A changed responsiveness to oestrogen in ewes with clover disease

N. R. Adams

Division of Animal Health, CSIRO, Institute of Agriculture, University of Western Australia, Nedlands, Western Australia 6009

Summary. When clover-infertile ewes are subsequently exposed to non-oestrogenic pasture, they have a reduced fertilization rate, due to an inability to store spermatozoa in the cervix, and the cervical mucus has a reduced spinnbarkeit, caused by a slower response to oestrogenic stimulation. Vaginal cell keratinization and oestrous behaviour occurred more slowly after treatment of affected ewes with oestrogen. Other changes in affected ewes suggest that phyto-oestrogens have permanent mild differentiating effects on adults. Sexual behaviour is masculinized, the cervix takes on a uterine-like appearance and the genital tract becomes permanently oestrogenized. The manner in which these changes relate to the altered responsiveness to oestrogen remains to be clarified.

Introduction

Severe abnormalities of the reproductive system may occur in sheep exposed to subterranean (Trifolium subterraneum L.) or red (Trifolium pratense L.) clover pastures containing high concentrations of phyto-oestrogens (Bennetts, Underwood & Shier, 1946). These problems constitute the syndrome known as clover disease (Shier & Rossiter, 1949) and include infertility, dystokia and uterine prolapse. Wethers may show enlargement of the bulb-urethral glands, but rams appear to be unaffected (Bennetts et al., 1946). In recent years, prolapse and dystokia have become uncommon in Western Australia and the term clover disease is now used to describe the permanent infertility in ewes after grazing on pastures of this type.

There are several phyto-oestrogenic compounds. Coumestans are the main phyto-oestrogens in the medicines like lucerne (Medicago sativa). In clovers, the isoflavones are more important, the main compounds being genistein, biochanin A and formononetin (Beck, 1964). The oestrogenic activity of clover in ruminants depends mainly on the content of formononetin (Millington, Francis & McKeown, 1964), a substance which itself has little oestrogenicity (Shutt & Cox, 1972). However, several lines of work, reviewed by Cox & Braden (1974), have shown that formononetin is metabolized in the rumen to the more active isoflavon, equol, while genistein and biochanin A are metabolized to inactive derivatives.

The sheep are not only infertile while grazing oestrogenic pasture but remain so even after the ewes have been removed from the pasture for several years (Schinckel, 1948). Oestrus and mating are close to normal in clover-affected ewes (Smith, 1971; Lightfoot et al., 1974), but sperm transport is impaired and fewer ova are fertilized (Bennetts et al., 1946; Turnbull, Braden & George, 1966; Lightfoot, Croker & Neil, 1967). There may also be a greater mortality of embryos in ewes with clover disease (Turnbull et al., 1966), though not all authors agree (Kaltenbach & Davies, 1970).
Most studies on permanent infertility have been carried out on ewes which have grazed oestrogenic pasture for at least three seasons, and then non-oestrogenic pastures for a minimum of 6 months.

Lightfoot et al. (1967) showed that poor sperm transport in clover-affected ewes is due to a failure to form a normal reservoir of spermatozoa in the cervix. Spermatozoa are stored in the cervix after mating (Mattner, 1963), and ascend to the oviducts over a period of time. The cervix of clover-affected ewes does not store spermatozoa efficiently, and 24 h after insemination such ewes have less than 5% of the expected number of spermatozoa (Lightfoot et al., 1967). This impaired sperm storage is associated with a change in the cervical mucus. Smith (1971) and Lightfoot et al. (1974) found that cotton swabs placed in the vagina took up a greater amount of cervical mucus in clover-affected ewes, than in controls at oestrus, although Smith (1971) found that the amount of dry matter taken up in the swabs was not altered. A more direct study on mucus aspirated by pipette from the cervical os (Adams, 1976a) showed that mucus from affected ewes did not have a higher proportion of water content, but had a lower spinnbarkeit. The total amount of mucus was not increased, but because it had a lower viscosity it could penetrate the cotton swabs more readily. Tampion & Gibbons (1962) showed that the structure of mucus orientates the spermatozoa for migration, and it is suggested that the network structure of cervical mucus (Chretien, Cohen & Psychoyos, 1974) is necessary for formation of the sperm reservoir. The reduced spinnbarkeit of mucus from ewes with clover disease reflects an absence of the normal molecular structure and this prevents the effective orientation for sperm migration (Text-fig. 1).

The cervical folds are reduced in size and cervical glands increase in clover-affected ewes (Adams, 1976b). This change in architecture may also affect maintenance of the sperm reservoir. In addition, Lightfoot et al. (1967) found that fewer of the spermatozoa recovered from the cervix of affected ewes were motile. Adams (1976a) could not find any clear-cut toxic effect of mucus from affected ewes on spermatozoa in vitro, and it is possible that the loss of viability seen in vivo was due to a failure of the mucus to separate the spermatozoa from the flow of leukocytes and fluid from the uterus (Mattner, 1968).

Text-fig. 1. Relationship between the spinnbarkeit of cervical mucus (y) and the proportion of spermatozoa guided to swim along a strand of the mucus in vitro (x); y = 0.106x - 1.77; r = 0.60, P < 0.001. The mucus was obtained from clover-affected and control ewes at oestrus, as described by Adams (1975).
Hormonal changes

Both oestrogen and progesterone influence cervical mucus production (Lindsay & Francis, 1969), and both hormones have been studied in clover-affected ewes.

**Oestrogen**

The ratio of RNA to DNA in the uterus is dependent on oestrogen (Little & Lambourne, 1976), but no differences have been found between affected and control ewes in the ratio of RNA to DNA in uterine biopsies taken at oestrus or dioestrus. The duration of oestrous behaviour was not altered in clover-affected ewes (Smith, 1971; Lightfoot et al., 1974). The ovulation rate is slightly higher in clover-affected ewes (Adams, Oldham & Heydon, 1979), and because an increased ovulation rate is generally accompanied by a greater production of oestrogen (Wheeler, Baird, Land & Scaramuzzi, 1977) this may be reflected in a slight increase in the amount of oestrogen circulating in such ewes, although there are no differences in ovarian vein concentrations of oestrogens in affected and control ewes (Rodgers et al., 1980).

Taken altogether, the evidence suggests there may be a slight increase in the amount of endogenous oestrogen in affected ewes, but it appears insufficient to cause the large changes seen in cervical mucus (Lindsay & Francis, 1969).

**Progesterone**

Smith (1975) found no difference between clover-affected and control ewes in peripheral plasma concentrations of progesterone throughout the oestrous cycle. The duration of the cycle was a little more variable in affected ewes (Adams, Lightfoot & Charlick, 1975). The variability probably results from an altered control of the corpus luteum by the uterus, as a result of hydrops uteri or endometritis (Adams, Hearnshaw & Oldham, 1981) which are seen only in severely affected animals and are probably incidental to the major cause of infertility. Obst & Seamark (1975) found reduced plasma progesterone at the end of the oestrous cycle in ewes grazing oestrogenic pasture, but it is more likely that this reflected the concurrent intake of phyto-oestrogens than the development of permanent infertility.

**Luteinizing hormone (LH)**

Findlay et al. (1973) found that ovariectomized ewes with clover disease did not give a positive feedback surge of LH in response to oestradiol-17β, although the pituitary response to synthetic gonadotrophin-releasing hormone was unimpaired. Attempts to repeat this work in intact ewes have been unsuccessful (Rodgers et al., 1980; W. A. Chamley, N. R. Adams, R. P. Hooley & R. Carson, unpublished). Furthermore, the duration of the oestrous cycle is only slightly altered (Adams et al., 1975), indicating that a surge of LH sufficient for ovulation does occur normally. Ewes with clover disease have more frequent episodic releases of LH, and higher baseline values of LH (Rodgers et al., 1980). These findings may explain the higher ovulation rate in affected ewes (Adams et al., 1979), but the sensitivity of the ovaries of these ewes to gonadotrophins has not been studied.

**Abnormal responses to oestrogen**

Considerable effort has been made to determine why clover-affected ewes produce abnormal mucus. Smith (1971) found that after ovariectomy and treatment with progesterone and a single dose of oestradiol benzoate, ewes produced mucus similar to that of controls. This result implied
that the cervical response to hormonal stimulation was normal, but other studies indicated that hormonal levels in affected ewes were also normal. The manner in which cervical mucus responds to treatment with oestrogen was therefore studied. Normal ovariectomized ewes were treated daily with 12.5, 40 or 100 µg oestradiol benzoate and the cervical mucus collected. The volume of mucus and the proportion of water it contained depended mainly on the dose of oestrogen used, but the spinnbarkeit depended primarily on the length of time the ewe had been exposed to oestrogen (Adams & Tang, 1979). These results suggested that the abnormal mucus seen in clover-affected ewes was not caused by a different level of circulating oestrogen, because in ewes with clover disease the volume of mucus and the water content were normal (Adams, 1976a).

Ovariectomized ewes with clover disease were therefore treated with oestrogen for a longer period of time. The spinnbarkeit of mucus was lower in the affected ewes at oestrus, but after ovariectomy followed by 11 days treatment with 10 mg progesterone daily and then a single dose of 25 µg oestradiol benzoate, this difference was less pronounced (Adams, 1979a). Repeated daily treatment with oestradiol benzoate increased the spinnbarkeit in the control ewes, but the increase was much less in those with clover disease, and a significant difference between groups was apparent after treatment for 3 days (Text-fig. 2). Thus the functional lesion appears to be not a different degree of hormonal stimulation, but a decrease in the perceived duration of stimulation. This conclusion is supported by other studies. The degree of keratinization of the vaginal epithelium did not increase as rapidly in affected ewes treated with oestradiol benzoate (Adams, 1979a). Furthermore, when 14 clover-affected and 14 control ovariectomized ewes were treated daily with 40 µg oestradiol benzoate, there was little difference between the groups in the total amount of oestrous behaviour, but the affected ewes were slower to exhibit oestrous behaviour ($P < 0.05$) and to become refractory to oestrogen (Text-fig. 3). This result differs from that of Scaramuzzi, Lindsay & Shelton (1972) who treated normal ewes repeatedly with oestradiol benzoate; the doses of oestrogen which hastened the onset of oestrus also delayed the onset of refractoriness, except at a very high dose. The behavioural change in clover-affected ewes therefore again suggests a slower rate of response to oestrogen or a failure to be 'primed' by a previous oestrogen treatment.

Text-fig. 2. Mean spinnbarkeit (±s.e.m.) of cervical mucus from 10 control and 10 clover-affected ewes at oestrus, and after ovariectomy and progesterone priming, during daily treatments with 25 µg oestradiol benzoate. Data from Adams (1979a).
Preliminary results suggest that cervical refractoriness to oestrogen is not enhanced in affected ewes. The ovariectomized ewes studied in the experiment on oestrous behaviour were treated for 2 days with 40 μg oestradiol benzoate commencing 13 days after the end of the preceding experiment. Cervical mucus was collected on cotton swabs. The amount of mucus from control ewes declined significantly over the 2-day test period (0.61 ± 0.08 compared with 0.30 ± 0.05 g; \( P < 0.05 \)) but was unchanged in clover-affected ewes (1.05 ± 0.19 compared with 0.87 ± 0.19 g).

It seems unlikely that the altered responsiveness depends on a changed ability to metabolize oestrogen. Tang & Adams (1978a) found that clearance rates and hepatic conjugation of oestradiol-17β were normal in affected ewes. Furthermore, a changed metabolism of oestrogen might be expected to affect responses to the dose level of oestrogen as well as responses to the duration of exposure.

**Developmental changes**

The reason why ewes with clover disease respond more slowly to oestrogen is not yet clear. Other changes also occur, which seem best understood as reflecting a permanent differentiation caused by prolonged exposure to phyto-oestrogens even though they are brought about in ewes exposed after puberty.

**Cervical histology**

Cystic hyperplasia of the endometrial glands is observed in ewes with clover disease (Bennetts et al., 1946), and the cervix may become more like the uterus (Adams, 1976b). The cervical folds fuse so that the crypts are lost, and the lamina propria is filled with cells similar to those seen in uterine stroma. Glands, normally absent from the cervix, are abundant and their histochemical reactions are similar to those of uterine glands (Heydon & Adams, 1977). The surface epithelium has more columnar but fewer goblet cells, and less stratified squamous epithelium (Lightfoot & Adams, 1979). This appearance of the cervix is the opposite of that seen when ewes are first treated with phyto-oestrogen (Adams, 1977). Similar changes have recently been produced in the cranial third of the cervix of 3 ewes treated for 12 weeks with three 3 cm
implants containing oestradiol-17β of the kind described by Karsch et al. (1973). The histological change is therefore not due to some aberrant effect of phyto-oestrogen but is a response to oestrogen itself.

The occurrence of uterine-like cells in the cervical fornix and anterior vagina has been described for mice treated with oestradiol-17β during the first 5 days of life (Forsberg, 1969). A similar change is reported for women whose mothers were treated with diethylstilboestrol during pregnancy (Herbst, Kurman & Scully, 1972). These findings show that oestrogen can cause the type of change seen in ewes with clover disease, although such changes occur only during organogenesis in other species.

**Masculinization and defeminization of behavioural responses**

The most specific type of differentiation caused by oestrogens or aromatizable androgens is a defeminization and masculinization of the sexual behavioural response to hormonal treatment later in life (Gorski, 1973). To see if this occurred in ewes with clover disease, 20 ovariectomized clover-affected ewes and 19 controls were injected daily for 30 days with 5 mg testosterone propionate in oil. The ewes were observed daily with 3 rams and their behaviour recorded. The clover-affected ewes showed a delay to first mount by the ram (3.14 ± 0.51 compared with 1.73 ± 0.15 (s.e.m.) days, \( P < 0.05 \)). The number of days on which ewes were mounted was not significantly different (affected 7.6 ± 1.8 days; control 10.3 ± 2.0 days). Clover-affected ewes showed aggressive behaviour sooner (15.7 ± 1.6 days) than did controls (21.8 ± 2.1 days, \( P < 0.05 \)).

Male-like behaviour was measured by testing each ewe with 3 oestrous ewes in a small pen after 21 and 28 days of testosterone treatment. Clover-affected ewes showed more courting behaviour sooner (15.7 ± 1.6 days) than did controls (21.8 ± 2.1 days, \( P < 0.05 \)).

**Permanent oestrogenization of the genital tract**

In mice treated neonatally with a large dose of oestrogen or an aromatizable androgen, the vaginal epithelium becomes permanently keratinized and this persists after ovariectomy (Terenius, Meyerson & Palis, 1969). There is a higher rate of cell turnover (Mori, 1975) and greater RNA, DNA and protein synthesis in the reproductive tract, even after ovariectomy (Kohrman & Greenberg, 1968). Vaginal flushings taken during dioestrus from ewes with clover disease indicated that the ewes appeared to be under oestrogenic stimulation (Tang & Adams, 1978b). Flushings from affected ewes had greater amounts of glycoprotein, and lower levels of enzymes. Further studies on ovariectomized ewes showed that clover-affected ewes have a thicker, more keratinized vaginal epithelium and increased protein and glycoprotein synthesis in the cervix (Tang & Adams, 1981).

**Other changes**

Affected ewes start to show oestrus at the normal time of year, but oestrous cycles are more erratic in the first half of the breeding season (Adams, 1979c). The amount of cervical mucus produced in response to oestrogen varies with the time of year but is similar in affected and control ovariectomized ewes (Adams, 1980).

Infertile ewes have permanent masculinization of the vulva, including growth of the clitoris and fusion of the ventral commissure (Adams, 1979b). These changes persist after removal from oestrogenic pasture, which again is consistent with a developmental type of change. Therefore, except for the failure to observe clearly defined differences of LH secretion in affected ewes, all of the changes brought about by oestrogen in the fetal or neonatal rodent have their counterpart in
ewes with clover disease, suggesting that the permanent infertility is a result of mild differentiation occurring in the adult.

The manner in which differentiation brings about a changed responsiveness to oestrogen is not yet clear. Both sexes have similar numbers of cytoplasmic oestrogen binding sites in the hypothalamus and pituitary gland, (Barley, Ginsburg, MacLusky, Morris & Thomas, 1977). The principal difference is that, after binding, translocated receptors remain in the nucleus for a shorter time in the male (Whalen & Massicci, 1975; Marrone & Feder, 1977). Because the duration of response is related to the duration of nuclear occupancy by the oestrogen–receptor complex (Bouton & Raynaud, 1979), the altered rate of responsiveness in affected ewes may result from changes in the ability of the nucleus to bind the oestrogen–receptor complex.

I thank M. R. Sanders for assistance and the Western Australian Department of Agriculture for the clover-diseased ewes.

References

Adams, N.R. (1975) Relationship between cervical mucus spinbarkheit and the direction of sperm migration in ewes with clover disease. J. Reprod. Fert. 43, 391–392.

Adams, N.R. (1976a) Cervical mucus changes in infertile ewes previously exposed to oestrogenic subterranean clover. Res. vet. Sci. 21, 59–63.

Adams, N.R. (1976b) Pathological changes in the tissues of infertile ewes with clover disease. J. comp. Path. 86, 29–35.

Adams, N.R. (1977) Morphological changes in the organs of ewes grazing oestrogenic subterranean clover. Res. vet. Sci. 22, 216–221.

Adams, N.R. (1978a) Altered response of cervical and vaginal epithelia to oestradiol benzoate in ewes after prolonged exposure to oestrogenic pasture. J. Reprod. Fert. 56, 611–613.

Adams, N.R. (1979b) Masculinisation of the external genitalia in ewes with clover disease. Aust. vet. J. 55, 22–24.

Adams, N.R. (1979c) Depressed incidence of oestrus in ewes with clover disease at the beginning of the breeding season. Aust. vet. J. 55, 481–484.

Adams, N.R. (1980) Seasonal variation in the nature of cervical mucus response to oestradiol benzoate in normal and clover-affected ovariotomized ewes. Biol. Reprod. 21, 1203–1207.

Adams, N.R. & Tang, B.Y. (1979) Changes in ovine cervical mucus in response to oestrogen treatment. J. Reprod. Fert. 57, 261–266.

Adams, N.R., Lightfoot, R.J. & Charlick, A.J. (1975) The duration of the oestrous cycle of ewes affected with clover disease. Aust. vet. J. 51, 306–309.

Adams, N.R., Oldham, C.M. & Heydon, R.A. (1979) Ovulation rate and oocyte numbers in ewes after prolonged exposure to oestrogenic pasture. J. Reprod. Fert. 55, 87–89.

Adams, N.R., Hearnsaw, H. & Oldham, C.M. (1981) Abnormal function of the corpus luteum in some ewes with permanent phyto-oestrogen infertility. Aust. J. Biol. Sci. 34, 61–65.

Barley, J., Ginsburg, M., MacLusky, N.J., Morris, I.D. & Thomas, P.J. (1977) Sex differences in the distribution of cytoplasmic oestrogen receptors in rat brain and pituitary: effects of gonadectomy and neonatal androgen treatment. Brain Res. 129, 309–318.

Beck, A.B. (1964) The oestrogenic isoflavones of subterranean clover. Aust. J. agric. Res. 15, 223–230.

Bennett, H.W., Underwood, E.J. & Shier, F.L. (1946) A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Aust. vet. J. 22, 2–12.

Bouton, M.M. & Raynaud, J.P. (1979) The relevance of interaction kinetics in determining biological response to estrogens. Endocrinology 105, 509–515.

Chretien, F.C., Cohen, J. & Psychoyos, A. (1974) A scanning electron microscope study of changes in baboon cervical mucus during the menstrual cycle. J. Reprod. Fert. 40, 447–449.

Cox, R.I. & Braden, A.W. (1974) The metabolism and physiological effects of phyto-oestrogens in livestock. Proc. Aust. Soc. Anim. Prod. 10, 122–129.

Findlay, J.K., Buckmaster, J.M., Chamley, W.A., Cumming, I.A., Hearnsaw, H. & Godding, J.R. (1973) Release of luteinizing hormone by oestriadiol-17β and a gonadotrophin-releasing hormone in ewes affected with clover disease. Neuroendocrinology 11, 57–66.

Forberg, J.G. (1969) The development of atypical epithelium in the mouse uterine cervix and vaginal fornix after neonatal oestriadiol treatment. Br. J. exp. Path. 50, 187–195.

Gorski, R.A. (1973) Perinatal effects of sex steroids on brain development and function. Prog. Brain Res. 39, 149–163.

Herbst, A.L., Kurman, R.J. & Scully, R.E. (1972) Vaginal and cervical abnormalities after exposure to stilbestrol in utero. Obstet. Gynec., N.Y. 40, 287–298.

Heydon, R.A. & Adams, N.R. (1977) Histochecmical studies on cervical glands in ewes with clover disease. J. comp. Path. 87, 353–361.

Kaltenbach, C.C. & Davies, H.L. (1970) Fertilization,
sperm transport, and early embryonic loss in ewes grazed on cultivars of subterranean clover (T. subterraneum). Aust. J. agric. Res. 21, 107–114.

Karsch, F.J., Dierschke, D.T., Weick, R.F., Yamaji, T., Hotchkiss, J. & Knobil, E. (1973) Positive and negative feedback control by estrogen of lutetinizing hormone secretion in the rhesus monkey. Endocrinology 92, 799–804.

Kohrmann, A.F. & Greenberg, R.E. (1968) Permanent effects of estradiol on cellular metabolism of the developing mouse vagina. Devl Biol. 18, 632–650.

Lightfoot, R.J. & Adams, N.R. (1979) Changes in cervical histology in ewes following prolonged grazing on oestrogenic subterranean clover. J. comp. Path. 89, 367–373.

Lightfoot, R.J., Croker, K.P. &Neill, H.G. (1967) Failure of sperm transport in relation to ewe infertility following prolonged grazing on oestrogenic pastures. Aust. J. agric. Res. 18, 755–765.

Lightfoot, R.J., Smith, J.F., Cumming, I.A., Marshall, T., Wrost, R.H. & Hearnshaw, H. (1974) Infertility in ewes caused by prolonged grazing on oestrogenic pastures: oestrus, fertilization and cervical mucus. Aust. J. biol. Sci. 27, 409–414.

Lindsay, D.R. & Francvis, C.M. (1969) Effect of progesterone and duration of pasture intake on cervical mucus response to phyto-oestrogens in sheep. Aust. J. agric. Res. 20, 719–724.

Little, D.A. & Lambourne, L.J. (1976) Changes in the nucleic acid content of the uterus of sheep during involution following ovarioectomy and following oestrogen stimulation. Aust. J. agric. Res. 27, 669–674.

Marrone, B.L. & Feder, H.H. (1977) Characteristics of [3H]oestradiol and [3H]progesterin uptake and effects of progesterone on [3H]oestradiol uptake in anterior pituitary and peripheral tissues of male and female guinea pigs. Biol. Reprod. 17, 42–57.

Mattner, P.E. (1963) Spermatozoa in the genital tract of the ewe. II. Distribution after coitus. Aust. J. biol. Sci. 16, 688–694.

Mattner, P.E. (1968) The distribution of spermatozoa and leukocytes in the female genital tract in goats and cattle. J. Reprod. Fert. 17, 253–261.

Millington, A.J., Francis, C.M. & McKeeown, N.R. (1964) Wether bioassay of annual pasture legumes. I. The oestrogenic activity of nine strains of Trifolium subterraneum L. Aust. J. agric. Res. 15, 527–536.

Mori, T. (1975) Effects of postpartum estrogen injections on mitotic activity of vaginal and uterine epithelial cells in mice treated neonatally with oestrogen. J. Endocr. 64, 133–140.

Obst, J.M. & Seamark, R.F. (1975) Hormone studies on ewes grazing on oestrogenic (Yarloop clover) pasture during the reproductive cycle. Aust. J. biol. Sci. 28, 279–290.

Rodgers, R.J., Clarke, I.J., Findlay, J.K., Brown, A., Cumming, I.A., Muller, B.D. & Walker, S.K. (1980) Plasma LH and FSH in ewes that were either fertile or infertile after long-term grazing of oestrogenic pasture. Aust. J. biol. Sci. 33, 213–220.

Searamuzzi, R.J., Lindsay, D.R. & Shelton, J.N. (1972) Effect of repeated estrogen administration on oestrus behaviour in ovarioctomized ewes. J. Endocr. 52, 269–273.

Schnickel, P.G. (1948) Infertility in ewes grazing subterranean clover pastures. Observations on breeding behaviour following transfer to "sound" country. Aust. vet. J. 24, 289–294.

Shier, F.L. & Rossiter, R.C. (1949) Clover disease. Practical findings and recommendations for control. J. Dep. Agric. West. Aust. 26, 111–116.

Shutt, D.A. & Cox, R.L. (1972) Steroid and phyto-oestrogen binding to sheep uterine receptors in vitro. J. Endocr. 52, 299–310.

Smith, J.F. (1971) Studies on ovine infertility in agricultural regions of Western Australia: cervical mucus production by fertile and infertile ewes. Aust. J. agric. Res. 22, 513–519.

Smith, J.F. (1975) Studies on ovine infertility in agricultural regions of Western Australia: plasma progesterone levels of fertile and infertile ewes. Aust. J. biol. Sci. 28, 85–88.

Tampion, D. & Gibbons, R.A. (1962) Orientation of spermatozoa in mucus of the cervix uteri. Nature. Lond. 194, 381.

Tang, B.Y. & Adams, N.R. (1978a) Changes in oestradiol-17β binding in the hypothalamus and pituitary glands of persistently infertile ewes previously exposed to oestrogenic subterranean clover: evidence of alterations to oestraadiol receptors. J. Endocr. 78, 171–177.

Tang, B.Y. & Adams, N.R. (1978b) Enzyme activities and protein and carbohydrate concentrations in cervical secretions at dioestrus in normal ewes and ewes with permanent phyto-oestrogen infertility. Aust. J. biol. Sci. 31, 241–246.

Tang, B.Y. & Adams, N.R. (1981) Oestrogen receptors and metabolic activity in the genital tract of ovarioctomized ewes with permanent infertility caused by exposure to phyto-oestrogens. J. Endocr. 89, 365–370.

Terenius, L., Meyerson, B.J. & Palis, A. (1969) The effect of neonatal treatment with 17β-oestradiol or testosterone on the binding of 17β-oestradiol to mouse uterus and vagina. J. Endocr. 62, 671–678.

Turnbull, K.E., Braden, A.W.H. & George, J.M. (1966) Fertilization and early embryonic losses in ewes that had grazed oestrogenic pastures for 6 years. Aust. J. agric. Res. 17, 907–917.

Whalen, R.E. & Massicci, J. (1975) Subcellular analysis of the accumulation of estrogen by the brain of male and female rats. Brain Res. 89, 255–264.

Wheeler, A.G., Baird, D.T., Land, R.B. & Scaramuzzi, R.J. (1977) Genetic variation in the secretion of oestrogen in the ewe. J. Endocr. 75, 337–338.