AUSTRALIAN SOCIETY
for
REPRODUCTIVE BIOLOGY

PROCEEDINGS
OF THE
EIGHTH
ANNUAL CONFERENCE

BRISBANE
August 18–20, 1976
AUSTRALIAN SOCIETY FOR REPRODUCTIVE BIOLOGY

EIGHTH ANNUAL CONFERENCE

Department of Physiology Lecture Theatres
University of Queensland
St Lucia

August 18, 19, 20, 1976

PROGRAMME and ABSTRACTS OF PAPERS

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**AUSTRALIAN SOCIETY FOR REPRODUCTIVE BIOLOGY**

August 1976

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ACKNOWLEDGEMENTS

The Society acknowledges with thanks the following agencies who have assisted in organizing the Eighth Annual Conference -

The University of Queensland,
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**AUSTRALIAN SOCIETY FOR REPRODUCTIVE BIOLOGY**

Eighth Annual Conference
August 18-20th, 1976

Physiology Lecture Theatres,
University of Queensland, St. Lucia.

**PROGRAMME**

Wednesday, August 18

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<td>J.D. O’shea, M.G. Nightingale &amp; W.A. Chamley.</td>
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<td>Vascular Changes During Luteal Regression in Sheep.</td>
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<td>Ultrastructure of Luteal Cells in Several Mammalian Species with Reference to the Secretion of Progesterone.</td>
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<td>Corpus Luteum Activity During the Oestrous Cycle of the Hopping Mouse Notomys Alexis.</td>
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R.C. Jones. The Nature of Ultrastructural Changes Induced by Exposure of Spermatozoa to Lysolecithin.

VASCULAR CHANGES DURING LUTEAL REGRESSION IN SHEEP

J.D.O'Shea*, M.G.Nightingale* and W.A.Chamley.# Departments of *Veterinary Preclinical Sciences and #Physiology, University of Melbourne, Victoria.

It has been shown that a dramatic fall in luteal blood flow occurs during luteolysis in sheep, but the mechanism has not been established. Changes in luteal capillaries in regressing corpora lutea (CL) are reported here.

Nineteen CL from sheep at Days (D) 1, 3, 11, 12, 13, 14, 15 and 16 of the cycle, and D14 and 17 of pregnancy, were examined by light and electron microscopy. A radioimmunoassay for progesterone was performed on luteal tissue from 17 of these CL.

Capillaries in all CL earlier than D14, and during pregnancy, appeared normal. A gap of ≈10nm separated the apposing plasma membranes at most intercellular junctions, with foci of closer contact. Larger gaps were occasionally seen, but the endothelium of most capillaries was not fenestrated. The progesterone content of the CL not showing capillary changes, including 4 from D14 or 15 of the cycle, was 19.74 ± 6.72 μg/g (n = 12).

Capillary abnormalities were first seen in 1 CL at D14, and were constantly present and widespread by D16. In these CL, many capillaries contained cellular debris, incorporating numerous basophilic, Feulgen +ve, chromatin granules. This debris first appeared at a time when most luteal cells showed little evidence of degeneration. Electron microscopy showed it to consist of numerous membrane-bounded electron-dense cellular fragments closely resembling the "apoptotic bodies" described by Kerr et al. (1972) as a feature of cell deletion in many tissues. These contained recognisable, cytoplasmic organelles and many dense nuclear remnants. Erythrocytes and occasional leucocytes were interposed between these fragments, but there was no evidence that platelets or leucocytes contributed significantly to the debris itself. Similar debris was less frequently present outside blood vessels at the time of its first appearance within capillaries.

Many endothelial cells of affected capillaries appeared normal, although endothelial gaps were increased in number and size. Some endothelial cells encircled, and may have engulfed, debris similar to that in the lumen. Others showed degenerative changes, and in some cases appeared to be in direct continuity with intraluminal debris. Pericytes also showed degenerative changes, and some capillaries appeared to be disintegrating.

In CL with capillary abnormalities, the luteal cells showed progressive degenerative changes. By D1 of the following cycle, luteal cell degeneration was advanced, with a reduction in the number of identifiable capillaries. Some remaining capillaries contained cell debris. The progesterone content of the CL showing capillary changes was 4.09 ± 2.56 μg/g (n = 5).

Thus at an early stage of regression, many luteal capillaries were filled with degenerating cellular debris, at least some of which appeared to be of endothelial origin, together with trapped blood cells. The precise significance of these capillary changes remains to be established: however, it is suggested that they may represent steps in the process by which many capillaries rapidly disappear from the regressing CL. Furthermore, these changes are presumably indicative of, and possibly causative in, a severe reduction in luteal capillary flow.

Reference

In eutherian mammals a correlation has been reported between the presence of venous and arterial pathways common to the ovary and uterus, and the occurrence of a unilateral uterine luteolytic mechanism. This paper reports observations on the blood vessels of the female genital tract in some marsupials in which the duration of the oestrous cycle exceeds that of pregnancy, and in which a uterine luteolytic mechanism would not be predicted.

Latex and/or silicone rubber casts of the genital blood vessels of 15 adult female brush possums (Trichosurus vulpecula) and up to 3 specimens of each of the macropods Megaleia rufa, Macropus giganteus, M. eugenii, M. agilis and Thylogale billardieri, were examined. Histological studies were performed on blood vessels from formalin-perfused specimens of T. vulpecula.

In T. vulpecula the genital tract is supplied by 3 major paired arteries and drained by corresponding paired veins, here named the ovarian and anterior and posterior urogenital vessels. In addition the cloaca is supplied by branches of the internal pudendal artery and vein.

The ovarian arteries arise from the aorta and, on approaching the ovary, give rise to a leach of small branches supplying the ovary, oviduct and anterior end of the uterus. Six to twelve of these branches supply the ovary: these are tortuous, anastomose with one another, and entwine with branches of the venous plexus draining the ovary. The ovarian vein, which is formed by the junction of corresponding branches, runs closely alongside the artery before joining the posterior vena cava.

The anterior and posterior urogenital arteries arise from the internal iliac artery. The anterior supplies the hind-part of the uterus, the lateral and median vagina, the anterior part of the urogenital sinus, and the bladder. The posterior supplies the remainder of the urogenital sinus. Corresponding veins draining the areas supplied by these arteries drain into the internal iliac veins.

In both arteries and veins, substantial anastomoses occur between branches of the ovarian and anterior urogenital, and branches of the anterior and posterior urogenital, respectively. Across-the-midline anastomoses also occur. Finally the ureteric vessels, which arise from or close to the renal vessels, anastomose with branches of both the ovarian and anterior urogenital vessels. Histologically, the ovarian artery and vein were of conventional structure and locally in close surface-to-surface contact.

The genital vessels in the macropods examined were essentially similar to those of T. vulpecula. However, the uterine branches of the ovarian vein appeared proportionately larger, and in some specimens the anterior and posterior urogenital vessels arose by common trunks.

There are marked similarities between the ovarian vessels of these marsupials, and those of many eutherian mammals. These similarities include the close venous-arterial relationships, suggestive of a specialization for countercurrent exchange, and the association with uterine vessels. A difference is seen in the large numbers of ovarian arterial branches in marsupials, which provides an interesting parallel with the testicular arteries in these species.
ULTRASTRUCTURE OF LUTEAL CELLS IN SEVERAL MAMMALIAN SPECIES WITH REFERENCE TO THE SECRETION OF PROGESTERONE
R. T. Gemmell and B. D. Stacy
C.S.I.R.O., Division of Animal Production,
P.O. Box 239, Blacktown, N.S.W., 2148.

In a previous ultrastructural study it was shown that there is a correlation between the formation and secretion of densely-staining granules and the known pattern of progesterone secretion in the cycling ewe (1). It was of interest to know whether similar features could be detected in other species, and this report deals with the examination of luteal tissue from the goat, cow, pig, rat, rabbit and guinea pig. Tissues from ovaries with actively secreting corpora lutea were fixed by intravascular perfusion with glutaraldehyde.

Granules, 0.2 μm diameter, were observed in the cytoplasm of luteal cells in all species but the presence of secretory granules in the surrounding intercellular space was noticed only in tissue from the sheep, cow, goat and pig. Agranular endoplasmic reticulum was prevalent in all species. Whorls of endoplasmic reticulum enveloping lipid droplets were present in luteal cells of the pig, rabbit and guinea pig, sheets of endoplasmic reticulum were also seen in these three species as well as in the cow. In the sheep, goat and rat the endoplasmic reticulum was not arranged in obvious, discrete structures. The sheep and goat were the only species in which the luteal cells were characteristically devoid of lipid in the secretory phase of the cycle; lipid droplets in these species were only prominent in the regressive phase. Densely-staining granules were found in close proximity to the lipid droplets within the whorls of agranular endoplasmic reticulum in the pig, rabbit and guinea pig. It is difficult to reconcile this structural finding with the pathways of synthesis and secretion of progesterone that have been proposed for the luteal cells in the sheep (1).

From this study of various species it is concluded that all luteal cells secreting progesterone share similar basic structural features characterized by the presence of agranular endoplasmic reticulum, Golgi regions and densely-staining granules.

REFERENCES
The length of the oestrous cycle of the Hopping mouse is about 8 days (1,2) and mucification of the vaginal epithelium occurs late in dioestrus (2), thus the corpus luteum may be functional especially as pseudopregnancy of mice, hamsters, and voles is of similar length.

This has now been investigated by (a) measuring progestin levels in peripheral blood, obtained from lightly ether anaesthetised animals by heart puncture, using the rapid competitive protein binding method (3) except that cyclohexane was used for extraction and dog plasma as binding protein, (b) determination of whether follicles could be ovulated during dioestrus by exogenous administration of 5 iu HCG, and (c) determination of whether a uterine decidual cell response could be obtained by unilateral intraluminal injection of 0.2 ml of arachis oil.

Serial sections of ovaries showed that usually 2 or 3 sets of corpora lutea occurred, thus indicating that these remain histologically visible for about 20 days, since mean cycle length is 7-9 days. Progestin levels during the cycle were: pro-oestrus, 129 ± 39 ng/ml (n=6); oestrus, 76 ± 24 ng/ml (n=4); dioestrus 1 and 2, 20 ± 7 ng/ml (n=5); dioestrus 3 and 4, 29 ± 10 ng/ml (n=7); dioestrus 5 and 6, 94 ± 21 ng/ml (n=6); dioestrus 7 and 8, 15 ± 4 ng/ml (n=3). Thus it appears that the highest levels of progestin occurs at pro-oestrus, but there is a second smaller peak late in dioestrus. Since seven samples from male Hopping mice had 13 ± 2 ng/ml of progestin, it may be that progestin early in dioestrus is of extraovarian origin.

Ovarian histology indicated small vesicular follicles only on day 1 of dioestrus (mean maximum size 440 ± 20 μm), whereas from dioestrus 2 follicles 600 μm were usually present. Only 1 out of 5 experimental animals given 5 iu HCG on dioestrus 1 ovulated, whereas 8 individuals given HCG between dioestrus 2 and 5 invariably had eggs in cumulus clot in their fallopian tubes 48 hours later. This indicates that "ovulable" follicles are present throughout most of dioestrus.

Unilateral injection of oil into uterine horns on either dioestrus 2, 3, 4 or 5 did not result in enlargement of that horn compared to the uninjected contralateral control horn 2-4 days later (n=2 in all cases). However, the placing of a vasectomised male with females, that had previously had regular cycles, resulted in a subsequent cycle length of 13-16 days (n=4).

Thus it is concluded that no functional corpus luteum is present, during the normal oestrous cycle of the Hopping mouse in spite of a small rise in progestin and mucified vaginal epithelium late in dioestrus, and pseudopregnancy may be 13-16 days. The reason for the comparatively long cycle in this and probably other Pseudomyids compared to common laboratory rodents is still unknown. Further investigations are continuing and these may shed some light on fundamental concepts underlying the control of ovarian follicular dynamics.

In six cyclic Merino ewes, Doppler ultrasonic probes (1) with luminal diameters of 1.5 or 2.0 mm were implanted around each ovarian artery either immediately below or within the vascular cone region. Subsequently, total blood flow (TBF) to each ovary (expressed as velocity (cm/sec)) and the concentration of progesterone in peripheral plasma was monitored over several oestrous cycles.

In both ovaries, the TBF was at base level from Day -1 until Day +3, inclusive, of the oestrous cycle (oestrus = Day 0); the mean flows ranged from 4.7 to 5.8 cm/sec in the ovulatory ovary and from 4.1 to 4.7 cm/sec in the non-ovulatory ovary. A similar order of flow was maintained in the non-ovulatory ovary during the remainder of the cycle. However, the flow to the ovulatory ovary gradually increased to a maximum level at about day 13 (mean, 17.9 cm/sec; range 13.2 to 25.1) and then declined sharply over the next 2 to 3 days to base levels. The changes in the TBF occurring in the ovulatory ovary during the oestrous cycle followed a similar pattern to that for the concentration of progesterone in the peripheral plasma. The mean values for the plasma progesterone levels and the prevailing mean TBF levels during the cycle were highly correlated (r = 0.980, P < 0.001).

While timed collections of ovarian venous outflow in anaesthetized ewes (2) indicated that the TBF in either ovary was elevated during the luteal stage of the oestrous cycle, the procedures adopted markedly affect ovarian flow, especially in the ovary without a corpus luteum (3). The present results indicate that there is a local control of ovarian blood flow in ewes and not a humoral control as suggested previously (2).

REFERENCES


HISTOLOGICAL OBSERVATIONS OF THE OVARY OF THE
CUNNINGHAM'S ROCK SKINK, *Egernia cunninghami*

G.S. Kesby
School of Anatomy
University of New South Wales
Kensington, N.S.W. 2033

In reptiles, the mode of reproduction varies from oviparous or egg-laying to truly placental or viviparous. In *Egernia cunninghami*, as in most ovoviviparous Australian skinks, the embryo develops within the uterus with a yolk-sac placenta initially and then a fairly well developed chorioallantoic placenta until birth. The appearance of a shell membrane, a diagnostic feature of ovoviviparity, is only brief and in the early part of the pregnancy. As an integral part of an overall study of reproduction and ovoviviparity in this species, the development of the ovum, with primary and secondary egg membranes, was studied. Adult female specimens were sampled at regular intervals during the yearly breeding cycle.

The ovaries of the Cunningham's skink can be considered to be typically reptilian in most respects. Each is an elongated sac, with 6-18 developing ova protruding into the central cavity from a thin mantle of cortical stroma. Primary oogonia are situated in a germinal epithelial bed on the medial aspect of the ovary.

The central mass of the developing and mature follicle is the large ovum which rapidly accumulates yolk droplets in its cytoplasm. The cytoplasm is contained within a distinct vitelline membrane. This is surrounded by a two-layered zona pellucida. The growing oocyte completely fills the follicle at all stages, with no evidence of a fluid-filled antrum. In small follicles the follicular epithelium is a single layer of low cuboidal cells which soon become columnar. With further growth the follicular epithelium differentiates into a pseudostratified epithelium with three morphologically different cell types: small, intermediate and pyriform or large cells. The large cells are nutritive in function and pass material through the zona pellucida and vitelline membrane into the oocyte cytoplasm. Towards ovulation, the granulosa epithelium again becomes flattened and single-layered.

The membrane granulosa is supported by a membrana propria. External to this is the theca, which in the early stages is uniform and poorly developed. By the time the ova are 5-8 mm in diameter the theca has differentiated into interna and externa. These layers remain identifiable until ovulation occurs when the mature ovum is 15-20 mm in diameter.

The results obtained show that the development of the ovum in the Cunningham's skink is similar to most other reptiles, whether oviparous or viviparous. There does not seem to be any single ovarian feature which could be correlated with the phenomenon of ovoviviparity.
BIOCHEMICAL STUDIES ON SHEEP OVARIAN FOLLICLES MAINTAINED IN ORGAN CULTURE.

R.F. Seamark, F. Amato, Susan Hendrickson, Leila Mak and R.M. Moor,
Department of Obstetrics and Gynaecology, University of Adelaide,
South Australia 5000.

The development of techniques for organ culture has made possible detailed biochemical studies on the response of individual ovarian tissues to gonadotrophic stimulation. In this study we report on the effects of human chorionic gonadotrophin (hCG 20 iu ml⁻¹) on oxygen uptake, glucose utilization, lactate production and cellular content of adenine nucleotides of sheep follicles isolated and maintained under the conditions of culture described by Moor (1).

The results, expressed as nmols per mg wet weight tissue per hr, (mean value ± SEM, No. of observations in parentheses) are summarised below.

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<tr>
<th>Condition</th>
<th>Entire follicles</th>
<th>Theca</th>
<th>Granulosa</th>
<th>+hCG</th>
<th>Entire follicles</th>
<th>Theca</th>
<th>Granulosa</th>
<th>+hCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen consumption</td>
<td>560±60 (9)</td>
<td>1080±164 (6)</td>
<td>52±14 (6)</td>
<td></td>
<td>530±44 (12)</td>
<td>1307±221 (6)</td>
<td>110±22 (6)</td>
<td>1307±221 (6)</td>
</tr>
<tr>
<td>Glucose utilization</td>
<td>8.5±0.7 (6)</td>
<td>9.6±0.8 (6)</td>
<td>9.7±1.1 (6)</td>
<td></td>
<td>5.8±0.4 (6)</td>
<td>7.2±1.1 (6)</td>
<td>5.9±0.6 (6)*</td>
<td></td>
</tr>
<tr>
<td>Lactate production</td>
<td>16.1±2.0 (6)</td>
<td>14.2±3.0 (6)</td>
<td>11.6±2.9 (6)</td>
<td></td>
<td>11.6±2.9 (6)</td>
<td>21.1±1.7 (6)*</td>
<td>9.0±1.7 (5)</td>
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<tr>
<td>Tissue ATP (2)</td>
<td>1350±220</td>
<td>2130±161 (7)</td>
<td>75±33</td>
<td></td>
<td>1612±240 (4)</td>
<td>153±33 (7)</td>
<td>12±10</td>
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<tr>
<td>Tissue ADP (3)</td>
<td>30±20</td>
<td>75±33</td>
<td>75±33</td>
<td></td>
<td>20±50</td>
<td>185±33 (7)</td>
<td>4.5</td>
<td></td>
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<tr>
<td>Tissue AMP (3)</td>
<td>20±50</td>
<td>20±50</td>
<td>20±50</td>
<td></td>
<td>185±33 (7)</td>
<td>185±33 (7)</td>
<td>4.5</td>
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<tr>
<td>RCR**</td>
<td>0.97</td>
<td>0.86</td>
<td>0.86</td>
<td></td>
<td>0.75</td>
<td>0.75</td>
<td>0.99</td>
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** Energy charge ratio = ATP + ADP + AMP

The results indicate that the tissues are well maintained in culture. The increase in lactate production observed with hCG coincides with a marked increase in progestin secretion, and a transfer of steroidogenic function from the theca interna to membrana granulosa. It is tempting to speculate that the increase in glycolytic activity of the follicles is due to the granulosa cells as they assume their new role.

References:
This study concerns an investigation of lipogenesis in intact sheep ovarian follicles in culture following exposure to hCG. Ovarian follicles 4-6 mm in diameter were obtained from ewes between days 4-14 of the oestrous cycle, and set up in organ culture as described by Moor (1). Lipogenesis was investigated by following the incorporation of radioactivity into the various lipid classes following inclusion of Acetate-1,2-14C (1 uCi ml⁻¹) or 32P inorganic phosphate (2 uCi ml⁻¹) into the media.

Studies with 14C acetate showed active incorporation throughout the 3 day period of culture into all lipid classes. Interestingly, the percentage distribution of incorporation remained constant throughout: phospholipid 55%, cholesterol 22%, triglycerides 15%, cholesteryl ester 6% and free fatty acids 2%. Inclusion of hCG (20 i.u. ml⁻¹) in the media significantly (P<0.05) increased 14C incorporation into all but the cholesteryl ester fraction.

In more extensive studies with 32P, active uptake into phospholipids was shown to occur within minutes of exposure to isotope. Separation of theca and granulosa tissues by microdissection, revealed that greater than 90% of the uptake, even after prolonged exposure, was in thecal lipid. hCG stimulated incorporation of 32P into both thecal and granulosa lipids as early as 5 mins (P<0.05) after exposure.

It is hoped that with further refinement of experimental approach, the relationship of the increase in phospholipid turnover to cyclic AMP formation, induced by gonadotrophin, may be elucidated.

References:
1. Moor, R.M. Oestrogen production by individual follicles explanted from ovaries of sheep.
HUMAN GRAAFIAN FOLLICLES IN TISSUE CULTURE: CORRELATIONS BETWEEN OESTROGEN AND PROGESTERONE PRODUCTION AND THE SITE OF $\Delta^5$-$3\beta$-HYDROXYSTEROID DEHYDROGENASE ACTIVITY.

J.F.P. Kerin, R.M. Moor and R.F. Seamark, Department of Obstetrics and Gynaecology, University of Adelaide, South Australia 5000.

This study concerns an investigation of the feasibility of maintaining isolated human ovarian follicles in organ culture. Follicles (4 to 8 millimetres in diameter) were removed at laparotomy, isolated from stromal tissue and set up in culture using essentially the same approach and conditions described by Moor (1). Assessment of endocrine function was made by daily analysis of the culture media for steroid hormones by using validated radioimmunoassay procedures or mass fragmentography.

Following varying periods of culture the follicles were subjected to both histological and histochemical examination. The histochemical method to detect the distribution and activity of the enzyme $\Delta^5$-$3\beta$-hydroxysteroid dehydrogenase ($3\beta$-HSD) was used according to Wattenberg (2). This enzyme has a well defined position in steroid biosynthesis in which it catalyses the conversion of pregnenolone to progesterone.

It was found that the follicles maintained endocrine function and their histological integrity for at least six days in culture. Furthermore they retained their capacity to respond to gonadotrophins. This was demonstrated by the addition of human chorionic gonadotrophin (HCG) (20 i.u. ml$^{-1}$) to the media which resulted in a sustained stimulation of both oestrogen and progesterone production as well as inducing morphological changes in the theca interna and granulosa layers.

Untreated follicles from both follicular and luteal phases of the menstrual cycle exhibited moderate to marked $3\beta$-HSD activity, predominantly in the theca interna layer. The treated follicles however, showed a similar degree of enzyme activity in the theca interna but in addition a marked increase in activity in the granulosa layer.

Two sets of controls were used in the histochemical assessment. When tissue sections prior to incubation were boiled for 3 minutes no reaction product appeared. Secondly if the substrate was omitted from the incubation media then the reaction product was of minimal intensity in all layers of the follicle.

No discernable histochemical differences were observed in the thecal layers of the treated or untreated follicles when either of the three substrates pregnenolone, $17\alpha$-hydroxy pregnenolone or dehydroepiandrosterone (DHA) were used. However DHA appeared to be the preferred substrate for $3\beta$-HSD activity in the granulosa layer of treated follicles.

In conclusion the technique of Moor (1) developed for organ culture of sheep follicles appears to be well suited to studies of human follicular function.

References:


Oestradiol levels during the oestrous cycle: a comparison of two breeds of different fecundity

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Ovulation rates in Finnish Landrace, Scottish Blackface and Tasmanian Merinos are 2.94, 1.26 and 1.03 respectively (1). The following measurements were made to determine any associations which may exist between oestradiol levels and ovulation rate in sheep.

Twelve ewes of each breed were subjected to laparotomy on day 9 of the oestrous cycle (oestrus = day 0). Twenty ml of ovarian venous blood was collected bilaterally by needle puncture (23G) of the ovarian vein midway between the ovary and the junction of the utero-ovarian vein. In a second experiment 20 ewes of the Finnish Landrace and Scottish Blackface breeds were bled (50 ml, jugular venipuncture) daily for 18 days during the breeding season. The plasma was removed and stored at -20°C. Daily oestrus records were obtained using vasectomised rams kept with the flock. Oestradiol was measured by radioimmunoassay (2) adapted for sheep ovarian or jugular venous plasma.

The concentration of oestradiol in ovarian venous plasma of Merinos (42.8 ± 8.3 pg/ml, n = 15; M ± SEM) was significantly lower than that in Finns (66.8 ± 10.0 pg/ml, n = 21) or Blackface (73.4 ± 10.8 pg/ml, n = 17) ewes. The Finn and Blackface did not differ significantly from each other. However, differences in secretion rates might exist because of breed variation in ovarian blood flow. There were no differences in mean concentration of oestradiol produced by right and left ovaries. The concentration of oestradiol in the peripheral circulation did not differ between breeds; the mean ± SEM concentration over all stages of the oestrous cycle was 1.79 ± 0.10 pg/ml (N = 160) and 1.75 ± 0.19 pg/ml (N = 59) for the Finn and Blackface breeds respectively. During the pre-ovulatory period (days -3 to 0) the oestradiol concentrations were 2.07 ± 0.25 pg/ml (N = 34) and 1.90 ± 0.27 pg/ml (N = 36) in Finn and Blackface ewes respectively.

These results suggest that in breeds with different ovulation rates the gross pattern of oestradiol levels are not necessarily different. The small differences in peripheral concentration (10%) may be real and sufficient to modify ovulation rate.

We wish to thank Marjorie Fordyce, Sandra Henderson, W.G. Davidson, D.W. Davidson and R.D. Preece for technical help.

REFERENCES


IMMUNIZATION OF EWES TO OESTROGENS: EFFECTS ON THE OESTROUS CYCLE AND PARTURITION

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Oestradiol feedback to the hypophysis and pituitary is a key part of the homeostatic control of ovarian function, while at parturition, there is a major rise of oestradiol in maternal blood. Antibodies against oestradiol and oestrone were raised in ewes to further elucidate the physiological functions of these hormones in respect to the oestrous cycle and parturition.

Adult ewes were actively immunized to oestradiol-17β and oestrone using conjugates of their 6-carboxymethoximes with bovine serum albumin. Oestrus was detected using vasectomised rams.

In all 18 ewes immunized against oestradiol late in 1974, there was a complete absence of oestrous behaviour in the 1975 breeding season. Control animals exhibited regular oestrous cycles. Laparotomy in July 1975 showed that the immunized animals had numerous ovarian follicles but were not ovulating. Administration of 120 μg diethylstilboestrol intramuscularly resulted in oestrous behaviour and laparotomy showed that ovulation had occurred. Although no further immunizations were given, oestrus occurred occasionally in 2 of the 18 ewes during 1976.

After immunization to oestrone in 1975, oestrus occurred occasionally in 2 of the 9 ewes in that year. The ovaries contained numerous follicles and multiple ovulations had occurred in 3 of the 5 animals examined. In the following year, 5 of the 9 ewes exhibited oestrus. Clearly, immunization against either oestradiol or oestrone had marked but differing effects on the ovarian-hypothalamic feedback system (spontaneous multiple ovulation occurred in some oestrone immunized ewes). Modification of the system induced by immunization could provide methods for manipulating ovulation rate.

All 8 ewes immunized to oestradiol during the third to fifth month after mating developed moderate antibody titres. Seven lambed at the expected time and parturition was of normal duration; no gross effect of immunization was evident. Only one of these ewes displayed occasional oestrous behaviour subsequently - an indication of the effectiveness of the immunization in blocking one aspect of reproductive function. Thus, any action of oestradiol in the maternal compartment near term may not be obligatory for normal parturition.
OCULAR ADMINISTRATION OF SYNTHETIC LUTEINIZING HORMONE
RELEASING HORMONE (LH-RH) IN THE RAT

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Synthetic luteinizing hormone releasing hormone (LH-RH) stimulates
the release of luteinizing hormone (LH) by the pituitary gland in many
species including man (1). Injection of LH-RH leads to elevation of
serum LH and subsequent ovulation in proestrus rats or hamsters whose
pre-ovulatory surge of endogenous LH had been blocked with barbiturates (2).

The rhythm method or periodic abstinence has widespread use among
those populations whose religious beliefs preclude the use of artificial
contraceptives. The incidence of failure of this method of birth control
is high, due in part to inadequate knowledge of the time of ovulation.
Use of LH-RH may greatly increase the reliability of the rhythm method (3).
Administration of LH-RH near the time when the natural endogenous pre­
ovulatory LH normally occurs may precipitate the LH surge, ensure ovul­
ation, and yield accurate information concerning the unsafe period. The
aim of this study was to develop a simple, convenient method of LH-RH
application in situations where long-term or periodical self-administration
is required. The principles underlying such a method might be extended to
use of a kit whereby a housewife can forecast her own time of ovulation.

Ovulation can be induced by placing a paper disc impregnated with
synthetic luteinizing hormone releasing hormone under the eyelid of the
cycling rat. The paper discs 2 mm in diameter were made of filter paper
using an ear punch. Such a paper disc can absorb a maximum of 1 micro­
liter of solution. LH-RH of varying doses were dissolved in 1 or 0.5
microliter of saline and delivered on these discs with a Hamilton micro­
liter syringe. Ocular administration of 10 µg LH-RH at 2:00 p.m. on the
second day of dioestrus advanced ovulation by 24 hours as examined by
dissecting the oviducts and identifying ova under microscope (4) on the
presumptive day of proestrus. Serum LH was determined by radioimmunoassay
1 hour after LH-RH administration and elevated LH level was observed in
LH-RH treated rats. Control animals receiving paper discs impregnated
with saline showed neither ovulation nor elevation of serum LH. Admini­
stration of a synthetic analog of LH-RH substituted in position 6 and 10
(Gly10–[D-Leu6] LH-RH-ethylamide) at doses of 30-50 nanograms by such an
ocular route on the second day of dioestrus induced ovulation in the
cycling rat. These observations provide evidence that LH-RH can be
absorbed by the ocular vascularization rapidly and in adequate quantity
to cause ovulation.

Although prostaglandin F$_{2a}$ (PGF) appears to be the natural uterine luteolysin in the ewe, its exact mode of action remains obscure. Not the least puzzling feature is the way that PGF manages to leave the uterus and reach the environs of the ovary without entering the general circulation. It has been suggested that due to the close apposition of the two vessels PGF is passively transferred from the uterine vein to the ovarian artery. To gain some idea whether PGF may be effectively transferred through vascular tissue proximal to the ovary in the ewe the following experiments were performed. In the mid-region between the ovary and the utero-ovarian venous anastomosis a surgical sponge of oxidized cellulose was placed in close contact with the ovarian vasculature. A catheter with a tip buried in the sponge was exteriorized to allow the controlled delivery of PGF to the outer surface of the blood vessels. Local lavage of the tissue was maintained for 6 hr and PGF was delivered at rates varying from 1 to 20 μg/hour. The effect of the treatment was observed on the structure and secretory function of the corpus luteum at the mid-luteal phase of the cycle. At all delivery rates PGF caused a lowering of the circulating levels of progesterone, and 48 hours after treatment the fine structure of the luteal cells showed changes characteristic of normal regression. The experimental procedure was repeated with tritiated PGF and blood samples were collected from the utero-ovarian vein (UOV). During the 6 hour administration of PGF the concentration of radioactivity in UOV plasma increased steadily and at the end of this period there was a significant accumulation of radioactivity in the corpus luteum. The results demonstrate that there is a ready transfer of PGF from perivascular tissue to the ovary, presumably via the ovarian arterial circulation. It cannot be decided from these studies whether luteolysis results from the action of PGF on ovarian blood vessels (1, 2) or on biochemical processes in the luteal cell (3).

REFERENCES

Prostaglandins F₂α is known to be luteolytic in the goat when administered into the uterine vein (1). Comparable doses of PGF₂α administered directly into the uterus in women have not provided evidence for a luteolytic action of PGF₂α in the human. These observations have now been extended to examine the effect of intraluteal injections of PGF₂α in the goat, and also the physiological changes in PGF concentrations in human luteal tissue during growth and regression.

Six non-pregnant and six pregnant goats were each given two intraluteal injections within one hour, of 60 μg PGF₂α in 0.1 ml saline, at 9-11 days following estrus or between 28-132 days of gestation. Three control goats were given two intraluteal injections of 0.1 ml saline.

Human corpora lutea from different stages of the menstrual cycle and from the 7th-12th week of pregnancy were collected and assayed for PGF content as previously described (2). Following the intraluteal injections of PGF₂α, there was a progressive decline in plasma progesterone levels to negligible levels over a period of 48 hours, in all goats, with no evidence of a return to normal levels within 96 hours of such treatment. There was no significant depression of progesterone levels in the control goats.

PGF levels in luteal tissue ranged from 1-9 ng/g in corpora lutea from 8 non-pregnant women during the mid secretory phase of the menstrual cycle and from 12 pregnant women. Higher levels of PGF (10-46 ng/g) were found in 3 out of 8 corpora lutea from women in the early secretory phase and in 5 out of 8 corpora lutea from the late secretory phase of the cycle.

The results show that PGF₂α at the dosage used was luteolytic in both non-pregnant and pregnant goats when injected directly into the corpus luteum. In human corpora lutea elevated levels of PGF in the luteal tissue were associated with both the growth and regression of the corpus luteum.

REFERENCES


A TECHNIQUE TO COUNT MOUNTS DURING FLOCK MATING IN SHEEP USING A METER HARNESSED TO RAMS.

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Recent work by Fowler (1) has indicated that if the number of mounts during a known period of mating can be counted, then the number of foetuses in the flock can be estimated. Such information can only be collected by direct observation which is time consuming and tedious. The work reported here describes the design and testing of a meter to count mounts.

The meter weighs 500 g and consists of a body and an articulating jaw. The body is attached to a ram mating harness and contains a counting mechanism. A plunger protrudes from the body and is depressed when the jaw closes. When thus depressed the plunger operates a counting mechanism, but only when the meter is elevated 10 degrees from the horizontal.

The meter was tested on four rams in pens 3 m square, where the mating activity of each ram with 5 spayed oestrous ewes was directly observed. The number of mounts was counted by the observer and the number registered by the meter recorded. The rams were 2½ years of age in store condition and had been joined for 5 weeks. They had been spelled for two weeks between the end of joining and the start of meter testing. The tests were carried out in March, and were of 1 or 3 hour's duration.

The rams reacted to the meter by rearing very high when mounting, often resulting in a mount which did not register on the meter. Where the number of mounts was less than 20, the regression of mounts registered by the meter on mounts observed was \( Y = 1.17 x - 2.01, r = 0.64 \). However where the number of mounts was high, the relationship was very good. The regression equation here was \( Y = 1.08 x - 4.41, r = 0.94 \).

The results indicate that if the meter is made lighter and more compact it will accurately record the number of mounts. This will allow prediction of the number of foetuses in a flock thereby being an invaluable tool to research workers, and also to private producers enabling prediction of numbers of multiple-bearing ewes and whether or not joining of weaner ewes in any one year is warranted.

REFERENCES

Merino rams aged 3 years were libido-tested (1) and castrated. Serves in 2 libido tests and aggression (scored 0 to 4) against entire rams (score, 3) were recorded fortnightly. When at base levels for libido and aggression (9% of original, entire level (OL) and 0.4, respectively), groups of 8 animals were given daily, intramuscular doses of 15 μg testosterone (T15), dihydrotestosterone (D15) or 100 μg oestradiol (E100) suspended in 2% methyl cellulose gel.

In 4 weeks, mean libido and aggression scores rose to maximal levels (92% OL and 2.8) in T15 animals. Treatment of these animals with 30 mg testosterone daily induced extreme aggression (>4) without increasing the libido. In group D15 and E100, aggression scores rose to 0.6 and 0.8 respectively, in 4 weeks while libido increased more slowly to 23% and 29% EL respectively over 14 weeks.

Groups of 8 castrates were then treated daily for 14 weeks with 5 mg testosterone (T5 and T5E) or 15 mg dihydrotestosterone (D15 and D15E) after which T5E and D15E animals were also given 100 μg oestradiol daily. After 2 weeks there were no changes in scores for T5 and D15 castrates while libido had increased marginally in T5E animals (73%, originally 64% OL) and markedly in group D15E (83%, originally 26% OL). In this time, aggression increased considerably in both T5E (3.4, originally 1.8) and D15E animals (2.9, originally 0.5).

Though dihydrotestosterone and oestradiol can evidently act synergistically to induce libido and aggression when given in relatively large doses to castrates, it is still possible that testosterone itself may be primarily responsible for the androgenic effects in entire rams. Nevertheless, plasma levels of dihydrotestosterone and oestradiol might prove to be useful parameters with which to select rams for libido.

REFERENCES

TESTOSTERONE TREATMENT OF RAM LAMBS: EFFECT ON ADULT LIBIDO.
P.E. Mattner, J.M. George and A.W.H. Braden,
Division of Animal Production, C.S.I.R.O.,
Blacktown, New South Wales, 2145.

Merino ram lambs treated from 2 or from 5 weeks of age (T2 and T5 rams) for 3 weeks with subcutaneous implants releasing 0.6 mg testosterone per day were reared with untreated controls (T0 rams). The 16 T0, 23 T2 and 20 T5 rams surviving to 2 years were given 3 pen libido tests (1) and 4 sequential samples of peripheral blood taken at half hourly intervals were collected from each ram.

The T0 and T2 rams did not differ significantly in respect to any characteristic examined. As a group, T0 and T2 rams differed significantly from T5 rams in respect to the mean number of services performed (7.8, S.E. 3.6 c.f. 10.5 ± 3.7, P < 0.001) and the mean of the individual mean plasma luteinizing hormone (LH) level (1.3 ± 0.6 c.f. 2.3 ± 0.3, P < 0.005 ng/ml) but not in regard to the mean of the individual mean plasma testosterone level (2.4 ± 0.6 c.f. 3.1 ± 0.8 ng/ml). Relative to the T0 and T2 groups, the T5 group contained a lower proportion of rams exhibiting a low level of libido.

Although the treatment given to T5 rams elevated both the libido and the plasma LH levels, the two effects evidently occurred independently of each other. For all T0, T2 and T5 rams there was an extremely low order of correlation between the number of services performed and either the lowest, the mean or the peak LH level; rS7 = 0.246, rS7 = 0.096 and rS7 = 0.103, respectively.

In a further study, ram lambs from the same flock were treated with testosterone implants for 3 weeks from 8 weeks of age. At 2 years of age, these rams did not differ significantly from untreated control rams in respect to libido or their plasma LH or testosterone levels.

It appears that in rams, there is a critical time in early life during which the level of circulating testosterone may play a role in predetermining the level of libido exhibited in adult life, possibly by acting on the area(s) of the central nervous system involved in the control of libido. Treatment of lambs with testosterone at this time might serve to reduce the incidence of rams exhibiting low libido.

REFERENCES

THE INFLUENCE OF ANDROGENS AND OESTROGENS ON MATING BEHAVIOUR IN MALE SHEEP.

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As a result of the report that testosterone (T) levels in bulls fluctuate during the day and are increased by sexual stimulation\(^1\), we have investigated whether a similar situation holds in rams, in order to establish if blood T levels might provide a practical index of an animal's mating potential.

Plasma T levels showed episodic daily fluctuations which varied greatly between rams, were different from day to day, and had no apparent diurnal rhythm. There was no correlation between a ram's plasma T level and his performance in a standard libido trial and the plasma level was not influenced by the contact with oestrous ewes.

We felt it possible that in entire rams, circulating levels of T may be well above a threshold required to elicit mating behaviour (learned experience may also play an important role) and therefore chose as an experimental model, male sheep castrated before puberty (wethers), which had had no previous sexual experience. Daily i.m. injections of T propionate revealed that 1 mg/day was insufficient to establish mounting of oestrous ewes, whilst doses in the range 2-10 mg elicited mounting behaviour after 10 days of treatment. The magnitude of the response appeared to be dose-related in this range, whilst doses above 10 mg did not further enhance behaviour.

To test the possibility that the T might not itself be acting directly on the brain but rather after aromatization to oestrogens\(^2\), 6 wethers were given subcutaneous implants of oestradiol (OE\(_2\)) in silastic tubing calculated to release daily, either 100 \(\mu\)g (3 wethers) or 200 \(\mu\)g (3 wethers). Fifteen days after implantation, one wether from each treatment mounted a ewe during the standard libido trial.

In a further experiment wethers received daily i.m. injections of steroids. The proportion of animals achieving mounting after 2 weeks was 1/3 for OE\(_2\) at 0.2 mg/day, 2/3 for OE\(_2\) at 1 mg/day and 1/1 for diethylstilboestrol at 1 mg/day. 10 mg/day of dihydrotestosterone (DHT) was ineffective on its own but given in conjunction with OE\(_2\) (0.2 mg/day) enhanced mounting activity over OE\(_2\) given alone.

These results are consistent with the effects of T on mating behaviour being mediated by prior aromatization to oestrogens. This was further supported by the lack of effect of DHT which cannot be converted to oestrogens. The ability to elicit mating behaviour in wethers with T or OE\(_2\) makes them potentially valuable as 'teaser' animals.

REFERENCES


TESTOSTERONE VARIABILITY AND FERTILITY IN BULLS

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The variability in plasma testosterone is one of several factors being examined in this laboratory for possible usefulness in predicting the fertility of bulls.

Plasma collected serially from 2-year old Africander cross (AX), Brahman cross (BX), and Hereford-Shorthorn cross (HS) bulls was assayed for testosterone by radioimmunoassay. Samples collected at 15-minute and 1-hour intervals for up to 8 hours established that all major testosterone peaks (4-14 ng/ml) in subsequent 24-hour studies could be detected by 2-hour sampling intervals.

Initially, 24-hour testosterone patterns were compared in two breeds of traditionally different fertility (AX 77%, BX 61%). The number of peaks per 24 hours in 6 AX bulls (3.7 ± 0.21) was higher (P < 0.01) than that (2.5 ± 0.22) in 6 BX bulls. The number of peaks in each bull remained the same when measured on 2 separate occasions in 3 bulls.

Relationships between testosterone patterns and fertility were examined more critically in 6 bulls that were each mated for 7 weeks with 35 cows (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Breed</th>
<th>Fertility (%)</th>
<th>Mean Level of ng/ml</th>
<th>Values Over 2 ng/ml</th>
<th>No. of Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>206</td>
<td>AX</td>
<td>83</td>
<td>3.8</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>234</td>
<td>AX</td>
<td>67</td>
<td>2.8</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>246</td>
<td>BX</td>
<td>71</td>
<td>4.1</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>300</td>
<td>BX</td>
<td>69</td>
<td>5.0</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>300</td>
<td>BX</td>
<td>10</td>
<td>4.7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>151</td>
<td>HS</td>
<td>21</td>
<td>5.5</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Compared to the first 4 bulls, which impregnated 67-83% of their cows, bulls 300 (10% preg.) and 151 (21% preg.) were remarkably lower in mean level of testosterone (13 2-hourly samples), in the number of values over 2 ng/ml, and in the number of peaks per 24 hours. These 2 infertile bulls were not identified as such by semen and libido tests before or after mating.

If additional data confirm these findings then the measurement of testosterone patterns may be a valuable addition or alternative to conventional semen and libido tests for detecting infertile bulls before they exert deleterious effects on the reproductive efficiency of a herd.
OESTRADIOL AND PROGESTERONE: SOLUBLE RECEPTOR LEVELS AND METABOLISM IN THE UTERUS OF THE OVARIECTOMIZED EWE.

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As part of a programme to examine the hormonal regulation of the ewe uterus during early pregnancy a model system using the ovariectomized animal has been developed. The effects of progesterone prior to oestrus (A), oestradiol at the time of oestrus (B) and progesterone subsequent to oestrus (C) on endometrial metabolism and on the survival of transferred embryos has been described (1,2).

Using this system we have measured soluble oestradiol (E) and progesterone (P) receptor levels and E and P metabolism in whole uterine tissue from animals killed at times which, in the case of ewes receiving treatments A, B and C, were equivalent to Days 1, 4 or 7 of pregnancy (Table I). Forty five Merino ewes were used with 3 animals per group. 'Total' soluble E receptor was measured in charcoal treated cytosols following 30°C heat exchange with excess 3H-E and agar gel electrophoresis. Total soluble P receptor was measured in the same cytosols using an initial incubation with excess cortisol followed by excess 3H-P for 18 hr at 0°C and electrophoresis as above. E and P metabolism was studied by in vitro incubation of 3H steroids with uterine minces.

E receptor was highest at Day 1 and P receptor was highest at Days 1 and 4 in animals which received oestrous E. For each receptor the level was not significantly influenced by omission of treatments A or C. E and P metabolism was low and not affected by steroid treatment.

<table>
<thead>
<tr>
<th>Soluble receptor levels (pmol steroid bound/mg tissue DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestradiol</td>
</tr>
<tr>
<td>Day after oestrus*</td>
</tr>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>ABC</td>
</tr>
<tr>
<td>AB-</td>
</tr>
<tr>
<td>A-C</td>
</tr>
<tr>
<td>-BC</td>
</tr>
<tr>
<td>--C</td>
</tr>
</tbody>
</table>

* in ewes which received treatment B

The results suggest that, in normal pregnancy, P secreted during the previous oestrous cycle does not alter the ability of the ewe uterus to synthesize E and P receptors. The low steroid metabolism may play a local role in maintaining the activity of these two steroids in the uterus.


HORMONE RECEPTOR LEVELS AND HORMONE, RNA AND PROTEIN METABOLISM IN THE GENITAL TRACT DURING THE OESTROUS CYCLE IN THE EWE.

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In the ewe the pattern of ovarian secretion of oestradiol and progesterone during the oestrous cycle has been well established. We have examined concentrations of soluble oestradiol and progesterone receptor proteins and several metabolic activities in the genital tracts of ewes during the oestrous cycle. These studies were an attempt to determine how ovarian hormones regulate pregnancy in the ewe during the first 10-14 days after mating.

Thirty three mature Merino ewes were killed at about 09.00 h on Days 0 (oestrus), 2, 5, 10 or 14 of the oestrous cycle. RNA:DNA ratios and in vitro rates of synthesis of protein were determined in slices of endometrium and sections of oviduct. Total oestradiol and progesterone receptors in endometrium and whole uterus were measured by agar gel electrophoresis at low temperature following in vitro labelling of cytosols. The in vitro metabolism of 3H-oestradiol and 3H-progesterone by whole uterus minces was also examined.

In caruncular endometrium and in whole uterus the concentrations of oestradiol receptor (pmol steroid bound/mg tissue DNA) were highest at oestrus and declined steadily thereafter to minimal values at Day 14. The concentrations of progesterone receptor were highest on Days 0-5, then declined to low levels on Days 10-14. At each stage of the cycle, receptor concentrations were similar in endometrium and whole uterus. There was little metabolism of either 3H-oestradiol or 3H-progesterone in minces of whole uterus and with either steroid the pattern of metabolism did not change at any stage of the cycle. The in vitro rates of synthesis of protein in caruncular endometrium and isthmic oviduct were highest at or shortly after oestrus (Days 0-2), then declined to low levels on Days 10-14. RNA:DNA ratios in these two tissues were also greatest at oestrus and subsequently fell to minimal values at Day 14.

The results suggest that in the endometrium levels of transcription and translation and oestradiol receptor concentrations are maximal at around oestrus, decline steadily during the period when progesterone secretion is increasing, and are lowest during the time of maximal progesterone secretion. Further, the substantial surge of oestradiol secretion which occurs on Days 3-4 of the oestrous cycle appears to have little stimulatory effect on these parameters on Day 5. The more prolonged elevation of progesterone receptor levels suggests a difference in the regulation of the two receptor populations.
ULTRASTRUCTURAL OBSERVATIONS ON THE EPITHELIUM OF THE VAGINAL CAECAE OF THE MARSUPIAL Perameles nasuta WITH PARTICULAR REFERENCE TO OESTRADIOL INDUCED SECRETORY ACTIVITY

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The anterior extremity of the vaginal complex of all Peramelid marsupials is characterised by the presence of two thin walled vaginal caecae. These lie ventral to the two uteri and at oestrus become distended with seminal fluid so as to equal or even exceed the size of the unevacuated urinary bladder.

The material was derived from a sexually mature female Perameles nasuta (body weight 630 g) captured at Tamborine Mountain on the 17th March 1976. Two pouch young with head lengths of 24.6 mm were removed at capture and specimens of one vaginal caecum were obtained for electron microscopy two days later, when the animal was ovariectomised. The first of four daily subcutaneous injections of 100 μg of oestradiol valerate (Schering) commenced on the 10th day after ovariectomy and a second series of specimens from the intact vaginal caecum were obtained on the 14th day post ovariectomy.

Two cell layers were present in the epithelium of the vaginal caecae. A basal layer of non-secretory cells was characterised by cytoplasmic tonofibrils and the presence of wide intercellular spaces. A luminal layer was present with secretory cells closely united by junctional complexes. Before and after hormone treatment these secretory cells contained variable amounts of membrane bound glycoprotein-like secretory product.

A marked increase in synthetic activity within the cytoplasm of the secretory cells followed the hormonal injections. This was evidenced by: increased amounts of endoplasmic reticulum; formation of cisternae within the endoplasmic reticulum; accumulation of an electron dense secretory product within the cytoplasm and its incorporation into vesicles; production of secretory vesicles by the golgi; the bulging of the cell surface into the lumen of the caecae; elongation of surface microvilli; fusion of secretory vesicles with the apical surface of the cell followed by the release of secretory product into the lumen.

Deletion of single cells occurred by apoptosis with both the epithelium of the caecae and invading macrophages contributing to their removal. Migration of leucocytes, particularly lymphocytes, was common in the first series of samples, however, their numbers were markedly reduced following hormonal treatment.

These observations indicate that the epithelium of the vaginal caecae is a target tissue for oestrogenic hormones and that the vaginal caecae are more than passive storage sacs for semen. Further observations can be expected to lead to a greater understanding of the survival and the transport of sperm within the female genital ducts of mammals.
EFFECT OF ESTROGEN SUPPLEMENTATION ON UTERINE, PLACENTAL AND OVARIAN BLOOD FLOW IN MID AND LATE TERM RABBITS

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Western Australia.

Estrogen administration increases total uterine blood flow in a number of species. But whether it increases maternal placental blood flow, with possible advantage to the fetus, or blood flow to non-placental tissues, such as the myometrium and vagina, is poorly understood. In the present work we examined the influence of estrogen on blood flow to these organs in mid term (day 16) and late term (day 28) pregnant rabbits. We also measured ovarian blood flow since estrogen is known to be luteotrophic in the rabbit. Radioactive microspheres, 25μm diameter, were used to measure organ blood flows: the rabbits had previously been anesthetized with an intravenous injection of sodium pentobarbitone, 40 mg/kg. Blood flows were expressed in ml/min per 100g of tissue.

A total of 45 rabbits, 24 control (Group 1) and 21 estrogen treated (Groups 2 to 5), were examined on day 16 of gestation. Group 2 (5 rabbits) were injected intravenously with 5 μg/kg estradiol-17β, 30 min before measurement; Group 3 (6 rabbits) with 10 μg/kg estradiol, 30 min before; Group 4 (6 rabbits) with 10 μg/kg estradiol, 180 min before; and Group 5 (4 rabbits) with 150 μg/kg conjugated equine estrogens (Premarin, Ayerst) 45 min before blood flow measurement.

Vaginal blood flow, in all estrogen treated groups, was significantly greater than that in the control group: 70%, P < 0.05 (Group 2), 107%, P < 0.001 (Groups 3 to 5 respectively). Myometrial blood flow was also greater, by 159%, 102%, 118% and 144%, P < 0.001 (Groups 2 to 5 respectively). Estrogen treatment appeared to have no influence, however, on maternal placental blood flow. Corpora luteal blood flow was greater in the estrogen treated Group 2 (66%, P < 0.01), Groups 3 and 4 combined (27%, P < 0.05) and Group 5 (47%, P < 0.05). Ovarian stromal blood flow was increased in Group 2 (292%, P < 0.01).

At day 28 of gestation blood flows were measured in 10 control rabbits (Group 6) and 10 rabbits (Group 7) injected intravenously with 2 to 10 μg/kg estradiol 17-α, 30 min previously. Estrogen treatment significantly increased blood flow to the vagina (95%, P < 0.05), myometrium (127%, P < 0.001) and corpora lutea (48%, P < 0.05) but, again, placental blood flow was little changed (1% decrease, not significant).

These results suggest that in the rabbit, estrogen treatment has little beneficial effect on maternal placental blood flow, but that it does have a role in the control of blood flow to the vagina, myometrium and ovaries.
EFFECT OF AN ANALOGUE OF LH-RH (D-Ser(TBU)\textsubscript{6}-EA\textsubscript{10}; Hoe 766, Hoechst AG)
ON GONADOTROPIN SECRETION, OESTRUS AND OVULATION RATE IN SHEEP

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Reproduction Research Section, University of Melbourne and
Department of Agriculture, S.S. Cameron Laboratory, Werribee, 3030
and *Muresk Agricultural College, Muresk, 6401.

Hoe 766 (2 µg s.c. in 0.5% gelatin) was approximately 25 times
more potent than LH-RH (50 µg s.c. in 0.5% gelatin, Hoechst, Op 62)
in releasing both LH and FSH in 4 ewes on Days 10-12 of the oestrous
cycle (Day 0= day of oestrus).

At Werribee, the effect of Hoe 766 on ovulation rate was
examined in 151 ewes treated with prostaglandin (125 µg ICI 80996 i.m.)
to synchronize oestrus. The ewes were allotted to 4 groups of equal
liveweight; 3 groups were injected with 2 µg Hoe 766 (s.c. in 0.5%
gelatin) on Day 1 (n=51), Day 12.5 (n=48) and Day 14.5 (n=39) of the
oestrous cycle and the fourth group acted as control (n=23). One to
six days after the next oestrus or expected day of oestrus the
ovaries were examined by laparoscopy and the number of corpora lutea
(CL) determined. Treatment on Day 12.5 increased (p<0.025) mean
ovulation rate (1.37 CL/ewe) compared to the control group (1.10)
and the group injected on Day 1 (1.14), but not compared (p>0.05) to
the group injected on Day 14.5 (1.26). Fewer ewes expressed oestrus
following treatment with Hoe 766 on Day 14.5 (35%) than on Day 12.5
(64%) or than on Day 1 (90%) compared with control group (82%).

In Western Australia, 1700 ewes on a private property were
joined with teaser rams on December 4 and allocated 17 days later
to prostaglandin or cronolone sponge synchronisation treatments in
preparation for artificial insemination (AI) (1). Each group was
subdivided into 3; one sub-group was treated with 2 µg Hoe 766 on
January 18 (5 days before AI), the second sub-group was treated with
2 µg Hoe 766 on January 19 (4 days before AI) and the third sub-group
was not treated (control). Daily incidence of oestrus was determined
with teaser rams. Ten days after Hoe 766 treatment, 47-58 ewes in
each of the sub-groups underwent laparoscopy and the ovulation rate
was assessed. Fewer ewes expressed oestrus following treatment with
Hoe 766 at 4 (34%) or 5 days (43%) before AI compared with controls
(60%). However, ovulation rates were higher in those ewes treated
4 days (1.30 CL/ewe) prior to AI compared to those treated 5 days
(1.17) before AI or not receiving Hoe 766 treatment (1.07).

These results suggest that Hoe 766 might prove useful in
increasing fecundity in animals, especially where AI is carried out
in conjunction with synchronised oestrus and ovulation.

REFERENCE
Proc. 53rd Annual Conference Australian Vet. Association,
Melbourne, pp. 186-189.
SEASONALITY OF OVULATION RATE IN MERINO EWES DIFFERING IN FECUNDITY

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It is of interest to establish if other components of reproduction rate are altered when selection has altered a character such as fecundity. This was examined using monthly endoscopy for 12 months to assess seasonality of ovulation rate in ewes from the '0' (low fecundity) and 'B' (high fecundity) flocks of the CSIRO fecundity selection experiment. The same animals (19 x '0'; 28 x 'B') were examined each month and they remained always in the presence of vasectomised rams to allow monthly assessment of occurrence of oestrus.

The main features of the data are presented in Fig. 1 which shows monthly values for the percentage of ewes ovulating and the mean ovulation rate of those ewes ovulating.

Low fecundity ewes showed the expected seasonal decline in ovarian activity in mid-summer. A high proportion (60%) of 'B' ewes continued to ovulate during this period. Mean ovulation rate of these animals was consistently higher than that of the '0' ewes and declined only slightly in November and December.

Seasonal occurrence of oestrus, not shown in Figure 1, also favoured the high fecundity ewes. The anoestrous period for '0' ewes extended from September to January inclusive. Fewer 'B' ewes experienced anoestrus and this was confined to November, December and January.

These results demonstrate that Merino ewes selected for fecundity continue to ovulate during the period when inhibitory seasonal factors normally suppress ovarian activity in Merinos of this region. This may represent additional reproductive merit of the 'B' Merino.
OVULATION RATE IN HIGH FECUNDITY MERINO CROSSES

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*CSIRO, Division of Animal Production, Armidale, N.S.W. **N.Z. Department
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++University of W.A., Nedlands.

Controlled increase of reproduction rate remains a high priority aim
for most species of farm livestock. Accordingly, considerable attention
has been directed to testing the efficiency of exogenous hormone manipu­
lations for achieving controlled increases in ovulation rate but thus
far these efforts have not been conspicuously successful.

Animal breeders have also been interested in this problem and have
been demonstrably successful in chickens and sheep. In this paper we
report on ovulation rates in ewes bred by crossing existing strains of

<table>
<thead>
<tr>
<th>Strain of Merino</th>
<th>Location of Study</th>
<th>No. of Ovulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine Wool X</td>
<td>N.Z.</td>
<td>62 12 0 0 0</td>
</tr>
<tr>
<td>Booroola X</td>
<td>N.Z.</td>
<td>33 41 19 6 2</td>
</tr>
<tr>
<td>Collinsville X</td>
<td>W.A.</td>
<td>27 3 0 0 0</td>
</tr>
<tr>
<td>Booroola X</td>
<td>W.A.</td>
<td>16 37 11 0 0</td>
</tr>
<tr>
<td>Peppin X</td>
<td>W.A.</td>
<td>9 4 0 0 0</td>
</tr>
<tr>
<td>Booroola X</td>
<td>W.A.</td>
<td>12 12 8 0 0</td>
</tr>
<tr>
<td>Collinsville X</td>
<td>Riverina</td>
<td>30 8 0 0 0</td>
</tr>
<tr>
<td>Booroola X</td>
<td>N.S.W.</td>
<td>28 10 2 0 0</td>
</tr>
<tr>
<td>Medium Non-</td>
<td>New</td>
<td>16 2 0 0 0</td>
</tr>
<tr>
<td>Peppin X</td>
<td>England</td>
<td>8 8 5 0 0</td>
</tr>
<tr>
<td>Booroola X</td>
<td>N.S.W.</td>
<td>8 8 5 0 0</td>
</tr>
</tbody>
</table>

*Mean BX ovulation rates exceeded those of the comparable local Merino in all cases with the increase ranging from 12 to 76 percent. Ovulation rate distribution was also altered in BX ewes but only in the case of the N.Z. work were values higher than three observed.

Preliminary data on litter size at birth of BX ewes from N.Z. (1.70) and W.A. (1.49) indicate that the observed increases in ovulation rate are accompanied by real increases in litter size. However, litter sizes of three or more were observed only in the N.Z. study where 14% of BX ewes had triplets.
A study was undertaken in 1975-76 to investigate factors affecting ovulation rates of mature Border Leicester x Merino ewes in northern Victoria. A similar study was undertaken in 1974-75 (1) and the results are compared. The ewes in the 1975-76 study were of similar breeding, age and history to those of the 1974-75 study and the treatments differed only in that the control ewes in 1974-75 were fed a supplement of wheat in addition to annual pasture, whilst in 1975-76 no supplement was offered. In both years lupin grain was fed at the rate of 0.5 kg per head per day for 14 days prior to mating and for the following 20 days mating period. Seasonal conditions during spring and early summer were similar in both years. Ovulation rates were determined by counting corpora lutea at laparoscopy and oestrous activity was measured after matings to vasectomized rams fitted with harnesses and crayons.

TABLE 1. Number, live weight, matings and ovulation rates of ewes in the 1975-76 experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Month</th>
<th>n</th>
<th>Weight (kg)</th>
<th>Mated (%)</th>
<th>Mean Ov. Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>Nov.</td>
<td>70</td>
<td>58.2 (66.1)</td>
<td>0.9 (100)</td>
<td>0.01 (1.27)</td>
</tr>
<tr>
<td></td>
<td>Dec.</td>
<td>66</td>
<td>58.0 (61.9)</td>
<td>21.2 (100)</td>
<td>1.02 (1.35)</td>
</tr>
<tr>
<td></td>
<td>Jan.</td>
<td>68</td>
<td>55.0 (62.3)</td>
<td>36.8 (100)</td>
<td>1.40 (1.40)</td>
</tr>
<tr>
<td></td>
<td>Feb.</td>
<td>62</td>
<td>57.9 (61.5)</td>
<td>52.2 (100)</td>
<td>1.39 (1.66)</td>
</tr>
<tr>
<td>Lupin</td>
<td>Nov.</td>
<td>103</td>
<td>56.5 (63.7)</td>
<td>0.9 (100)</td>
<td>0.02 (1.36)</td>
</tr>
<tr>
<td>0.5 kg/bd</td>
<td>Dec.</td>
<td>101</td>
<td>58.1 (63.1)</td>
<td>6.9 (100)</td>
<td>0.58 (1.48)</td>
</tr>
<tr>
<td></td>
<td>Jan.</td>
<td>100</td>
<td>54.2 (61.1)</td>
<td>74.3 (100)</td>
<td>1.21 (1.37)</td>
</tr>
<tr>
<td></td>
<td>Feb.</td>
<td>102</td>
<td>54.1 (60.1)</td>
<td>51.5 (100)</td>
<td>1.10 (1.49)</td>
</tr>
</tbody>
</table>

The onset of oestrus was later in 1975-76 and at no stage in this year did oestrous activity reach that recorded in 1974-75. In 1975-76 the ovulation rates were lower, except in the ewes fed lupins in January (Table 1). However, mean ovulation rates of the ewes fed lupins were higher than the unsupplemented ewes in December, January and February (P<0.01). In 1974-75 this effect of lupins occurred only in February.

The delayed onset of oestrus, the lower level of oestrous activity, the lower ovulation rates and the earlier significant difference in ovulation rates between the ewes fed lupins and the unsupplemented ewes indicate further the number and complexity of factors governing the successful mating of ewes in Victoria, particularly during the late spring and early summer. Further indicated is the need for farmers who mate their ewes at this time to use some form of mating indicator to be sure that the ewes have mated during the 6 to 8 weeks period that they are joined to the rams.

REFERENCE

EFFECT OF FEEDING LEGUME GRAIN SUPPLEMENTS ON OVULATION RATE IN BORDER LEICESTER x MERINO EWES.

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Department of Agriculture,
S.S. Cameron Laboratory,
Warrinbee, Victoria, 3030.

Live weight, ovulation rate and wool growth rate were compared at Warrinbee, Victoria, during March and April 1976, in mature Border Leicester x Merino ewes. The ewes were individually fed iso-energy supplements of 500 g of either peas, lupins, soya bean pellets (soya bean meal : barley; 4:1) or lucerne pellets (lucerne : barley; 1:1). The basal diet fed to all ewes was 500 g of the lucerne/barley pellets. The flock was allotted at random to four groups each of 42 ewes. They were weighed weekly. Oestrous cycles were synchronized by two injections given 12 days apart, each of 100 μg, of the prostaglandin P F6-analogue 'Estrumate' (ICI Macclesfield U.K.). Ovulation rates were determined at laparoscopy 28 days after the second prostaglandin treatment. The ovulation studied had occurred 30 days after the commencement of feeding of the supplements. Wool growth rate was determined in 10 ewes per treatment using two dye bands applied 28 days apart.

TABLE 1. The effects of feeding legume grain supplements to ewes.

<table>
<thead>
<tr>
<th>SUPPLEMENT</th>
<th>Basal</th>
<th>Peas</th>
<th>Lupins</th>
<th>Soyabean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight at ovulation (kg)</td>
<td>53.3</td>
<td>51.5</td>
<td>52.6</td>
<td>51.4</td>
</tr>
<tr>
<td>Live weight change during supplement feeding (kg)</td>
<td>+ 0.9</td>
<td>+ 0.9</td>
<td>+ 0.8</td>
<td>+ 0.9</td>
</tr>
<tr>
<td>Intake (g/day)</td>
<td>870</td>
<td>864</td>
<td>858</td>
<td>873</td>
</tr>
<tr>
<td>Organic matter</td>
<td>114</td>
<td>157</td>
<td>231</td>
<td>244</td>
</tr>
<tr>
<td>Protein</td>
<td>14</td>
<td>11</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>Oil</td>
<td>514</td>
<td>525</td>
<td>528</td>
<td>528</td>
</tr>
<tr>
<td>Wool growth (μ/day)</td>
<td>51 ab</td>
<td>36 b</td>
<td>39 ab</td>
<td>38 ab</td>
</tr>
<tr>
<td>Number of ewes eating supplements</td>
<td>1.84 ab</td>
<td>1.78 b</td>
<td>1.97 a</td>
<td>1.94 a</td>
</tr>
</tbody>
</table>

Mean B + P and L + S 1.81 e 1.95 d
a,b,c,d,e Treatments with unlike superscripts differ (P<0.05) X².

Although all ewes ate similar amounts of organic matter daily those fed lupins or soya bean meal had ovulation rates higher (P<0.05) than those of ewes fed peas or the basal diet. There was no difference in wool growth rate between groups. The major characters which differ between the supplements were the levels of protein and oil. Ewes in each group were in good condition and increasing in live weight. Observations were made during the peak of the breeding season and high ovulation rates could be expected. A supplement of soya bean meal produced an increase in ovulation rate identical to that produced by a supplement of lupins which have been shown, in a number of experiments, to increase ovulation rate in ewes. Peas were not an effective alternative to lupins as a supplement to increase ovulation rate in ewes. There may be a level of protein intake above which responses in ovulation rate occur. Site of digestion of the protein may be important since at least 40% of the protein in lupin and soya bean meal is insoluble in the rumen.
Ewes which have been exposed to the plant estrogens found in subterranean clover for several seasons may become permanently infertile. The infertility is due mainly to altered function of the cervix, but other organs, including the hypothalamus, are also affected. The following work examines the hypothesis that there is also an alteration in ovarian function in affected ewes.

Ewes were drawn from the flock described by Rossiter and Marshall (1). Seven ewes had grazed the highly estrogenic Dinninup cv. subterranean clover for 4 years and had reduced lambing, and 8 ewes had grazed the very lowly estrogenic Northam A cv. and were of normal fertility. Ewes were run on non-estrogenic pasture, with raddled, vasectomized rams and examined daily at 1000 hours for estrus. At estrus, the left ovary was removed, the number of large follicles and ovulation sites counted, and the ovary weighed. After 5 weeks, the ewes were run again with vasectomized, raddled rams and 7 days after estrus was observed, the remaining ovary was removed and weighed. Results are shown on Table I.

Table I. Weights (g ± s.e.m.) of ovaries and corpora lutea in clover affected and control ewes before and after unilateral ovariectomy.

<table>
<thead>
<tr>
<th></th>
<th>Control affected (N=7)</th>
<th>Control (N=8)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary 1 (left)</td>
<td>1.75 ± 0.61</td>
<td>1.72 ± 0.15</td>
<td>-0.03</td>
</tr>
<tr>
<td>Ovary 2 (right)</td>
<td>2.26 ± 0.15</td>
<td>3.13 ± 0.43</td>
<td>0.87**</td>
</tr>
<tr>
<td>Difference</td>
<td>0.51 ± 0.15</td>
<td>1.41 ± 0.31**</td>
<td>0.90*</td>
</tr>
<tr>
<td>Corpora lutea (right)</td>
<td>0.51 ± 0.08</td>
<td>0.76 ± 0.11</td>
<td>0.25</td>
</tr>
<tr>
<td>Difference, less C.L.</td>
<td>0.003 ± 0.12</td>
<td>0.65 ± 0.29</td>
<td>0.65*</td>
</tr>
</tbody>
</table>

*P<.05  **P<.01

Studies on another 7 clover affected and 9 control ewes from the same flock showed no significant difference between the weights of left and right ovaries (1.88 vs 2.03 ± 0.19g) nor in total ovarian weight between clover affected and control ewes. Thus the results of the present study may be reasonably ascribed to a failure of ovarian compensatory hypertrophy in clover affected ewes.

Other unpublished work (Adams & Oldham, 1976) has shown that the ovulation rate is altered in entire, cycling ewes with clover disease. It seems likely that the hypothalamic/ovarian axis is permanently altered in ewes with clover disease. This may contribute to the infertility, which is widespread in West Australian sheep flocks.

Reference

INCREASE IN MELATONIN CONTENT OF FETAL SHEEP PINEAL TISSUE APPROACHING TERM.

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The pineal methoxyindole derivative melatonin has been thought for many years to have an endocrine function. We have previously shown (1) that HIOMT, a key enzyme in the synthesis of methoxyindoles is induced in the last 4-5 days of intrauterine life of the sheep foetus.

This paper reports the melatonin content of foetal sheep pineal glands in relation to HIOMT activity during the last 30 days of gestation. Pineal melatonin content was estimated using a specific radioimmunoassay (Kennaway, Frith, Phillipou & Seamark, manuscript in preparation). Glands were homogenised in isotonic KCl and part assayed for HIOMT (2). The remainder was extracted with borate buffer and chloroform and the chloroform extract subjected to chromatography on Lipidex 5000 to separate melatonin from other acetylated indoles prior to assay.

Fetuses 120-143 days of gestation contained 60±14 pg melatonin per gland (range 0-230, n=21), whereas fetuses 143-150 days had a mean melatonin content of 777±224 pg (range 0-5000, n=30), (p<0.02). Elevated HIOMT, however, was not consistently associated with high melatonin content. Pineal gland monoamine oxidase activity (3) assayed in a similar series of animals was found to be present from 100 days of gestation. Thus the discrepancy between HIOMT activity and melatonin content may be a result of methoxytryptophol synthesis.

The synthesis, and presumably release of methoxyindoles from the pineal gland, is influenced by estrogens and catecholamines in rats (4). The last few days of pregnancy in the sheep is a period of marked endocrine change including increased estrogen secretion and sympathetic maturation. Whether the changes in melatonin content and secretion simply reflect changes induced by the enhanced endocrine activity, or play a fundamental role in effecting the general changes observed, remains to be determined.

References:
PRODUCTION AND CHARACTERIZATION OF ANTISERA TO PREGNANCY SPECIFIC ANTIGENS IN THE SHEEP, COW AND PIG

L.D. Staples, R.A.S. Lawson, Mildred Cerini*, Marion Sheers* and J.K. Findlay*

Department of Agriculture, and *Reproduction Research Section, University of Melbourne, S.S. Cameron Laboratory, Werribee, 3030.

Antigens specific to ovine pregnancy have been described using antisera raised in rabbits (1).

This paper describes the production and characterization of antisera to ovine, bovine and porcine embryos and the use of these sera to investigate the occurrence of pregnancy specific antigens in the sheep, cow and pig.

Antisera were obtained from two calves (849 and 822) following repeated challenge with an extract of 16 day ovine embryos. These antisera were titred against a standard embryo extract by agar gel double diffusion, both before and after absorption with a number of tissue preparations including liver and kidney from pregnant and non-pregnant sheep. These tests, together with immunofluorescent antibody tracing, showed that the antigen(s) were located in embryonic, uterine and luteal tissues from pregnant sheep only. There were no cross-reactions against bovine, porcine or canine embryos or against hCG, PMSG or ovine placental lactogen (OPL). Conversely, antisera raised against bovine, porcine or canine embryos or against OPL did not form precipitin lines against ovine embryos in agar gels.

Preliminary haemagglutination tests on peripheral venous blood were carried out in trays using antiserum from calf 849 absorbed with liver and kidney. In 15 separate tests, an average accuracy of 86.4% (accuracy range 70-100% over 198 samples) was achieved in samples collected between days 11-19. In five 19 day pregnant ewes from which venous blood was taken at up to hourly intervals following surgical removal of the embryo, haemagglutination tests for pregnancy became negative within 1-2 h of surgery.

These calf antisera are also being used to monitor the in vitro production of antigens in tissue culture and to identify the immunologically active fraction(s) of ovine embryos after fractionation procedures.

Antisera were also raised against bovine embryos in 3 rabbits (BE1-BE3). All antisera were specific for pregnancy antigens present in bovine embryos when tested by immunofluorescent antibody tracing and agar gel double diffusion following absorption of the antisera with non-pregnant liver and kidney. Similarly, 2 rabbits (PE1 and PE2) immunized with porcine embryos produced pregnancy specific antisera when tested by immunofluorescent antibody tracing.

REFERENCE

PROTEIN COMPOSITION OF UTERINE FLUSHINGS OF RATS IN DIFFERENT ENDOCRINOLOGICAL STATES

K. Umapathysivam and W.G. Breed
Department of Anatomy & Histology
University of Adelaide, South Australia 5001

A major protein component of uterine fluid has been described for the rabbit in early pregnancy and pseudopregnancy (1, 2). Its function may be related to uptake of steroids and/or nutrients by the unimplanted embryo (3). This component has not been described for rats, although secretory activity by uterine epithelial and glandular cells early in pregnancy has been claimed (4) but this has recently been challenged (5, 6).

We have, therefore, used SDS-gel electrophoresis, originally developed by Maizel (7) and modified by Schnaitman (8), to analyse blood free uterine flushings obtained at either pro-oestrus, oestrus, dioestrus or day 5 pseudopregnancy, after the protein level has been measured by Lowry assay (9). Electrophoretic profiles of the gels were subsequently recorded with an optical densometer.

At pro-oestrus (n=4), 13-16 bands occurred of which large peaks had Rf values of 0.5, 0.6, 0.9 (post albumin) and 1.0 (albumin). At oestrus (n=5), 11-14 bands occurred, Rf values of large peaks being same as pro-oestrus. At dioestrus (n=5) and on day 5 pseudopregnancy (n=5) uterine flushings has lower total protein so these were pooled and concentrated. 11-17 and 12-14 bands occurred respectively. There were only two large peaks which had Rf values of 0.9 and 1.0.

Ovariectomized animals indicated that replacement therapy with 1.0 μg of 17 β-oestradiol/day for 10 days (n=3) with or without 1 mg of progesterone/day for the last 5 days (n=3), but not with 5 mg of progesterone/day (n=3), resulted in electrophoretic patterns with 11-15 and 10-12 total number of bands respectively. Large peaks had Rf values of 0.5, 0.6, 0.9 and 1.0. In serum (n=8) there were 10-12 bands of which the dominant peaks had Rf values of 0.2, 0.3, 0.9 and 1.0.

In conclusion, therefore, peaks were visible that had Rf values of 0.1, 0.2, 0.3, 0.4, 0.5, 0.9, 1.0 and 3.0 in both serum and uterine fluid, irrespective of endocrine states, but peaks with Rf values of 0.6, 0.7, 0.8 and 1.4 were present only in uterine flushings, while a peak with Rf value of 2.0 was only present in serum.

In uterine flushings obtained at pro-oestrus and oestrus, two protein components (Rf ϑ values 0.5, 0.6) occur in greater amounts than at dioestrus and day 5 of pseudopregnancy. By comparison with protein standards, the molecular weights of these components are about 68,000 and 49,000 daltons respectively. No extra protein bands were found in day 5 pseudopregnant flushings. Thus it may be that oestrogen induces the increase of two, proteins in uterine fluid, whereas it appears that progesterone does not induce any extra protein components.

References:
CARBONIC ANHYDRASE ISOENZYMES IN THE ERYTHROCYTES AND UTERUS OF THE SHEEP.

J.E.A. McIntosh, Dept. of Obstetrics and Gynaecology, University of Adelaide, Adelaide, South Australia 5000.

Carbonic anhydrase (CA), the enzyme catalysing the reversible hydration of CO₂, occurs in the female reproductive tract of several mammalian species, and the endometrium is one of the main loci of activity (1). Endometria of both non-pregnant and pregnant sheep are rich in CA activity but, unlike the rabbit, this level is independent of ovarian function (1,2). The sheep enzyme can, however, be obtained in large amounts, facilitating its purification. The aim was to characterize endometrial CA and to compare it with CA isolated from sheep red blood cells so as to gain information on the properties of extra-erythocytic CA as a first step in determining whether this enzyme is involved in implantation and maintenance of pregnancy.

CA was isolated from sheep erythrocytes using CHCl₃-EtOH (3) and then chromatographed on DEAE Sephadex with 0.1M-tris-HCl (pH 9.3 at 4°C) (3). Two isoenzymes were found, the major fraction emerging first from the column. Repeated chromatography yielded two fairly pure isoenzymes as judged by polyacrylamide gel electrophoresis (3). Endometrial tissue (97g wet wt.) from ten non-pregnant sheep in the luteal phase was homogenized (Waring Blender) with 0.05M-tris-HCl and centrifuged. The supernatant was negligibly contaminated with haemoglobin and the CA content of the endometrium was 1500 units/g wet wt (4). There was no difference in content between tissue from animals in the luteal or estrous phases. CA activity was precipitated from the supernatant with (NH₄)₂SO₄ (35-60% saturation, pH 7.7, 4°C), redissolved in water, dialyzed and lyophilized. The residue was chromatographed on DEAE-Sephadex in 0.1M-tris-HCl to yield a single peak of CA activity. The activity was pooled and subjected to isoelectric focusing (3) when a single peak of activity was found. Ampholytes were removed by gel filtration; the overall yield of CA was 82%.

Table I. Properties of endometrial (EN) and erythrocyte (ER1, ER2) isoenzymes. Methods and conditions are given in (3).

<table>
<thead>
<tr>
<th>General Properties</th>
<th>EN1</th>
<th>ER1</th>
<th>ER2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular wt (kDa)</td>
<td>29000</td>
<td>29000</td>
<td>29000</td>
</tr>
<tr>
<td>E₂₈₀nm</td>
<td>17</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>g atom Zn/mol</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>7.0</td>
<td>6.6</td>
<td>-</td>
</tr>
<tr>
<td>Kinetic properties as CO₂ hydratases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kₐ (μM)</td>
<td>6.3</td>
<td>9.0</td>
<td>14</td>
</tr>
<tr>
<td>10⁻⁴ V/E₀ (sec⁻¹)</td>
<td>2.6</td>
<td>5.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Type of inhibition by acetazolamide non-comp.</td>
<td>mixed</td>
<td>mixed</td>
<td></td>
</tr>
<tr>
<td>Kᵢ (acetazolamide; approx.) (μM)</td>
<td>9</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>E₀/Kᵢ (acetazolamide; approx.)</td>
<td>0.7</td>
<td>0.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Sheep erythrocytes thus lack a low specific activity form of CA (3), and the endometrial isoenzyme is a typical high specific activity type which differs in its kinetic properties from the erythrocyte isoenzymes.

References:
CYCLIC AMP RELEASE BY SHEEP OVARIANS FOLLOWING GONADOTROPHIN STIMULATION IN VIVO.

T.J. Weiss, F. Amato and P.O. Janson, Department of Obstetrics and Gynaecology, University of Adelaide, South Australia 5000.

A study has been made of changes in cyclic 3,5-adenosine monophosphate (cAMP) and cyclic 3,5-guanosine monophosphate (cGMP) levels in ovarian venous plasma in vivo before and after human chorionic gonadotrophin (hCG, 500 i.u. i.v. Pregnyl : Organon, Morden, Surrey, U.K.).

Anaesthesia was induced with sodium pentobarbitone and maintained with a halothane oxygen mixture. Following a low midline laparotomy, one of the utero-ovarian veins was cannulated and connected by means of a loop to the jugular vein in order to maintain ovarian venous flow. Heparin (25,000 i.u.) was introduced intravenously. The brachial artery was cannulated in order to collect concurrent arterial samples. The vascular connections to the uterus were ligated to avoid uterine contributions.

Samples were collected in chilled tubes, immediately spun down and the plasma added to two volumes of chilled ethanol, centrifuged and the extract assayed immediately or frozen at -80°C until assayed. The saturation assay of Brown et al. (1) was used to determine cAMP as previously described (2). Cyclic GMP was determined with a cGMP RIA kit (The Radiochemical Centre, Amersham, Bucks. U.K.).

Seven ewes were examined at intervals ranging from day 1 to 13 of the estrous cycle. The mean ±SEM (No. of determinations) ovarian venous plasma content of cAMP in unstimulated ewes was 124±9.1 (17) pmol/ml, very similar to the mean arterial level of 131±12.2 (17) pmol/ml. Injection of hCG resulted within 30 min in a marked increase in the secretion of cAMP from ovaries with a well developed corpus luteum (c.l.) of up to 1.9 nmol/min.

Ovaries with very fresh corpora lutea, that is day 1 or day 2, failed to respond to the hCG during the 40 min collection period, the ovarian venous concentration in these animals not differing significantly from circulating levels.

There was no significant difference between the mean venous concentration of cGMP of 31.7 pmol/ml and the mean arterial concentration of 37.2 pmol/ml. Treatment with hCG did not alter cGMP levels.

In conclusion, hCG can elicit a release of cAMP from ovaries with a mid-cycle c.l., but no release was observed from ovaries with an early, or late c.l. No evidence was found of ovarian release of cGMP.

References:
As early as 1919, Corner observed that the corpus luteum of the sow is composed of cells of both granulosa and thecal origin, an observation which has since been extended to the domestic species in general (1). The ultrastructural differences between these two luteal cell types in the corpus luteum of the sow were then documented (2,3), but the function of the thecal lutein cells has been little studied and remains unclear.

The technique of superfusion in vitro (4) was used to demonstrate that the porcine corpus luteum, in the form of slices, secretes progesterone and small quantities of oestradiol-17β. In this study we have combined several techniques such that dispersed porcine luteal cells were separated into populations of cells of different sizes by sedimentation at unit gravity, and each luteal cell type was superfused separately in an attempt to define its secretory role.

Corpora lutea from sows at 30, 60 and 90 days' gestation were dissociated using a combination of treatments with collagenase and hyaluronidase, and trypsin and DNase. The resulting cell suspension was separated into categories of cells of different sizes by sedimentation at unit gravity. Two populations of luteal cells were obtained, the larger ones of 30-50 μm diameter, and the smaller ones of 15-20 μm diameter. Samples of the two cell types were fixed for electron microscopy, which revealed ultrastructural differences between them.

The two populations of luteal cells were superfused for up to 18 hr with Dulbecco's modified Eagle medium, the cells being supported in a column in a matrix of Biogel, and the medium passed through the column at 10 ml/hr. Fractions were collected every 30 min and assayed for progesterone and oestradiol-17β.

At 30 and 60 days of gestation, the large luteal cells produced progesterone at an initial rate of approximately 100 ng/hr/10⁵ cells, which decreased gradually after 8-10 hr. At 90 days, the initial rate of progesterone release was 50-60 ng/hr/10⁵ cells. The smaller cells also released progesterone into the medium at approximately 15-20 ng/hr/10⁵ cells at all stages of gestation, gradually decreasing over the period of superfusion. At 30 days of gestation, neither cell type released significant amounts of oestradiol-17β, but at 60 days, 10-20 pg/hr/10⁵ cells were measured in the superfusates from the larger cells, increasing to 50-100 pg/hr/10⁵ cells at 90 days.

Both cell types were perfused for 20 min with 10 μg porcine LH at the three stages of gestation, and both showed an immediate response in terms of progesterone release which decreased in magnitude with increasing age of gestation. The response by the smaller cells was greater than that by the larger cells. There was no response by either cell type in terms of an increase in oestradiol-17β.

REFERENCES
In this study virgin QS female mice aged 8-10 weeks and weighing 25-30 gr were used. Blood samples were collected from the tail vein into micro-cap centrifuge tubes. After centrifugation the plasma was stored at -20°C until assayed for LH content using a solid phase radioimmunoassay technique. The day of the morning on which the copulatory plug was found was designated day 1 of pregnancy. Parturition in these mice occurred on day 20.

Daily LH values throughout pregnancy. Samples were collected at 0800 hr on days 1-20 of pregnancy. The results for 8 mice sampled over this time period show that LH values which are low on day 1 (5-6 ng NIH LH S-16/ml) rise to 18-25 ng/ml prior to implantation, which occurs late on day 5. After this levels drop slightly and then rise to a peak of 30-40 ng/ml around mid-pregnancy. Values drop sharply after this and remain relatively stable around 10-15 ng/ml.

Daily LH values during days 1-14 of suckling delayed pregnancy. Samples were collected at 0800 hr on days 1-14 of post partum pregnancy from mice that were each suckling 8 pups. Results of 8 mice sampled over this time showed a similar pattern of LH release to that found in normal pregnancy.

Daily LH values during normal and delayed pregnancy. A group of 7 mice were bled at 0800 hr for 42 days following mating. These mice littered and mated again on day 20. Following the post partum mating each animal suckled 8 pups. The results confirmed the findings of the previous two experiments.

Comparison of LH values during pregnancy and pseudopregnancy. Eight mice mated to intact males and 8 mated to vasectomised males had blood samples collected daily at 0800 hr during days 1-9 and twice daily at 0800 and 2000 hr during days 10, 11, 12 and 13. Titres of LH in pregnant and pseudopregnant animals show a similar pattern after mating. However LH levels in pseudopregnant mice reached peak values significantly higher (P < 0.05) than found in pregnant mice around days 10-11. Days 10 and 11 represent mid pregnancy in the mouse, the time after which removal of the pituitary will not interrupt pregnancy. At around this time pseudopregnancy is terminated in the pseudopregnant animal.

It is suggested that the release of LH after copulation in the mouse is independent of the presence of the foetus and represents an endogenous rhythm of release. However the ovulatory release of LH which terminates pseudopregnancy is partially suppressed in pregnant mice.
LH-RELEASING HORMONE ACTIVITY IN THE OVINE PINEAL

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Department of Obstetrics and Gynaecology, University of Adelaide, 4000
and Medical Research Centre, Prince Henry's Hospital, Melbourne, 3004.

Early reports that the pineal organ was able to exert an anti-gonadal effect in vivo (1), and that this was achieved at least in part by an inhibition of LH-releasing hormone (LH-RH) release from the hypothalamus (2), are in contrast to the detection of significant amounts of immunological and biological LH-RH activity in ovine pineal tissue (3). This study represents an attempt to resolve this difference by examining the immunological and biological LH-RH activity in the ovine pineal.

A lyophilized acid-soluble fraction was prepared from lamb pineal. Immediately prior to radioligand and biological evaluation, the lyophilized material was redissolved in 2.0M acetic acid, neutralised, and subsequently diluted in phosphate-buffered saline (PBS, pH 7.4). Immunological LH-RH activity was assessed by double-antibody radioimmunoassay (4). Solutions of ovine pineal extract (2.0 mg/ml) and synthetic LH-RH (1.0 ng/ml in 0.2% bovine serum albumin PBS) were processed as stated above, and compared to untreated synthetic LH-RH (1.0 ng/ml in 0.2% BSA PBS).

Acid-treated synthetic LH-RH exhibited comparable immunopotency to that of untreated LH-RH, whereas the ovine pineal extract was unable to displace 125I-LH-RH from the anti-LH-RH serum even when incubated at up to 0.29 mg/ml of assay medium.

Biological potency of the pineal extract (2mg/ml), was compared to that of 0.5 ng and 1.0 ng synthetic LH-RH or vehicle alone (0.2% BSA saline) on the basis of LH secretion in the ovariectomised, oestrogen and progesterone pretreated (OEP) female rat (5). Net changes in rat gonadotrophin levels following injection of 0.5 ng synthetic LH-RH (39.7 ± 6.0 ng LH/ml) and 1.0 mg ovine pineal extract (~2.1±2.6 ng LH/ml) were significantly different (p<0.05) from that of vehicle alone (6.1±2.8 ng LH/ml).

These results indicate an absence of immunological LH-RH activity in extracts of ovine pineal tissue, although this same material contained one or more factors capable of inhibiting LH secretion in vivo. This finding is contrary to that of White (3) but remains consistent with the proposed anti-gonadal properties of the pineal.

REFERENCES:
EFFECTS OF ICE SEEDING AND OF FREEZING AND THAWING RATE ON THE
DEVELOPMENT OF SHEEP EMBRYOS STORED AT -196°C.

R.J. Bilton & N.W. Moore
Department of Animal Husbandry, University of Sydney, Camden, N.S.W.2570

Two factors likely to affect the survival of frozen/thawed embryos are freezing and thawing rates, and the rate and temperature at which crystallisation occurs. In two experiments the viability of Day 6 sheep embryos (morulae and early blastocysts) after storage in liquid N2 was assessed by their development in culture. Storage and culture were carried out in Dulbecco phosphate buffer containing 25% sheep serum (DB + S) and 1.5M dimethylsulphoxide (DMSO) was used as a cryoprotectant. DMSO was added and diluted at 30°C over a 20 min. period. Embryos were cooled to 0°C, and warmed from 0 to 30°C after thawing, at rates of
0.7°C/min. Freezing and thawing were carried out at pre-determined rates measured over the range 0°C to -50°C. Following dilution of DMSO, the embryos were cultured in vitro for 24-36 hours.

Expt. 1. A single freezing rate (0.3°C/min.) and two thawing rates (2.2 & 4.6°C/min.) were used. During freezing crystals of frozen DB+S were added at -2.5, -5.0, -7.5 or -10°C to induce crystallisation. There was no effect of thawing rate on subsequent development in culture, nor was there any difference in development between embryos seeded at -2.5, -5.0 and -7.5°C (8 of 22, 16 of 31 and 8 of 24 embryos developed). After seeding at -10°C none of 30 developed.

Expt. 2. Embryos were frozen and thawed as shown in Table 1 and the storage medium was seeded at -2.5°C.

| TABLE 1 |
| Development of embryos in culture |

<table>
<thead>
<tr>
<th>Rate (°C/min.)</th>
<th>Freezing</th>
<th>Thawing</th>
<th>Number of embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stored</td>
</tr>
<tr>
<td>0.15</td>
<td>1.2</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>0.15</td>
<td>2.2</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>0.30</td>
<td>2.2</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>0.30</td>
<td>4.6</td>
<td>13</td>
<td>8</td>
</tr>
</tbody>
</table>

No embryo developed following thawing at the slowest rate, whereas around half survived other freezing/thawing combinations. Seven embryos which developed in culture were transferred to recipient ewes and three developed to lambs.

With the procedures used it would seem that substantial damage to embryos results from slow thawing and from seeding at a relatively low temperature.
EFFECTS OF PROGESTERONE AND OESTRADIOL ON ENDOMETRIAL METABOLISM AND EMBRYO SURVIVAL IN THE OVARIECTOMIZED EWE

B.G. Miller & N.W. Moore
Department of Animal Husbandry, University of Sydney, Camden, 2570

The environment in the uterus in which early embryonic development occurs remains poorly described. Results from previous studies employing ovariectomized ewes suggested that progesterone secreted before oestrus and oestrogen secreted around the time of oestrus influenced uterine metabolism during early pregnancy and the subsequent survival of embryos 25 days after mating. In this experiment we have examined the effects of these components of ovarian secretion and also of oestrogen secreted during days 3-4 of pregnancy on uterine metabolism and the shorter-term survival of embryos. 126 ovariectomized ewes received one of eight treatment regimes (Table 1). Each component of the regimes was an attempt to simulate a phase of ovarian steroid secretion during the oestrous cycle and subsequent early pregnancy. Ewes from each group were killed on Days 21, 27 and 34 of the experiment; 4, 10 and 17 days after oestrus in ewes which received Oestrous E. Ewes killed on Days 27 and 34 received 4-day old embryos on Day 21. In vitro rates of synthesis of protein and RNA:DNA ratios in the endometrium and the amounts of protein in uterine flushings were determined on Days 21 and 27.

Table 1 Treatment regimes

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base E</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Priming P</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oestrous E</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Maintenance P</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maintenance E</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Normal embryos were recovered on Days 27 and 34 from 21 of 27 ewes which received both Priming P and Oestrous E (Groups 1 and 2). Omitting Maintenance E (Groups 2, 4, 6, 8) reduced the rate of protein synthesis and the luminal content of protein on Day 21 and RNA:DNA ratios on Days 21 and 27, but had no effect on the survival and development of embryos. Omitting Priming P (Groups 5 & 6) did not change endometrial metabolism or amounts of luminal protein, but normal embryos were recovered from only 4 of 25 ewes. Omitting Oestrous E (Groups 3 & 4) reduced protein synthesis and amounts of luminal protein on Day 21 and RNA:DNA ratios on Days 21 & 27; and no normal embryos were recovered. In ewes which received neither Priming P nor Oestrous E (Groups 7 & 8) normal embryos were recovered from only of 25 ewes. The effects of deleting different components of the treatment regime on survival of embryos were the same on Days 27 and 34. Thus the effects of omitting hormone treatments must have been mediated before Day 27. It seems that Priming P and Oestrous E change the uterine environment in early pregnancy in ways which affect embryo development, whereas the substantial metabolic effects of Maintenance E appear largely irrelevant to the survival of embryos.
THE EFFECTS OF AGE OF EGG AND SITE OF TRANSFER ON SURVIVAL OF TRANSFERRED EGGS IN THE EWE

I. D. Killeen
Department of Agriculture,
Agricultural Research Station, Leeton

The effects of age of egg, degree of synchronisation between donor and recipient and the site of transfer on the survival of transferred sheep eggs was examined by Moore and Shelton (1964). In their study eggs were recovered 2 to 3 days after oestrous onset so that precise information is not available on the fate of eggs recovered later than this. In the present study, the survival of eggs recovered from donor ewes 2, 3 or 4 days after oestrous onset and transferred to the tubes or uterine horns of recipient ewes was examined.

Border Leicester x Merino ewes were used for the experiment. Donor ewes were treated with 1200 i.u. pregnant mare serum gonadotrophin and joined to Dorset Horn rams. The recipient ewes were joined to vasectomised rams and both donors and recipients were inspected twice daily (0700 and 1700 hrs) for occurrence of oestrus.

Eggs were recovered by flushing a portion of the uterine horns and fallopian tubes with sterile sheep serum and one or two fertilized eggs were transferred to each recipient ewe. The eggs were transferred to recipient ewes which had been recorded in oestrus at the inspection before, at the same inspection or at the inspection after the particular donors. At lambing, lambs and their recipient mothers were identified.

TABLE I. The effects of site of transfer and age of egg transferred on survival

<table>
<thead>
<tr>
<th>Site of Transfer</th>
<th>Age of Egg*</th>
<th>Number of Recipient Ewes</th>
<th>Number of Pregnant Eggs Transferred</th>
<th>Number of Lambs Born</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus</td>
<td>2 days</td>
<td>20</td>
<td>12 (60%)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
<td>23</td>
<td>20 (87%)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>21</td>
<td>14 (67%)</td>
<td>30</td>
</tr>
<tr>
<td>Tubes</td>
<td>2 days</td>
<td>21</td>
<td>15 (71%)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
<td>20</td>
<td>11 (55%)</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>20</td>
<td>13 (65%)</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>125</td>
<td>85 (68%)</td>
<td>183</td>
</tr>
</tbody>
</table>

* Time of egg recovery after oestrous detection.

Overall there was no significant difference between site or time of transfer on either the percentage of pregnant ewes or the percentage of lambs born but the quadratic component of the interaction for both variates was significant (P<0.05). Maximum survival rates were achieved when eggs were transferred into the uterus at three days. At two days, survival rates tended to be higher with tubal transfers but site of transfers appeared to have little effect at 4 days.

THE USE OF EMBRYO TRANSFER IN THE ANGORA GOAT

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Department of Animal Husbandry,
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The use of embryo transfer for the rapid multiplication of animals carrying particular genetic characteristics has been well demonstrated in sheep\(^1\). In the present study, which forms part of a programme of rapid build up in numbers of selected Angora goats, embryos collected from Angora does mated to Angora bucks were transferred to feral does. The feral does were mature animals of mixed breeding (crosses between animals of Cashmere, Angora and milch breed origin) and they had been collected from the semi-arid areas of western New South Wales 1-2 years prior to the study.

Forty Angora does were treated with progesterone, daily for 17-20 days (12 mg/day, i.m.), and an equine anterior pituitary extract\(^2\) (HAP). A total dose of 40 or 45 mg HAP was given as 3 equal s.c. injections on consecutive days commencing on the day before the final injection of progesterone. All but one were served 24-48 hours after the final HAP injection and 3\(\frac{1}{2}\)-5\(\frac{1}{2}\) days after mating, 35 of the 40 does provided 312 embryos. Unfertilized ova, or no embryos, were recovered from the remaining 5. The majority of the embryos were of 8 cells to morulae and 310 were transferred, at a rate of one or two per animal, to the uterine horns of 280 feral does which had been first detected in oestrus by vasectomised bucks within 48 hours of their respective donors. 166 of the feral does kidded (59%) producing 179 Angora kids (58% of embryos transferred).

There was no effect of age of embryo, number transferred, or degree of synchronisation between respective donors and recipients, on the survival of transferred embryos.


In the goat limited information is available on control of oestrus and ovulation and in the present experiment mature feral does harvested from western N.S.W. were treated with polyurethane intravaginal pessaries containing 20 mg Cronolone (G.D. Searle). The pessaries were left in situ for 17 days and 2 of the 4 groups involved received PMSG (400 IU, S.C.) the day before removal of pessaries (Table 1). Group 1 treatment commenced some 2-3 weeks before breeding activity commenced while does in Groups 2, 3 and 4 experienced at least 2 regular oestrous cycles before treatment. Following treatment the ovarian status of the does was assessed by laparotomy and progesterone levels in peripheral plasma.

**TABLE I**

<table>
<thead>
<tr>
<th>Number of does</th>
<th>Served - Days after pessary removal</th>
<th>Returned to service</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>1-3</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td>Treated</td>
</tr>
<tr>
<td>1</td>
<td>Pessary/PMSG - Anoestrus</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>Pessary/PMSG - Breeding</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Pessary Alone - Breeding</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Nil - Breeding</td>
<td>0</td>
</tr>
</tbody>
</table>

Twenty-eight of the 35 treated does were served after treatment and 14 of those in Groups 2 and 3 returned to service after an interval of 19-24 days. No doe in Group 1 returned to service and laparotomies and progesterone levels revealed that they had become anoestrous at a time when untreated animals were starting to show regular oestrous cycles. Three does in each of Groups 2 and 3 were served 6-10 days after treatment. All 6 had ovulated within the first few days after treatment but their corpora lutea (CL), even though they commenced producing progesterone, failed to persist for more than 2-3 days. Oestrus in these animals was associated with a new ovulation. CL failed to persist in a further 2 does in Group 1, but they did not ovulate again. Normal cyclic activity was observed in 11 of the 12 control does. The remaining doe, although mature, had an infantile genital tract.

Results suggest that treatment late in anoestrus does not hasten the onset of breeding activity. Further, CL formed after treatment, at any time, may have a markedly shortened life.
LIVESTOCK AND FERTILITY IN HEREFORD HEIFERS AND MATURE LACTATING HEREFORD COWS

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During a period of five months, 21 groups containing 8 Hereford heifers were held in feedlot pens. Either a maintenance ration of hay or a production ration of hay and oats was fed so that a range of live-weights of between 200 and 350 kg was achieved. At the end of this period 12 groups were held on a maintenance ration of hay and 9 groups fed the production ration of hay and oats, commencing 3 weeks before joining and continuing for the 6 weeks of joining which was by artificial insemination. The average weight at the first oestrus detected during the experimental period was 260 ± 5 kg. The following relationship between liveweight and pregnancy rate was derived:

\[ \text{Pregnancy rate} = 54.44 + 0.00137 \text{pre-mating wt}^2 -0.00163 \text{pre-mating wt} \times \text{gain during mating} \]

\[ (100R^2 = 89.6\%) \]

Liveweight gain during mating reduced pregnancy rate, apparently through a reduction in the conception rate to first service. To achieve a pregnancy rate of 90% in groups of Hereford heifers held on a maintenance ration, an average liveweight of 330 kg would have been required.

To examine the relationship between pre-mating liveweight and fertility in mature lactating Hereford cows, data from two herds grazing at the Pastoral Research Station was pooled for the years 1973, 1974 and 1975. Both herds calved during autumn and were mated by artificial insemination using teaser bulls as the main method of oestrus detection. One herd was in a stocking rate experiment and the other was in a breed comparison experiment. During the period from calving until the end of mating, mean liveweight changes were generally small. Data from 260 cows was classified into pre-mating liveweight intervals of 50 kg and a linear regression line fitted. The regression obtained was:

\[ \text{Pregnancy rate} = 18.6 + 0.11 \text{pre-mating liveweight} \]

\[ (100R^2 = 92\%) \]

This information would be useful in planning the nutritional management of beef cattle for optimal reproductive performance.
EVIDENCE FOR HYPOTHALAMIC DYSFUNCTION IN SUCKLED BEEF COWS
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C.S.I.R.O., Division of Animal Production,
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Two groups of 7 crossbred beef cows were fed on a high quality feed (60% lucerne chaff; 40% rolled oats) ad libitum for 3 months post-partum. The first group suckled their calves whilst the second had their calves removed within 48 hours of birth.

At 6 weeks post-partum, by which time all of the non suckled cows, but none of the suckled cows, had experienced regular ovarian cycles, all cows were injected s.c. with a luteolytic dose of PGF$_2$$_a$ analogue (Cloprostenol, I.C.I., U.K.) and 27 hours later were injected i.m. with 500 $\mu$g oestradiol benzoate (ODB). Jugular blood samples were collected 3 hourly for 30 hours after the ODB injection and were later assayed for luteinizing hormone (LH). Progesterone was assayed in blood samples collected daily.

Luteal regression, followed by oestrus, occurred in 5 of the 7 cows without calves. A fresh corpus luteum formed in all 5 although a surge in LH secretion was detected in only 4 of them. The other 2 cows had experienced oestrus within 5 days hence were refractory to Cloprostenol and ODB treatment. In contrast, in the suckled cows there were no active corpora lutea at the time of treatment, oestrus did not result from the ODB injection, and only one cow exhibited a surge in LH secretion.

ODB induced LH release is not dependent on prior experience of progesterone (1), and 4 of the 5 suckled cows which had still not experienced an ovarian cycle did exhibit oestrus after a similar injection of ODB 8 weeks later. It is concluded that the earlier failure of ODB to induce LH release or oestrus in the suckled cows is indicative of hypothalamic dysfunction in these animals at that particular time post-partum. It is possible that this failure in positive oestrogen feedback is the central basis for anoestrus in the suckled cow.

REFERENCES

1. Nancarrow, C.D., and Radford, H.M. Responses in ovariectomized cows to repeated injections of thyrotrophin releasing hormone (TRH) and to oestradiol benzoate (ODB). Theriogenology (ASRB abstract this meeting) (1976).
The role of prolactin in reproductive processes, in particular the post-partum anoestrus syndrome in cattle, requires investigation. An initial experiment has been carried out to determine the effects of repeated prolactin surges upon oestrus and LH release induced by oestrogen.

Five Hereford cows and one Shorthorn cow were ovariectomized and trained in stalls for several weeks whereupon jugular catheters were implanted. At 1200 hr on day 1, the cows were bled (10 ml) and 3 were injected i.v. with 200 μg TRH in saline. This sequence was repeated every 6 hours for 84 hours. All cows were injected i.m. with ODB (500 μg in peanut oil) at 1200 hr on day 3. Blood was taken every 15 min from 1200 to 1600 hr each day, and every 3 hours following ODB injection until termination of the experiment. Various oestrus detection methods were employed and plasma samples were assayed for LH, prolactin and progesterone.

Progesterone concentrations in all cows throughout the experiment were low (0.24 ng/ml ± 0.03 SE, n = 30). On 4 successive days of TRH treatment, peak concentrations of prolactin in 2 Herefords reached 42-114 ng/ml and were attained some 10-30 mins after injection. Basal levels were restored by 180 mins. Peaks of > 300, 114, 72 and 194 ng/ml were attained in the Shorthorn, the first being associated with venepuncture and recatheterization. Control cows maintained basal prolactin levels (< 20 ng/ml) except on day 4 when the first samples were elevated.

All cows exhibited oestrus and all but one (TRH treated) responded with surges in LH secretion. Times from ODB to onset of oestrus were 24, 29 and 33 hours (mean 25.3) and 27, 27 and 30 hours (mean 28.0) for control and treated cows respectively. Times from ODB to the LH peak were 27, 30 and 36 hours (mean 31.0) and 21 and 27 (mean 24) for control and treated cows respectively. Peak LH values ranged from 24.2 to 40.1 ng/ml.

It is apparent that surges of prolactin release in the cow neither affect the positive oestrogen feedback, nor alter the temporal relationships between these responses and oestrogen.
CORPUS LUTEUM INHIBITION BY PROLACTIN IN THE TAMMAR WALLABY

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During lactational quiescence in the first half of the year and seasonal quiescence subsequently, female tammars possess a quiescent corpus luteum and about 80% possess a blastocyst in diapause (1). Removal of the pouch young during lactational quiescence or removal of the whole pituitary at either period of the year results in immediate enlargement of the corpus luteum and blastocyst activation, progressing to full term pregnancy (2,3). The experiments reported here were designed to identify the pituitary factor responsible for the tonic inhibition of the corpus luteum. Because of the association with lactation, oxytocin and prolactin were considered to be the most likely factors.

In the first series, 19 intact, lactating females had the pouch young removed and were then injected thrice daily for seven days with either sheep prolactin, oxytocin, reserpine or saline. Saline did not inhibit reactivation, but the other three substances delayed resumption by 6-12 days.

In the second series, 8 females in seasonal quiescence were hypophysectomised and then injected on the same regime with either oxytocin, prolactin, oxytocin and prolactin together or saline. Four animals given prolactin showed delay, whereas oxytocin given alone did not delay resumption.

In the third series, only the anterior pituitary was removed from 8 animals followed by the same regime using prolactin or saline. Only the prolactin treated animals were delayed for a period equal to the period of injection.

Since only the anterior pituitary was removed and caused reactivation, oxytocin produced by the posterior part of the pituitary can be ruled out and prolactin, secreted by the anterior pituitary, would appear to be the inhibitory factor. Since the corpus luteum in these animals reactivated seven days after the anterior pituitary was removed it is unlikely that it is dependent upon gonadotrophins for growth and steroid synthesis. In both these respects the pituitary-corpus luteum interactions of the tammar wallaby are unusual and enlarge understanding of this aspect of reproductive biology.


THE EFFECT OF 2-BROMO-α-ERGOCRYPTINE (CB154) AND 2-CHLORO-6-METHYLERGOLINE-88-ACETONITRILE (LERGO) ON PROLACTIN SECRETION OF THE EWE

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Reproduction Research Section, University of Melbourne, Werribee, 3030 and *Department of Agriculture, N.S.W., Richmond, 2753.

The effect of two prolactin inhibitors CB154 (Sandoz) and LERGO (Lilly), on prolactin secretion in the lactating ewe was assessed by radioimmunoassay (1) expressing results as ng NIH-PRL-S8/ml plasma.

Blood was sampled from two lactating ewes around the time of hand-milking, both before and during CB154 treatment (30 mg i.m.). Two other lactating ewes received 30 mg LERGO (i.m.) at 18 hours and 1 hour before milking and then 60 mg LERGO 18 hours before milking on the following day. Blood was collected during the time of milking both before and during LERGO treatment.

The marked increase in prolactin concentration in response to the milking stimulus was blocked by both CB154 and LERGO treatment. Basal levels of prolactin were reduced from 55-110 ng/ml to very low levels (<10 ng/ml) in the CB154-treated ewes. In the LERGO-treated ewes, prolactin levels were suppressed from 80-150 ng/ml before treatment to 21-67 ng/ml just prior to the first milking and to 6-36 ng/ml prior to the next milking.

In another experiment, 3 groups of 3 ewes in the mid-luteal phase received injections of CB154 (20 mg i.m.) or LERGO (30 mg i.m.) at 24, 18 and 1 hour before receiving an injection (10 μg i.v.) of Thyrotrophin Releasing Hormone (TRH). A further 3 mid-cycle ewes were given 30 mg (i.m.) LERGO or saline, respectively, 18 hours before receiving 10 μg (i.v.) TRH. Blood samples were collected at regular (15 to 30 min) intervals under quiet conditions.

The saline treated animals had mean basal prolactin levels of 136±20 (SE) ng/ml and all responded to TRH (>400 ng/ml). CB154 treatment reduced basal prolactin levels to <12 ng/ml and markedly suppressed the prolactin response to TRH to <56 ng/ml. A single injection of LERGO 18 hour before TRH suppressed basal prolactin levels and the prolactin response to TRH in only one of 3 animals. In the animals which received 3 injections of LERGO, prolactin levels were suppressed to 11, 32 and 3.5 ng/ml respectively just prior to TRH and reached maximum values of 48, 38 and 79 ng/ml following TRH.

It is concluded that both CB154 and LERGO can inhibit both basal and TRH induced prolactin secretion and that their action is probably directed at the pituitary gland.

REFERENCE

THE IMPORTANCE OF THE MILKING STIMULUS AND PROLACTIN RELEASE IN THE ARTIFICIAL INDUCTION OF LACTATION IN COWS

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The importance of the milking stimulus and associated hormone release on the artificial induction of lactation was studied in 18 monozygotic twin cows which had failed to conceive. The sibling of each pair which had calved and lactated was used as a control. Lactation was induced artificially by a seven day scheme of oestradiol-17β (0.1 mg/kg live weight/day) and progesterone (0.25 mg/kg live weight/day) injections (1).

Milking was commenced 10 days after beginning treatment in nine treated cows and at 24 days for the other nine treated cows. Fifteen out of the eighteen non-pregnant cows were induced to lactate with milk yields ranging from 20 to 85 (mean=50) percent of their twin sisters. Once lactations were established, milk fat and milk protein compositions were similar in all 3 groups.

The lactose (2) and total nitrogen (Kjeldahl) contents of the mammary secretion and plasma prolactin (3) and progesterone (4) were measured in four sets of monozygotic twins. Lactogenesis, as indicated by both the surge in lactose and changes in the total nitrogen content of the mammary secretion, appeared to occur earlier in cows which had good induced lactations than cows which responded poorly to treatment. Progesterone levels fell and prolactin levels became elevated before lactogenesis. The cows with artificially induced lactations which were milked from day 24 had elevated prolactin levels between days 11-18 but these levels were much lower (30-40 ng/ml) than those occurring in their twin sisters at parturition (>100 ng/ml). There also appeared to be an association between the prolactin peak and the lactose surge (lactogenesis) in both normal and artificially induced lactations.

Milking of cows during the period when steroid hormone residues are high (days 10-24) (5), did not enhance yields following the artificial induction of lactation. Fifteen out of eighteen cows with induced lactations and seventeen out of eighteen of their twin sisters with normal lactations subsequently conceived when joined with the bull.

REGULATION OF ENZYME LEVELS IN THE RAT EPIDIDYMIS BY ANDROGENS.

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The potential use of androgenic manipulation as a form of fertility control in the male prompted this study on the effect of such treatment on metabolic processes in the epididymal epithelium. It is in the epididymis that sperm mature and develop the capacity for fertilization.

The specific activities of enzymes involved in the glycolytic sequence, glyceride formation and the pentose phosphate cycle were determined in extracts of the caput and cauda epididymis. The conditions for the assays were chosen to achieve maximum activity in vitro. In intact animals, the presence of sperm contributed to the specific activity of enzymes in the homogenate, particularly in the cases of hexokinase, phosphoglycerate kinase and phosphoglycerate mutase which were very active in sperm. In order to simplify the comparisons, two experimental treatments were compared, namely efferent duct ligation (EDL) and castration. EDL prevents sperm from entering the epididymis but androgen support is maintained, whilst castration removes the source of sperm and the major supply of androgens. Castration for 4 weeks caused a large decline in the specific activities of hexokinase, phosphofructokinase, aldolase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Several other enzymes showed smaller but statistically significant changes. The magnitude of the changes differed between the caput and cauda, being greater for phosphofructokinase and aldolase in the caput and greater for hexokinase in the cauda. Administration of either testosterone propionate or dihydrotestosterone benzoate at 1 mg/kg per day for 2 weeks to rats castrated for 2 weeks restored enzyme levels to near those measured in EDL rats. Equivalent doses of either oestradiol or progesterone were without effect. In the same experiment, EDL animals were given cyproterone acetate at 16 mg/kg per day for 2 weeks. Although epididymal weight was reduced, specific activities of enzymes were not altered.

The results demonstrate that the maximum potential flux through the glycolytic sequence and the pentose phosphate cycle is under androgenic control and this control is exerted on enzymes located near the beginning of these sequences. However, large quantities of an anti-androgen may need to be administered to intact animals in order to compete effectively with circulating androgens and thereby suppress enzyme activities.
Orally administered α-chlorohydrin causes reversible infertility in a number of species, and as the site of action is the epididymis it has many of the desirable features of a male antifertility agent. However, as toxic side-effects have precluded trials in man, development of a more innocuous derivative is of high priority.

Male rats (5 per compound) were injected daily for 14 days with the compounds shown below at a dose equivalent to 5 mg/kg α-chlorohydrin using propylene glycol as vehicle and control. Two female rats were caged with each male on day 7. The presence of spermatozoa in the vagina was taken as evidence of mating. Male rats were killed and weighed on day 15 and the thymus, spleen, adrenal, testis and epididymal weights recorded. The number and motility of spermatozoa flushed from the vas deferens was assessed. Embryo numbers were recorded on day 20 after mating and their presence taken as evidence of pregnancy.

Alpha-chlorohydrin (5 mg/kg x 14) and compounds I, III and IV caused complete infertility. Compounds V and VI reduced fertility from the control level of 100% to 88 and 78% respectively. Glycidol and compound II did not impair fertility. Only compound VI reduced the number of spermatozoa flushed from the vas deferens. The motility of spermatozoa was significantly reduced by α-chlorohydrin and compounds I, III and IV and glycidol. No significant change in testis, spleen or body weight occurred, however, hypertrophy of the thymus was evident in each treatment group, and elevated adrenal weight and leucocyte numbers resulted from dosage with compound III. Hypertrophy of the epididymis was noted with some compounds (I, II, V and glycidol) although there was no macroscopic evidence of a lesion.

Compound IV is of particular interest in view of its effectiveness and apparent low toxicity.

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{NH}_2 & \quad \text{CH}_2\text{O} & \quad \text{CH}_2\text{OPO}_3\text{Na}_2 \\
\text{CHOH} & \quad \text{CHOH.HCl} & \quad \text{CH} & \quad \text{CHOH} \\
\text{CH}_2\text{Cl} & \quad \text{CH}_2\text{Cl} & \quad \text{CH}_2\text{Cl} & \quad \text{CH}_2\text{Cl} \\
\alpha\text{-chlorohydrin} & \quad \text{I} & \quad \text{II} & \quad \text{III} \\
\text{CH}_2\text{O} & \quad \text{CH} & \quad \text{CH} & \quad \text{CH}_2\text{OH} \\
\text{CH}_2\text{Cl} & \quad \text{CH}_2\text{Cl} & \quad \text{CH}_2\text{Cl} & \quad \text{CH}_2\text{Cl} \\
\text{IV} & \quad \text{V} & \quad \text{VI} & \quad \text{glycidol}
\end{align*}
\]
STUDIES OF THE COMPOSITION OF HUMAN UTERINE RINSINGS
Suzanne Morris and I.G. White
Department of Veterinary Physiology, University of Sydney, N.S.W. 2006

Uterine fluid provides the environment for spermatozoa as they ascend to the oviducts and it is important to understand its nature in order that the fluid may be modified effectively for contraceptive purposes.

Uterine rinsings were collected from women by washing the endometrium in situ with 10 ml of isotonic saline using a micro-urine collecting tube inserted through the cervix. The rinsings were centrifuged to remove debris and samples which were obviously contaminated with blood were discarded.

The sugar and sugar alcohol content of the rinsings was examined by descending paper chromatography. Inositol and glucose were found in every sample and some unidentified sugars were found in several rinsings. The concentration of inositol, which was measured in 3 samples by elution from the chromatogram and subsequent periodate oxidation was \(30.3 \pm 4.3\ \mu g\) per ml of rinsing. This indicates a relatively high concentration of inositol in whole uterine fluid compared with blood plasma. Although glucose may act as a source of energy for spermatozoa the role of inositol is obscure as there is little evidence that it is metabolized directly by spermatozoa.

In order to determine if human uterine fluid contains a glycerylphosphorylcholine (GPC) diesterase, the rinsings were incubated with human seminal plasma, in the presence of antibiotics, at 37°C for up to 24 hrs. The GPC content of the incubation mixture was then estimated using cellulose thin layer chromatography. The results indicated the presence of a diesterase although the degree of breakdown of GPC varied with different rinsings. The GPC diesterase in human uterine fluid may provide an additional source of energy for spermatozoa in the female tract as the products of its action are possibly metabolized by human sperm.

The protein content of each rinsing was measured by a modification of the Lowry method. No significant change in protein concentration was apparent during the menstrual cycle.

The levels of 16 amino acids were measured in several uterine rinsings and corresponding blood plasmas. Although there was variation between samples, the amino acid content of the rinsings and blood plasma were different. Uterine rinsings contained high levels of glycine, which constituted 20% of the total amino acids, glutamic acid (15%) and serine (10%). Free amino acids may be of significance in relation to the copper intrauterine device (IUD) as glycine and histidine enhance the production of cupric ions from copper metal (1).

Each year, 70% or 7.7 million cows in France are artificially inseminated with a varying proportion of usage between breeds with more than 80% A.I. in dairy cattle and less than 30% in beef cattle. Semen of dairy bulls is used mostly on dairy cows (with 1 out of 3-4 of the A.I. with semen of progeny tested bulls), whereas that of beef bulls is largely used for cross breeding (more than half of the A.I. with semen of tested bulls). Some seasonality is seen in the activity of A.I. centres with a peak in Spring, April-May. The results of fertility are quoted as 60-90 day non-return rates after 1st service, with a mean around 68%. The overall calving rate for all services or mating is around 80-90%. Part of this loss can be attributed to the male and a higher selection of semen quality has been started by A.I. organizations with preselection of young bulls on sperm freezability before they enter in progeny testing, as well as selection of bulls on the breeding performance of their daughters. Laboratory tests have also been developed for assessing semen quality in the bull. For the females, breeding efficiency varies with the breed: dairy heifers mature earlier whereas in beef heifers puberty is delayed until 15 months old or later; most dairy cows show oestrus in the second month after calving whereas in beef cows, cyclicity (tested through progesterone in blood) occurs later, more than 2 to 3 months after calving, especially in nursing cows. Thus control of the oestrous cycle has been developed for two specific physiological situations: oestrus induction in heifers or in cows showing post-partum anoestrus, and oestrus synchronization in cyclic animals. Induction of oestrus and ovulation is obtained by subcutaneous implants of progestagens, SC 21009, plus injection of PMSG (600 to 800 I.U.) on the day of implant removal. Fertility after A.I. at 48 and 72 h after the end of treatment is higher with a shorter duration of treatment (7 vs 9 vs 11 days) and a higher dosage of progestagen (12 vs 9 vs 6 mg) (see Table 1). Fertility is the same as in control cows. In cyclic cows, oestrus synchronization is obtained either by association of progestagen implant (7 to 9 days) and PGF2α analogue, the latter being injected 2 days before implant removal, or by two successive injections of prostaglandins 11 days apart. Fertility is similar for both methods. After oestrus synchronization and with a combination of systematic A.I. at a predetermined time and pregnancy test (progesterone in blood plasma 20-22 days after A.I.), a programmed herd management has actually been tested in which oestrus detection is deleted. Preliminary results of 88% of pregnancy within 65 days indicate that the system would be of practical use. Work is also in progress in France to induce moderate superovulation for twinning in cows; an alternative is given in this field by egg transfer.
Table 1: FERTILITY OF LACTATING COWS TREATED WITH SC 21009

<table>
<thead>
<tr>
<th>Duration of treatment (days)</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calving rate (%)</td>
<td>58.8</td>
<td>57.5</td>
<td>45.6</td>
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</table>

<table>
<thead>
<tr>
<th>Dose of SC 21009</th>
<th>12</th>
<th>9</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calving rate (%)</td>
<td>60.3</td>
<td>55.5</td>
<td>43.4</td>
</tr>
</tbody>
</table>

PMSG
| Calving rate (%) | 53.1 | 37.5 |

REFERENCES

FERTILITY OF BOAR SEMEN FROZEN IN A CONCENTRATED STATE

W. M. C. Maxwell and S. Salamon
Department of Animal Husbandry, University of Sydney, N.S.W. 2006.

The preparation of large volumes of frozen-thawed semen required for cervical insemination of pigs involves prolonged processing, freezing and thawing procedures, and also limits efficient utilization of storage facilities. These disadvantages can partly be overcome by freezing a small volume of concentrated semen and extending the thawed semen before insemination (1). These workers have frozen semen at a concentration of 0.6 x 10⁹/ml. This paper reports the results of a fertility test of semen frozen at a sperm concentration of 0.9 x 10⁹/ml.

Semen was collected by the manual method and the sperm-rich fraction of ejaculates split into two portions for processing by two methods. Method A involved removal of seminal plasma and glycerolization after cooling, and in method B removal of seminal plasma and glycerolization occurred before cooling. The diluent used consisted of 250 mM tris, 111 mM fructose, 15 mM EDTA (disodium salt), 79.5 mM citric acid, 15% (v/v) egg yolk.

In method A, semen was diluted (1:1, semen:diluent) at 30°C with the diluent containing no glycerol. In method B, the semen was centrifuged (1000g, 10 minutes) to remove the seminal plasma, and the spermatozoa resuspended (3%) (v/v) glycerol.

The cooling method used was modified from that described by Paquignon and Courrot (2). Both the diluted semen (method A) and resuspended semen (method B) were cooled to 15°C in one hour, held at this temperature for 3 hours, then further cooled to 5°C in one hour. Before freezing, the semen processed by method A was centrifuged (1000g, 10 minutes), the supernatant removed, and the spermatozoa resuspended to a concentration of 0.9 x 10⁹/ml with glycerolated diluent. In both methods (A and B) the semen was frozen by pelleting on dry ice, and the frozen pellets (0.3 ml) were stored in liquid nitrogen.

For insemination, the pellets were thawed at 37°C, and 30 ml of thawed semen was extended by adding 50 ml of pre-freezing medium containing no egg yolk and glycerol. The volume of inseminant (80 ml) contained 10 x 10⁹ motile spermatozoa (27.0 x 10⁹ total). Oestrus was detected by teaser boars twice daily, and inseminations were performed 24 hours (single) or 12 and 24 hours (double) after detection.

Thirty-two animals were inseminated, of which 20 (62.5%) farrowed. The mean litter size was 7.7, and one animal returned to service 65 days after insemination. Farrowings after insemination with semen processed by methods A and B were 9/16 and 11/16, and for single and double insemination 10/16 and 10/16.

The results obtained in this study showed the possibility of freezing boar semen in a concentrated state.

References
THE METABOLISM OF BOAR SPERMATOZOA DURING COOLING TO AND AFTER STORAGE AT -196°C

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The aim of this work was to compare the metabolic roles of the various chemicals used in the storage of boar semen. Collection, storage and thawing of the spermatozoa were carried out by the routine used at the laboratory (1).

In the first experiment the metabolism of spermatozoa was measured during each stage of the storage process. While the semen was being cooled from 28°C to 15°C glucose was oxidized and incorporated into spermatozoal lipid. After the addition of glycerol at 15°C and subsequent further cooling metabolism of glucose was not significantly greater than that due to the egg yolk, but oxidation of glycerol and its incorporation into lipid took place during cooling from 15°C to 5°C.

Because high concentrations of glycerol were found to cause an increase in its oxidation by sperm, in a further experiment spermatozoa were incubated, with the thawing diluent used at Nouzilly, before and after storage at -196°C. The medium, therefore, contained high concentrations of glucose, glycerol, citrate and bicarbonate, as well as egg yolk. Citrate was not metabolized and carbon dioxide fixation was only just detectable. Incorporation of fatty acids into sperm lipids occurred both before and after storage.

After storage there were decreases in the oxidation of glucose and glycerol. The fall in glucose oxidation (1.16 to 0.14 μmoles CO₂/3h/10⁸ motile spermatozoa) was greater than that in glycerol (0.90 to 0.34). Oxygen consumption of thawed sperm fell precipitously during the first hour of incubation, in contrast to the oxygen uptake of fresh cells which rose slightly during the first two hours. Both types of sperm showed increased oxygen uptake on addition of caffeine. During 5 hours of incubation fresh spermatozoa showed a constant high level of oxygen utilisation after addition of caffeine, whereas the response seen with stored cells diminished.

These results indicate that the metabolism of glycerol may be one facet of its cryoprotective function, and that it may also be important to spermatozoa after thawing.

REFERENCE


(a) Present address: Department of Physiology, University of New England, Armidale, N.S.W. 2351.
Artificial insemination (AI) with neat semen allows about 20 ewes to be inseminated from one ejaculate. Attempts to increase this coverage by diluting the semen have met with little success despite the great numbers of spermatozoa in ram semen. A possible reason for this is that the seminal prostaglandins (PGs) present in high concentration in the neat semen, and which might be involved in sperm transport, are considerably diluted by this procedure.

However, before testing this hypothesis by supplementing diluted semen with PGs, the effects of PGs on sperm metabolism and motility, and the stability of PGs in semen have been examined.

The effects of PGs on the metabolism of fructose by spermatozoa were assessed using the Warburg technique. The effect of each PG was examined separately at a concentration of 20 μg/ml or as a mixture in the concentration found in semen (PGE1 28; E2 3.2; E3 2; F1α 5 and F2α 2.3 μg/ml) and the experiments were replicated 4-6 times. The following results were statistically significant at the 5% level at least. The mixture of PGs inhibited O2 uptake by 6% compared with controls and increased lactate accumulation (+5%) and PGE1 increased the latter by 11%, results indicating a slight inhibition of the TCA cycle. By contrast, PGF1α and PGE3 stimulated oxygen uptake (11 and 8% respectively) and increased CO2 production (28 and 18%) suggesting that the metabolism of endogenous substrates was stimulated.

To check the effects of PGs on motility and on acrosomal morphology, 50 μl semen was diluted with 0.2 ml of a solution containing the 5 seminal PGs at 5 times the concentration in semen, or a solution prepared similarly but lacking PGs, and with 0.8 ml MES inositol egg yolk (5% v/v) buffer. The mixtures were either incubated at 39°C for 4 h or chilled to 5°C for 48 h. No significant differences due to PGs were seen in the 4 replicates studied.

To check the stability of added PGs, diluted whole semen, washed spermatozoa (180 x 10⁶), dialysed seminal plasma or a mixture of the two, in MES inositol buffer, were added to tubes containing 0.5 μg PGE2. The mixtures were then treated as follows - no treatment, incubation at 39°C for 1 h, chilling to 5°C for 24 h or storage in liquid N₂ for 48 h following suitable preparation. Then the spermatozoa were removed and the samples stored at -20°C. The experiment was replicated 3 times and the PGE content was measured by radioimmunoassay. The amounts of PGE present did not differ significantly between the various storage treatments and the results indicate that neither added PGE2 nor the PGEs in whole semen are broken down under these conditions.

Thus, no adverse effects on sperm metabolism or motility have been detected in these studies. In addition, it seems unlikely that the PGEs will be broken down upon contact with spermatozoa and thus they can be added to the semen extender in AI trials if so desired.
The nature of ultrastructural changes induced by exposure of spermatozoa to lysolecithin

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The effect of lysolecithin on spermatozoa was studied to evaluate the suggestion by Lucy (1) that the compound may be involved in the fusion between acrosomal and plasma membranes during the acrosome reaction associated with the process of fertilization.

Boar semen was diluted in 4.96, 49.6 and 496 µg/ml lysolecithin in Krebs-Henseleit-Ringer and incubated for 10 seconds or 2 hours before fixing for electron microscopy. The general effect of the lysolecithin was to break or remove the membranes from the spermatozoa: the acrosome reaction described by Lucy (1) was not observed. An exposure to a concentration of 496 µg/ml for 10 seconds removed the plasma membrane over the acrosome and tail and caused some structural damage to the mitochondria. However, the plasma membrane over the postacrosomal region of the head and over the midpiece of spermatozoa containing a cytoplasmic droplet were not damaged. After 2 hours incubation the acrosomal contents of all spermatozoa were completely dispersed. A concentration of 49.6 µg/ml of lysolecithin for 10 seconds removed the plasma membrane over the acrosome of a small proportion of spermatozoa and caused breakage of a proportion. However, the membrane over the tail was less susceptible to damage. After 2 hours exposure the membrane over the acrosome and tail was either broken or removed from most spermatozoa. The lowest concentration of lysolecithin had no effect on the spermatozoa.

It was considered that if lysolecithin could be used to selectively remove the plasma membrane over the acrosome of spermatozoa, such preparations could be used in studies of fertilization to assess the significance of the acrosome reaction. The membrane removal was attempted using a 100-fold dilution of spermatozoa with Krebs-Henseleit-Ringer 10 seconds after exposure to lysolecithin. The dilution itself was damaging to spermatozoa. As Gabara et al. (4) reported that the addition of serum would prevent the membrane labilizing action of lysolecithin the effects of adding heated pig serum or heated foetal bovine serum 10 seconds after the addition of lysolecithin were tested. The serum alone did not affect spermatozoa, but neither did it prevent the mitochondrial damage and loss of motility.

It is suggested that the use of a short exposure of spermatozoa to lysolecithin may be a useful method for workers studying the chemical composition of the membranes of spermatozoa.

ACROSOME DEVELOPMENT DURING SPERMIOGENESIS
IN SOME AUSTRALIAN MARSUPIALS: AN ULTRASTRUCTURAL STUDY

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Differences exist between marsupial and eutherian sperm in the development and mature form of the acrosome. In addition, the role played by the acrosome in egg penetration would appear to differ in the two groups. The major part of the acrosome in eutherian sperm appears to be involved in dispersal of the egg cumulus, leaving only the inner membrane and the equatorial segment for involvement in penetration of the zona pellucida (1). A cumulus is not found in marsupial eggs, and this raises the question of whether the marsupial acrosome may be analogous to the inner membrane and equatorial segment of the eutherian acrosome. These observations suggest that study of the form and development of the marsupial acrosome may provide valuable comparative information which could lead to a better understanding of the role of the acrosome among mammalian sperm in general.

This paper provides preliminary information for such a study by comparing acrosome development during spermigenesis in a number of Australian marsupials with the information available for eutherians. The species examined include Phascolarctos cinereus, Macropus parma, Megaleia rufa, Potorous tridactylus, Isodon macrourus and Trichosurus vulpecula. With respect to the initial stages of acrosome formation, these species can be divided into three groups based on the mode of formation of the granular component of the acrosome. Each differs from the situation in eutherians in that a proacrosomal granule is not present in the acrosomal vacuole which adheres to the nuclear surface.

In spermatids of the 'Trichosurus group' (including M. parma, Me. rufa, P. tridactylus) and the 'bandicoot group' (P. nasuta, I. macrourus) the granular component forms as a narrow electron dense layer in contact with the section of acrosomal membrane apposed to the nucleus. However, the electron dense layer is centrally located and discrete in the bandicoots, but is spread over the entire length of this section of the acrosomal membrane in the 'Trichosurus group'.

Early acrosome development in Phascolarctos differs from the above and is notable for the extreme development of the Golgi complex and the large size of the granular acrosomal component. The latter develops rapidly after the adherence of the acrosomal vacuole to the nuclear surface.

The further development of the acrosome, at least in Perameles (2) and Trichosurus, also differs considerably from that in eutherians. In these marsupials a relocation of the acrosomal material occurs in late spermigenesis when it comes to occupy a greatly reduced area of the nuclear surface. In Trichosaurus this results in the release at spermiation of a spermatozoon in which the acrosome differs considerably from its definitive form; the latter being achieved only after epididymal transit.

References.

GLUCOSE METABOLISM IN THE RAT TESTIS

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Some metabolic and enzymatic profiles of glycolytic metabolism in the normal rat testis have been examined by the direct fluorometric analysis of pyridine nucleotides linked with the metabolite or enzyme (Table I) using the methods described by Lowry and Passonneau (1972).

<table>
<thead>
<tr>
<th>Metabolite Level</th>
<th>Enzyme Activity</th>
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<tr>
<td>m mol/kg wet wt</td>
<td>m mol/kg wet wt/hr</td>
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<tr>
<td>Glycogen</td>
<td>0.50 ± 0.03</td>
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<tr>
<td>Glucose</td>
<td>0.97 ± 0.10</td>
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<tr>
<td>Glucose-6-phosphate</td>
<td>0.027 ± 0.002</td>
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<tr>
<td>Fructose-1,6-diphosphate</td>
<td>0.094 ± 0.010</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.084 ± 0.004</td>
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<tr>
<td>Lactate</td>
<td>1.28 ± 0.08</td>
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The concentration of glycogen, glucose and lactate were low compared with other tissues but the levels of ATP were relatively high and approached those of nervous tissue. Enzyme activities, except for lactate dehydrogenase, were also low, particularly in relation to the nervous system. Preliminary experiments were performed to localize enzymes. Frozen sections (10 μm) of rat testis were dried at -40°C and individual seminiferous tubules weighing 0.15 to 0.25 g were dissected. The tubules were staged and analyzed for hexokinase. Enzyme activity was low in spermatogenic stages up to meiosis (2, 3 and 4) but rose during the period of spermatid maturation (stages 5, 6, 7 and 8) and was maintained until just after spermiation (stage 1).

Fluorometric methods are highly sensitive and may be useful in the biochemical assessment of testicular function from small biopsy samples.

Reference

The peculiar seminal sugar pattern of a number of Australian marsupials has now been characterized (1, 2). However, the metabolism of marsupial spermatozoa or the role of the two major seminal sugars, N-acetylglucosamine and glucose, has not been investigated.

In the present study semen was collected by electroejaculation from animals (Trichosurus fulpsus) held in captivity (1). The semen was washed twice in calcium-free Krebs Ringer phosphate and the washed spermatozoa incubated at 37°C in a micro Warburg system (Braun 1.5 ml reaction flasks). The washed spermatozoa were incubated in substrate-free Krebs Ringer phosphate to determine the endogenous respiration and with 2 mM glucose or N-acetylglucosamine added to the medium. In some experiments labelled substrates were used. Oxygen consumption under these various conditions is shown in the table, with the number of replicates in brackets.

### Oxygen Consumption of Spermatozoa

<table>
<thead>
<tr>
<th>Substrate</th>
<th>μl/10⁸ spermatozoa/3 hr mean ± SEM</th>
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<tr>
<td>Endogenous</td>
<td>97.8 ± 19.7 (6)</td>
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<tr>
<td>Glucose (2 mM)</td>
<td>110.6 ± 14.3 (5)</td>
</tr>
<tr>
<td>N-acetylglucosamine (2 mM)</td>
<td>80.4 ± 20.5 (4)</td>
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</table>

The endogenous respiration of possum spermatozoa appears to be higher than those of eutherian species (e.g. bull, ram and man) and is not further increased by exogenous glucose or N-acetylglucosamine. Chemical analysis has, however, shown that both sugars are utilized by possum spermatozoa and presumably have a sparing effect on the unknown endogenous substrate within them. The numerous prostatic spheres found in marsupial semen, in about equal numbers to the spermatozoa, do not utilize oxygen when incubated in the present system.


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