Improvement in gonadal function through modification of negative feed-back

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Summary. The response to the use of exogenous gonadotrophins to raise the ovulation rate of domestic animals is not sufficiently predictable for the technique to be acceptable for general use. Genetic variation in the ovulation rate of the sheep is however apparently independent of variation in the concentration of gonadotrophins in peripheral plasma, and is postulated to arise from variation in the sensitivity of the hypothalamus to negative feed-back. Changes in ovarian function following unilateral ovariectomy, treatment with œstrogen, immunization against ovarian steroids, and treatment with the antiœstrogen clomiphene all indicate that the ovulation rate can be changed by modifying « negative feed-back » without a change in the variance. It is suggested that the development of this approach to controlling gonadal activity offers greater potential than continued efforts to refine the use of exogenous gonadotrophins such as PMSG.

Introduction.

Current techniques to modify gonadal function in domestic animals tend to depend on the use of exogenous gonadotrophins to bring about quantitative changes (e.g. to raise ovulation rate) or steroids or compounds with steroid like actions, or luteolytic agents to bring about qualitative changes (e.g. to control the timing of ovulation) or combinations of the two to effect the occurence of ovulation. In that the use of these techniques in general and of exogenous gonadotrophins in particular tend to be limited to special circumstances, such as embryo transfer in cattle or sectors of the sheep industry where high prices compensate for their inadequancies, it may be concluded that they are not satisfactory. The variation among the responses of individual animals to exogenous gonadotrophins is such that not only is it difficult to predict the mean response of a group of individuals, the high variance is itself a disadvantage. There is the option to pursue the development of these techniques or to consider alternatives. It will be argued that the modification of steroid feed-back offers a more promising means of raising gonadal function and for inducing ovulation at a time when it would not otherwise occur. This argument is based on three premises :

a) the theoretical consideration that such an approach modifies existing buffering systems through changing their equilibria while maintaining them, rather than over-riding them as with the use of exogenous gonadotrophins.

b) variation in the sensitivity of the hypothalamus/hypophysis to the negative feed-back effects of gonadal hormones on gonadotrophin release has been postulated to underly genetic variation in ovulation rate among some breeds of sheep i.e. it appears to be the cause of natural variation.

c) there are several documented cases of large changes in gonadal fuction through changes in negative feed-back without a change in the variance.
These will be considered in turn.

a) *Theoretical considerations.*

It is accepted that normal gonadal stimulation is buffered. Gonadotrophic hormo-nes from the pituirary gland stimulate the gametogenic and hormonal activities of the gonad and in turn the gonadal hormones feed-back on the activity of the hypothalamus/hypophysis to depress gonadotrophin release and hence their own production.

When exogenous gonadotrophins are used to raise the number of eggs shed it is equally well accepted that the variance of the ovulation rate increases. Although pregnant mares serum gonadotrophin (PMSG) is the gonadotrophin usually used in domestic animals, the use of follicle stimulating hormone (FSH) to induce superovu-lation in laboratory animals indicates that the increase in variance is not a characteristic of PMSG itself. It could be argued that the increase in variance is an obligate corollary to the change in the mean, but information from different experi-ments suggests that exogenous gonadotrophins may override endogenous buffering systems.

When the ovulation rate of sheep has been examined following the use of graded dose of PMSG it is invariably found that the dose-response curve is flat over small doses, or even, in the case of mice, that the ovulation rate may be reduced (Land, 1970). This may be interpreted to indicate that the additional ovarian negative feed-back hormones produced in response to PMSG have a greater depressant effect on endogenous gonadotrophin production in gametogenic terms than the extra gameto-genic activity of the PMSG introduced. Regardless of the balance between gametogenic and steroidogenic activity one could envisage that as the dose of PMSG gradually increased, the extra feed-back would progressively lead to the elimination of endoge-nous gonadotrophin support. This hypothesis is supported by the data of Bindon and Piper (1977), who showed that the removal of PMSG with excess anti PMSG serum 1 or 3 days later in cyclic sheep and cattle respectively led to the ovulation of the normal number of eggs. The data is compatible with collapse of all developed follicles on removal of the only gonadotrophic support, PMSG, followed by a resumption of balanced endogenous gonadotrophic stimulation leading to the ovulation of the normal number of eggs. In the absence of normal buffering systems, small differences in PMSG itself or in ovarian sensitivity, follicle populations, or PMSG clearance between animals could lead to the observed increase in variance. It is not necessarily a direct consequence of a change in the mean.

b) *Genetic variation in ovulation rate.*

Early studies of genetic variation in ovulation rate concentrated on the measure-ment of gonadotrophic hormones, but little association has been observed between

them and ovulation rate. The concentration of LH in peripheral plasma during the oestrous cycle was found to be similar in breeds of sheep differing three-fold in ovulation rate (Land et al., 1973). This observation has now been confirmed and extended to FSH (Bindon et al., 1978), and to environmental variation (Findlay and Cumming, 1976).

The observation that LH release in ewes of high prolificacy was less sensitive to the beed-back effects of œstradiol (Land, Wheeler and Carr, 1976) led to the argument that the variation in ovulation rate arose from variation in the sensitivity of the hypothalamus/hypophysis to negative feed-back. The hypophysis of prolific sheep could tolerate higher concentrations of feed-back hormones and hence more follicular development than could the hypothalamus of sheep of low prolificacy. This hypothesis is supported by the observation that the rate of secretion of œstradiol-17β is higher in Finn-Merino ewes which would be expected to have an ovulation rate around two than in Merino ewes which would be expected to shed only one egg (0.99 $+$ 0.19 vs. 0.37 $+$ 0.09 ng/ml ; Wheeler et al., 1977). It is further supported, by the observation that treatment with oestradiol during the œstrous cycle had less effect on the ovulation rate of highly prolific Finnish Landrace ewes than on the ovulation rate of less prolific Scottish Blackface ewes (Land, 1976). Bindon et al. (1978) did not however detect differences in the concentration of total œstrogen in peripheral plasma in their study of breeds of differing prolificacy.

More recently, the use of silastric implants of œstradiol 17β, has led to a consistent reduction in the ovulation rate of normally prolific Finnish Landrace ewes (Land, unpublished data), confirming the argument that exogenous œstrogen may supplement endogenous negative feed-back, and showing that the mean ovulation rate can be changed predictably without any change in the variance.

In the situations described natural variation in ovulation rate is apparently effected through changes in sensitivity to negative feed-back, rather than through changes in gonadotrophic stimulation, without any change in variability.

c) *Increased gonadal function following changes in negative feed-back.*

Increases in gonadal function are documented for several different situations, for example :

1° *Unilateral ovariectomy.* — Possibly the most dramatic demonstration of the potential of a reduction in negative feed-back to raise ovulation rate is the ovulation of the normal total number of eggs by the remaining ovary following unilateral ovariectomy, e.g. Fenton et al., (1970) show that pigs double the number of eggs shed per ovary when one is removed. The time taken to effect such a change may be very little, in the sheep, for example, the interval required is, at the most, four days ; the programming of the ovary is apparently sufficiently vague even as late as day 14 of the œstrous cycle for the ovulation rate to be doubled : (Land 1973), and with a barely detectable change in gonadotrophins (Findlay and Cumming, 1977).

Furthermore, the variability of the ovulation rate was no greater when the eggs were shed by one ovary with only four days notice than when they were shed by two ovaries with 17 days notice.

2⁰ *Active immunization.* — The possibility of increasing the ovulation rate of the sheep by removing potential feed-back hormones by active immunization of adult females has been reported by Van Look *et al.*, (1978). Immunization against androstenedione lead to an approximate doubling of the ovulation rate of Welsh Mountain ewes from 1.36 ± 0.14 to 2.60 ± 0.17, but greater embryo mortality in the immunized ewes left litter size unchanged. Nevertheless the potential for improvement is obvious.

3⁰ *Passive immunization.* — Temporary changes in ovulation rate could be expecte to result from passive immunization against feed-back steroids. Fairclough. Smith and Peterson (1976) treated ewes with antisera raised against œstradiol both before and throughout the expected time of ovulation but positive feed-back was apparently blocked and the animals failed to ovulate. Treatment whith anti œstrone antisera during the luteal phase of the œstrous cycle of sheep failed to change the ovulation rate of Welsh ewes (Baird and Land, 1976, unpublished). While it is possible that an intermediate « dose » of anti-œstrogen serum may reduce negative feed-back without blocking positive feed-back, it might well be more fruitful to consider passive immunization againt a hormone or hormones with a major role in negative but not positive feed-back.

4⁰ *Pharmacological agents.* — Weak-oestrogens such as clomiphene are widely used to induce ovulation in anovulatory women, but have not been successfully translated to the animal industry. Snook and Hansel (1971) reported a rise in the ovulation rate of sheep following treatment with clomiphene but did not pursue the possibility of developing a standard from of treatment. Further studies (Land and Scaramuzzi, 1978) confirmed that treatment with clomiphene may raise the ovulation of sheep, and indicated that it might have a role to play in the induction of ovulation during anoestrus, but the repeatability of the response was very low. Variation in negative feed-back among breeds and between seasons may account for the difficulty of developing a suitable technique with a compound which is so close to normal oestrogenic activity is the sheep. The potential for a weaker oestrogen is however demonstrated.

5⁰ *Testis growth.* — The growth of the testes of the sheep, both in lambs and between seasons in adults (Land and Carr, 1975 ; Hochereau-de-Reviers, Loir and Pelletier, 1976, respectively) is influenced by the testes themselves as indicated by the acceleration of the rate of growth of the remaining testis when one is removed. Immunization against oestrone and oestradiol by injection of antisera at 14, 16 and 18 weeks of age brought about an increase in the rate of testis growth in Merino lambs previously postulated to be particularly sensitive to negative feed-back (Land, Baird, Carr and McCoubrey, unpublished). At 26 weeks of age the diameter of the testes of lambs immunized against oestrone was 2.9 cm *vs.* 2.4 cm for controls. Again, as in the female, modification of negative feed-back raised gonadal function.

Discussion.

Variation in the equilibria between gonadotrophic stimulation and the feed-back control of gonadotrophin release causes variation in the number of eggs shed without

an increase in variance. While genetic variation and artificially induced decreases in the number of eggs shed can be predicted and demonstrated, artificial increases are a potential rather than a reality. Nevertheless the data available indicate that it may be possible to develop suitable systems. What generalisations may be made ?

Firstly, although steroid hormones have been the prime candidates for immunization, other feed-back links should not be neglected. The demonstration of a role and an identity for inhibin for example would give an obvious alternative antigen.

Secondly the merits of active versus passive immunisation are yet to be resolved. In that steroid hormones tend to have essential roles in the physiology of reproduction in addition to the control of gonadotrophin release these components would also be lost when active immunisation gives high titres, and this may prove to be a relative disadvantage. Passive immunisation might not only allow greater control of antibody titres, it might also more readily offer the opportunity of raise low titres against several feed-back hormones. The collective effect of small changes in such a group of hormones could be sufficient to achieve the desired change in the ovulation rate but the equilibria of none of the hormones would be perturbed to such an extent that other aspects of reproduction were adversely affected.

Thirdly, to avoid the loss of essential roles, it may be reasonable to consider the rule of thumb that individuals should be immunized against steroids of the opposite sex. The loss of androgens from females and oestrogens from males may have less serious side effects than vice versa. The necessity to maintain essential roles may put an upper limit on the potential increase.

Fourth, although clomiphene itself did not give consistent results, the use of other anti-oestrogens should not be neglected.

The preceding discussion has concerned the opportunities for direct intervention to change equilibria. The observation by Mattner, George and Braden (1976) that there is a narrow age window when treatment of ram lambs with testosterone caused quantitative variation in the libido of adults indicates an alternative approach. Such a window might also be present in females, when developing equilibria are sensitive to modification by steroid treatment to give a hypothalamus which is less affected by negative feed-back. We know that genetic variation in ovulation rate, for example, is accompanied by prepuberal LH peaks at different ages (Blanc et al., 1975). It may well be that these differences are associated with conditioning rather than simply responding to variation in the sensitivity of the hypothalamus/hypophysis to negative feed-back.

In view of these considerations, it would apparently be more fruitful to support the investigation of the opportunities for improving gonadal function by modifying steroid feed-back rather than further attempts to refine techniques based on the use of only exogenous gonadotrophins such as PMSG.

Résumé. La réponse à l'utilisation de gonadotrophines exogènes pour augmenter le taux d'ovulation des animaux domestiques est insuffisamment prévisible pour que la technique soit acceptable dans le cas d'un emploi généralisé. Cependant, une variation génétique du taux d'ovulation de la brebis est apparemment indépendante de la variation de la

concentration des gonadotrophines dans le plasma périphérique et elle est supposée venir de la variation de la sensibilité de l'hypothalamus au « feed-back » négatif. Des modifications dans la fonction ovarienne après ovariectomie unilatérale, traitement avec œstrogène, immunisation contre des stéroïdes ovariens et traitement avec un anti-œstrogène, le clomiphène, indiquent que le taux d'ovulation peut être changé en modifiant un « feed-back » négatif sans que la variance ne le soit. Il est suggéré que cette approche de contrôle de l'activité gonadique offre un plus grand potentiel que des efforts continus pour affiner l'utilisation des gonadotrophines exogènes telles que PMSG.

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