9880 impregnated sponges (30 mg) with 750 i.u. PMS in crossbred ewes. They found that the sponge treatment duplicated the much more tedious progesterone injection procedure and they obtained satisfactory lambing results. Robinson and Smith (unpublished data) later demonstrated that the breeding season of Dorset Horn, Southdown and Border Leicester ewes could be advanced by the use of SC-9880 impregnated sponges, used without PMS, in late December-early January.

Extensive field tests in Merinos were conducted in the January-May breeding season of 1965. Some ten thousand ewes were involved on nine properties in three States. It was found that sponges containing 10 mg SC-9880 effectively blocked ovulation and oestrus and that oestrus was released 36 to 60 hours after sponge withdrawal. Ovulation occurred 48 to 72 hours after withdrawal. With 20 mg SC-9880, oestrus and ovulation occurred some 6 to 12 hours later, and with 40 mg a little later again. In some but not in all experiments fertility was higher after 20 or 40 mg than after 10 mg. It was never lower at the higher doses.

Unlike the situation at "Cocketgedong", fertility has been exceedingly variable and this has been largely due to enormous variations between rams. Conception rates for individual rams at the first oestrus have ranged from 0.4% to 60%, with an overall mean of about 30% . It seems therefore, by comparison with the spring inseminations, that the use of pooled semen cancelled out the effect of the poorest rams, and that the mean of a pooled sample approached that of the better rams.

For natural mating, results depended on the intensity with which rams were used. When used sparingly, normal fertility was attained. When used excessively, so that their semen was self-diluted to an extent comparable with a sample used for A.I., fertility was suppressed.

The incidence of oestrus following sponge withdrawal has varied, generally between 80 and 98%, but in one case in Western Australia, where the average daily temperature was over 100°F it was only 20%.

Fertility at the second oestrus after sponge withdrawal ("second cycle") appears perfectly normal, with conception rates to A.I. of up to 76%. Oestrus is still well synchronized. If sponges are withdrawn over a three day period, over 90% of ewes will be in oestrus over a five day period commencing 16 days later.

In attempts to determine the reasons for the relative failure at first oestrus, the time of ovulation, rate of sperm transport and fertilization of ova have been studied. Ovulation relative to the onset of oestrus appears normal and, provided a large excess of spermatozoa is used, fertilization is normal. Sperm transport does appear somewhat abnormal. The numbers of spermatozoa recoverable from the Fallopian tubes at intervals after insemination are exceedingly variable. Curiously, the numbers appeared higher at 4 and 12 hours than at 24, and were high again at 36 hours. The variation was such, however, that none of these trends was statistically significant.

The most important general conclusion to be drawn from the work with the sheep is the extreme importance of the male, and the variability between males. There is no evidence that ova shed following a controlled ovulation, whether in the breeding season or in anoestrus, are not normally fertilizable. If a large excess of semen of suitably high fertilizing capacity is used, high fertilization rates can be expected. If a dose which is accepted as minimum for normal fertility in an uncontrolled cycle is used, fertility is reduced by about one half. Sperm transport may be implicated, as also may be survival of spermatozoa in the tract. Certainly the normally accepted figure of $100\text{-}150 \times 10^6$ spermatozoa for A.I. needs greatly to be exceeded in the ewe inseminated at the first oestrus. It is unlikely that the variable fertility is due to any change in the tract due to the sponge insert, as this is a characteristic of all form of progestagen treatment.

The extreme variability between males cannot be predicted simply by examination of the semen. Artificial insemination involving controlled oestrus automatically involves frequent collection of semen and dilution, and very little is known of the effects on fertilizing capacity of frequent ejaculation and of differences between rams in the capacity of their, semen to withstand dilution. By analogy with the bull, such differences could be enormous.

In short, any consideration of fertility following control of ovulation must take into account the male, and stresses placed upon him as a result of such control. This is a factor which is commonly overlooked and which perhaps has contributed a great deal to the variability which has characterized practically all work on the control of ovulation in the sheep and in the cow.

III. THE COW

There are many reasons why the use of progestagens poses more problems in the cow than in the ewe. They differ in many respects:

(i) Normally the cow is lactating when it is desired to impregnate her. There is considerable variability in the length and occurrence of oestrous cycles in lactating cows. Oxytocin causes regression of the corpus luteum when given early in the cycle of the cow but not of the ewe. It is conceivable that the let-down hormone and other hormones associated with lactation might influence the outcome of hormonal attempts at synchronization.

(ii) The significance of species-specificity in gonadotrophic hormones cannot be under-rated in the present state of knowledge. Bovine LH is luteotrophic in the cow, but sheep LH is not luteotrophic in the ewe. Bovine prolactin appears not to influence the cycle in the cow, whereas sheep prolactin is lutetrophic in the ewe. This suggests there may be unsuspected mechanisms of ovarian control in the bovine that will not be discovered until bovine preparations are assayed in the bovine.

(iii) Ovulation occurs after the end of oestrus in the cow. This may mean nothing more than that there are widely different neural thresholds for steroids for oestrus and ovulation in the cow. However, it might also mean that the mechanisms for triggering ovulation in the cow are more complex than in the ewe. Delayed ovulation is a problem in cattle and this could further indicate complexities in the cow compared with a simple system of control of follicular maturation in the ewe. In the same vein, excessive follicular development is common in the cow but not in the ewe. The usual explanation for these phenomena is that the cow produces only small amounts of FSH and is very sensitive to it but is relatively less sensitive to LH. PMS will not cause ovulation in the ewe in the presence of a corpus luteum, but will in the cow. These observations also agree with the idea that the cow is very sensitive to FSH.

(iv) There are two waves of follicular growth during the cycle in the cow, one wave commencing soon after ovulation and being replaced about the 12th-14th day after ovulation with a second wave, which presumably contains the follicle destined to rupture at the ensuing oestrus. Luteinization is associated with ovulation and atresia of some follicles at mid-cycle. It seems reasonable to suggest that these waves of follicular growth, atresia and luteinization are associated with fluctuating blood levels of oestrogen. Oestrogen and progesterone show a variety of interacting effects depending on the organ under consideration, the time relations, and the quantities. It seems possible that more will need to be known about the normal bovine oestrous cycle before a thorough understanding of the optimum hormonal treatments for synchronization can be determined.

(v) The cow is more sensitive' to progesterone than is the ewe, and the progesterone-oestrogen time relationships are different. Approximately 10 mg of progesterone per day is required to suppress oestrous cycles in the ewe (live weight approx. 45 kg) and to condition her to respond to oestrogen given at an optimum time of two days after cessation of progesterone treatment. Approximately 30 mg per day is sufficient to suppress oestrous cycles in cows (approx. 450 kg), while 10 mg will condition the spayed heifer (approx. 300 kg) to respond to oestrogen given three days after cessation of treatment (Carrick and Shelton, personal communication). The sensitivity to oestrogen appears somewhat less than that of the ewe on a weight basis. Some 400 μg oestradiol benzoate will induce oestrus in the primed mature cow (Melampy et *al.* 1957), while 200 μg is effective in the spayed heifer (approx. 0.75 μg/kg v. 0.5 μg/kg for the ewe). However variation between animals is considerable.

There is a lag of four to five days between the commencement of regression of the corpus luteum and oestrus in the cow. The comparable period in the ewe is about two days. These times correspond with those needed for optimum expression of the progesterone-oestrogen interaction. Clearly an extension of the study of endocrine actions, interactions, and blood levels is urgently needed.

(vi) The ewe has a well-defined seasonality in reproductive function. There is evidence of a seasonality in the bovine also, though this is not so marked as in the ewe. However, it could mean that optimum treatments for synchronization could differ between times of the year. The ewe has been shown also to be influenced by introduction of the ram. It would be of interest to know if the cow was as easily influenced by the bull.

It is possible that solution of the practical problem of synchronization in the cow may come either from commercial interests or from extrapolation of the work in the ewe, without contributing a great deal to our knowledge of how the method works. The use of progestagens in the human female to prevent conception provides a remarkable example of how a reliable and acceptable technique has preceded complete knowledge of the mechanisms that control the ovarian cycle and of the mode of action of the compounds used. Nevertheless scientists cannot accept the notion that there is little need to understand how a successful technique works. The need in the cow to obtain high fertility after synchronization puts an added burden on the physiologist. The problem is where should we start? Certain studies seem to have high priority.

(a) A thorough study should be made of changes in ovarian morphology throughout the oestrous cycle, using visual techniques.

(b) The steroids produced by the ovary should be identified and where possible measured and related to the observed changes in the genital tract. Oestrogen determinations in blood are difficult but progesterone can be measured more readily. Surgical techniques of cannulation and perhaps of exteriorization of the ovary will have to be developed.

(c) The detailed studies conducted with spayed ewes which have contributed to our understanding of the fine balance between the ovarian hormones and of the endocrine and social factors involved in oestrous behaviour need to be repeated with the cow.

(d) The effects in calves and cows of purified preparations of bovine gonadotrophins will have to be studied. Cost and organization are important problems in such work.

(e) There seems no reason why field trials using synthetic progestagens, administered orally or by implant, injection or plastic sponge, should not continue. A good deal of success has been obtained by oral administration of MAP which at present seems to offer a practicable means of synchronization but less successful results have been obtained with CAP and other progestagens. Injections are unreliable and depots that can be withdrawn at will, such as implants or intravaginal sponges, have not as yet proved reliable. Where synchronization is good but fertility is poor, one can expect normal fertility at the subsequent but less well synchronized oestrus.

(f) It has been assumed that control of the reproductive cycle in the cow is required in order to increase breeding efficiency by reducing costs and improving fertility. It is possible, however, that a wide use could be found for injection of long-acting progestagen preparations for long-term suppression of ovarian function to prevent conception. There seems no reason why this could not prove far more useful than spaying.

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