AUSTRALIAN SOCIETY
for
REPRODUCTIVE BIOLOGY

PROCEEDINGS
OF THE
SEVENTH
ANNUAL CONFERENCE

SYDNEY
August 18–20, 1975
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ACKNOWLEDGEMENTS

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

The University of Sydney
The Joint Organizing Committee
The Joint Programme Committee
Ansett Airlines of Australia,
who assisted financially with printing of Abstracts.

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AUSTRALIAN SOCIETY FOR REPRODUCTIVE BIOLOGY

Seventh Annual Conference
August 18 - 20, 1975

Carslaw Lecture Theatre, University of Sydney

PROGRAMME

Monday, August 18
9.00 - 9.30 ......................... Registration

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SESSION III

Chairman: Dr. I.G. White

2.00-3.00
Guest Lecturer - A.E.S. - Dr. W.D. Odell

Metabolic and ultrastructural changes in ejaculated spermatozoa induced by heating the testes of rams.

3.00 13

A comparison of spermatozoa numbers released in the urine and in ejaculates of a marsupial, the Brush-Tailed Possum.

3.15 14
J.C.Rodger, I.G.White

SESSION IV

Chairman: Professor G. Sharman

4.00-4.30
Guest Lecturer - B.P. Setchell

Blood circulation in the Testis: its control and significance.

4.30 15
C.H.Tyndale-Biscoe, J.Hawkins

The summer solstice and the onset of breeding in the Tammar Wallaby.

4.45 16
D.J.Kennaway, R.F.Seamark

Pineal gland changes during the period of blastocyst activation in the Tammar Wallaby, Macropus Eugenii.

5.00 17
G.P.M. Moore

Reactivation of RNA synthesis in macropod embryos after diapause.

5.15 18
R.L. Hughes

Preliminary observations on reproduction and embryonic development in the marsupial devil, Sarcophilus Harrisii.

6.00
Cocktail Party, Wentworth Building, University of Sydney.
### Tuesday August 19

#### Session V
**Chairman: Dr. B.P. Setchell**

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<td>M. Anne Denehy</td>
<td>Spermatogenesis in a passerine hybrid: phagocytosis by sertoli cells after absence of synaptonemal complexes.</td>
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<td>9.15</td>
<td>Suzanne Morris, Patricia Brown-Woodman, I. G. White</td>
<td>Decrease in the glycerolphosphorylcholine content of the epididymis after injecting α-chlorohydrin into rats.</td>
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<td>10.00</td>
<td>N. R. Frith, F. E. Ickowicz, P. B. Marley</td>
<td>Production of prostaglandin-like substances by mouse spermatozoa in vitro.</td>
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**CONCURRENT CONJOINT SESSION WITH A.E.S.**

#### Session VI
**Chairman: Professor N. W. Moore**

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<td>9.00</td>
<td>Margaret B. Parr, Earl L. Parr</td>
<td>Endocytosis in the uterine epithelium of the mouse.</td>
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<td>J. H. Stanton</td>
<td>Preliminary investigation into uterine epithelial cell types using cluster analysis.</td>
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<td>9.30</td>
<td>B. Buirchell, R. Hahnel</td>
<td>Estradiol 17-α metabolism: A control over human endometrial growth (Endocrine Society Paper)</td>
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<td>9.45</td>
<td>Leigh C. Murphy, Grant M. Stone</td>
<td>The uptake and metabolism of [3H] progesterone by the ovariectomized mouse uterus.</td>
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#### Session VII
**Chairman: Dr. J. K. Findlay**

**MORNING TEA**

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<tr>
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<td>W. G. Breed</td>
<td>The effect of differing environments on the oestrous cycle and ovulation rate in several species of native Australian rats.</td>
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<td>11.15</td>
<td>Carol Worthington, J. P. Kennedy</td>
<td>Primordial follicles in the ovary of the neonatal ewe lamb.</td>
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<td>11.30</td>
<td>Robin Tassell, W. A. Chamley, J. P. Kennedy</td>
<td>Gonadotrophins in the neonatal female lamb.</td>
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<td>11.45</td>
<td>J. K. Findlay, B. M. Bindon</td>
<td>Plasma FSH in Merino lambs selected for fecundity.</td>
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<td>12.00</td>
<td>I. A. Cumming, J. K. Findlay</td>
<td>FSH and unilateral ovarioectomy in the ewe.</td>
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<tr>
<td>12.30</td>
<td>P. E. Mattner, B. D. Stacy, B. W. Brown</td>
<td>Changes in total ovarian blood flow during anaesthesia and cannulation of utero-ovarian veins.</td>
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**LUNCH**

#### Session VIII
**Chairman: Professor C. W. Emmens**

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<td>M. J. Carrick, P. T. Cupps</td>
<td>Interactions between steroids and spasmogens on the myometrium of the ewe.</td>
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<td>10.15</td>
<td>W. J. Fulkerson, R. Hooley, G. H. McDowell, L. R. Fell</td>
<td>Progesterone and induction of lactation in ewes.</td>
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**Interactions between steroids and spasmogens on the myometrium of the ewe.**

**Progesterone and induction of lactation in ewes.**

**Plasma FSH in Merino lambs selected for fecundity.**

**FSH and unilateral ovarioectomy in the ewe.**

**Preovulatory gonadotrophin and ovarian steroid changes in French sheep breeds different in fecundity. Does FSH stimulate follicles? (Endocrine Society Paper) **

**Changes in total ovarian blood flow during anaesthesia and cannulation of utero-ovarian veins.**

**The role of follicles in Luteolysis**

**James Goding Memorial Lecture**

**Professor W. Hansel**

**The role of follicles in Luteolysis**

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*Note: The schedule and the order of the sessions seem to be slightly scrambled or out of sequence, which might cause confusion in understanding the content. The content is extracted from a scientific meeting agenda, focusing on various topics related to endocrinology, reproductive biology, and other related fields.*
3.00  36  R.J.Lightfoot
T. Marshall
Effects of lupin grain supplemen-
tation on ovulation rate and
fertility of Merino ewes.

3.10  37  D.J.Rizzoli, R.
Baxter, J.Reeve, I.A.Cumming
The effect of lupin grain supple-
mentation on ovulation rate in
Border Leicester X Merino ewes.

3.20  38  J.L.Reeve, P.A.
Kenney, R.Baxter, I.A.Cumming
Effect of lupin grain, wheat and
lucerne supplements on ovulation
rate in Maiden Border Leicester X
Merino ewes.

3.30  39  AFTERNOON TEA

Session IX
Chairman: Dr. R.I. Cox

4.00  40  T.J.Weiss, R.F.
Seamark, R.M.Moor
J.E.A. McIntosh
CAMP formation in the sheep ovarian
follicle in response to gonadotrophins;
changes in the sterol content of
sheep ovarian follicles in culture:
relationship to stereiodogenesis
and effects of gonadotrophins.

4.15  41  R.F.Seamark, R.P.
Hamilton, R.M.Moor
FSH and LH activities of PMSG,
and effects on serum and fetal
trophoblast cells maintained in
culture.

4.30  42  J.E.A. McIntosh,
Meredith Kaethner, Francesca Stewart,
R.M.Moor
The role of progesterone in
regulating the length of
oestrus cycles in sheep.

9.30  44  R.I.Cox, Patricia
A. Wilson
Effects of immunizing sheep to
steroid oestrogens and phyto-
estrogens.

9.45  45  D.G. Fowler
Predicting the number of corpora
lutea in Merino ewes.

10.00 46  R.T.Gemmell, B.D.
Stacy
Effects of colchicine on the
structure and function of the
corpus luteum in the sheep.

10.15 47  B.D.Stacy, R.T.
Gemmell
Morphological and functional
together luteum after
restriction of ovarian blood flow.

5.00  48  ANNUAL GENERAL MEETING, A.S.R.B.

7.30  Conference Dinner, Kei-Ron, 62 Carrington Road, Waverley.

Wednesday August 20

Session X
Chairman: Dr. P.E. Mattner

9.00  42  A.R.Bray, J.F.Hecker
M.Wodzicka-
Tomaszewski
The role of progesterone in
regulating the length of
oestrous cycles in sheep.

9.15  43  R.J.Fairclough, J.F.
Smith, A.J.Peterson,
L. McIlwain
The effect of oestradiol-17β,
progesterone and prostaglandin P2α
antiplasma on luteal function
in the ewe.

11.00 49  D.P.Hennessy,
P. Williamson
Plasma progesterone levels in
non-pregnant sows showing delayed
return to oestrus following mating.

11.15 49  D.A.Shutt, R.P.
Shearman, R.C.
Lynaham
Steroid and prostaglandin F con-
centrations in human corpora lutea
in relation to luteolysis.

11.30 50  L.P.Cahill, R.W.Knee,
R.A.S. Lawson
Induction of parturition in sheep
with a single dose of oestradiol-
benzoate.

11.45 51  C.A.Sloan
Comparison of dexamethasone
trimethylacetate (DTMA) and
prostaglandin (PG) F2α as abort-
ifacents in the cow.

12.00 52  J.P.Drinan, M.S.F.
Wong, R.I.Cox
Hormonal changes in cow and calf
about normal and induced calving.

12.15 52  Ann V. Ghisalberti,
P.Helen Pearce, P.N.
Di Marco, I.T.Oliver
Foetal postmaturity (Endocrine
Society Paper)

12.30 53  N.W.Bruce, R.K.
Bartholomeusz
Effects of medroxy-progesterone
acetate and oestradiol-17β on
pregnancy maintenance in ovari-
ectomized rats.
THE EFFECTS OF OLFATORY STIMULI UPON THE UNIT ACTIVITY OF ANTIDROMICALLY IDENTIFIED NEUROSECRETORY NEURONES IN THE PARAVENTRICULAR NUCLEI OF THE RAT.

P.M. Young

Department of Anatomy, The University of Adelaide, South Australia, 5000.

Olfactory afferents to the magnocellular neurosecretory nuclei have been demonstrated in several species (Hayward, 1973), and elevated plasma levels of oxytocin have been found in the male donkey prior to coitus (Walsley, 1963). If the posterior pituitary hormones are indeed involved in the contractile activity of male sex organs during coitus (Melin, 1970) then it may be assumed, particularly in animals in which coitus is completed in very much less time than the latent period of the neurohormonal reflex (sheep and ox) that the stimuli initiating the reflex liberation of the hormones must originate during the courtship preceding active coitus. These observations in part prompted this investigation.

Neurosecretory neurones in the paraventricular nuclei of urethane anaesthetised male rats were recorded with stainless steel microelectrodes following their antidromic identification by delivering pulses to the neurohypophysis. In a separate series of experiments multiunit activity was recorded from a 50 \( \mu \) electrode positioned in the pituitary stalk. The olfactory stimuli used were urine from individual females in oestrus, urine from ovariectomized females, and two analar grade chemicals amyl acetate and cineole.

Whatever the role of neurohypophysial hormones in sexual function may be, there are a wealth of observations involving gonadotrophin release, sexual behaviour, sperm transport and penile sensitivity, these findings demonstrate that at least in the male rat odour from the urine of a sexually receptive female is capable of activating a specific group of neurosecretory neurones in a characteristic way that is compatible with the depolarisation frequency necessary in these cells to release neurohypophysial hormones.

Session XII
Chairman: Dr. R.F. Seamark

2.00 54 I.L. Pike, R.G. Wales
The uptake and incorporation of \( \text{U}^{14}\text{C} \) glucose during culture of mouse blastocysts previously undergoing normal implantation and induced delay of implantation.

2.15 55 L.D. Staples, I.A. Cumming, R.A.S. Lawson, F.J. Morgan
Proteins of the uterine secretion in sheep.

2.30 56 R.A.S. Lawson, Mildred Cerini, J.K. Findlay
An immunological test for pregnancy in the ewe.

2.45 57 J.C. Cerini, Mildred Cerini, J.K. Findlay, R.A.S. Lawson
The distribution of prostaglandin F, progesterone and pregnancy-specific antigen in unilaterally pregnant ewes.

3.00 58 Mildred Cerini, J.C. Cerini, J.K. Findlay, R.A.S. Lawson
Preliminary characterization of pregnancy specific antigen(s) in the ewe.

3.15 59 A.A. Gidley-Baird, C.W. Emmens
The function of anterior pituitary hormones in the initiation of implantation in mice.

3.30 AFTERNOON TEA

Session XIII
Chairman: Dr. R. Lawson

4.00 60 B.G. Miller, N.W. Moore
The effects of progesterone and oestradiol on endometrial metabolism in the ovariectomized ewe.

4.15 61 N.W. Moore, B.G. Miller
Progesterone and oestradiol requirements for the survival of embryos in the ovariectomized ewe.

4.30 62 R.J. Bilton, N.W. Moore
The storage of cattle embryos.

4.45 63 S. Salamon, G.N. Pearse
Fertility of Boar semen frozen-stored for 24 years.
HYPOTHALAMIC NEURONES INVOLVED IN THE NEUROENDOCRINE REGULATION OF OVULATION

J. Priedkalns and G. Sobels
Harvard Medical School, and Department of Anatomy, University of Adelaide, Adelaide, South Australia, 5001.

Recent research (see Cross, 1973) has given new insight into the relationship of action potential activity in neurones and the discharge of hormonal products from their axonal terminations. To obtain further data on hypothalamic neurones which form the neurophysiological basis for ovulation the ultrastructure and biogenic amine content of axon terminals were examined in perivascular parts of the external zone of the median eminence, and some electrophysiological properties of neurones determined in the anterior hypothalamus, on the four days of the oestrous cycle in the rat.

Linear scanning techniques were employed to determine quantitatively the percentage volumes of tissue occupied by different types of axon terminals. The percentage volumes of terminals containing mainly large (~120 nm) granules (dense-core vesicles) increased daily from oestrus (6.2 ± 1.5%) to pro-oestrus (8.9 ± 1.4%), and of those containing mainly small (~40 nm) granules (resembling synaptic vesicles) decreased daily from oestrus (26.1 ± 2.6%) to pro-oestrus (23.1 ± 2.9%). Terminals with approximately equal representation of both types of granules were counted equally in the two groups. The increases and decreases were statistically not significant. The observations nevertheless suggest the possibility of utilization of the large granule content, and associated accumulation of small vesicles, during the period from proestrus to oestrus, i.e. late on proestrous day. Since the RF release related to the oestrous cycle and ovulation constitutes only one of the components of the hypothalamo-pituitary axis, only relatively small differences in the percentage volumes observed could be expected; morphological identification of the individual RF’s and monoamines involved will be necessary in further work. By the assessment of monoamine fluorescence intensity, increased amounts of catecholamines were recorded during pro-oestrus and metoestrus in the external-zone axons.

In the anterior hypothalamus, we demonstrated increased electrical responsiveness of neurones to an iontophoretically applied progestagen in pro-oestrus. Initial data (Priedkalns et al., 1975) showed that medroxyprogesterone acetate (U-21, 687E) increased the firing rates of 41% of anterior hypothalamic neurones studied in pro-oestrus, of 20% in oestrus, and of none in dioestrus. These pro-oestrus results correlate with our previous work demonstrating facilitatory effects on ovulation of progesterone implants applied to the anterior hypothalamic area 2-4 hours before the 'critical period'.

The present observations 1) support the hypothesis that oestrous cyclicity and ovulation are at least partly determined by neurones in the anterior hypothalamus, and 2) are consistent with hypotheses that some of the granules of axon terminals in the external zone of the median eminence contain gonadotrophin-RF’s and that monoamines are important in their pro-oestrous release.
Changes in the peripheral levels of LH, FSH and testosterone in rams have been studied from birth to sexual maturity (Lee et al., 1974), and the present study represents further investigations into the pituitary-testicular relationships in developing rams using the following manipulations - castration and oestradiol-17β treatment.

Twenty-one male crossbred Merino-Corriedale lambs were used in this study. Seven lambs in this group of animals were bilaterally castrated within 24 hours after birth. Three to 4 normal rams (Group 1) and wethers (Group 2) were bled at the ages of 2, 5, 15, 21 and 28 weeks, and on each occasion when the animals were bled, 12 bleeds of 8 - 10 ml of blood at 15 min intervals were taken by venepuncture from the jugular vein. Animals which were treated with 5 μg of oestradiol-17β intravenously were bled at hourly intervals for up to 24 hours after oestrogen injection. Two ewe lambs were included in this group. The plasma samples collected were assayed for LH, FSH and prolactin by specific radioimmunoassays.

Plasma FSH levels in the normal rams at the age of 2 weeks showed a mean value of 35 ng/ml, at 5 weeks the levels increased to 50 ng/ml and at 15, 21 and 28 weeks the values ranged between 29.0 and 49.2 ng/ml. LH levels were <0.5 ng/ml at 2 and 5 weeks but increased to levels which ranged between 1.4 to 3.5 ng/ml by the ages of 15, 21 and 28 weeks. Preliminary results of prolactin concentrations showed a mean value of 33 ng/ml at 2 weeks postnatally and this was increased to 245 ng/ml by the 5th postnatal week.

In the castrate lambs, plasma FSH levels at the age of 2 weeks were significantly higher (4 - 10 fold; mean value of 324 ng/ml) than that seen in normal ram lambs; this value was elevated to 803 ng/ml by the 5th postnatal week. At the ages of 15, 21 and 28 weeks plasma FSH concentrations tended to decrease and varied between 389 - 855 ng/ml. In contrast, there was no significant difference in the mean LH values observed at the various times studied. Episodic secretion of LH was seen in most of the animals and values ranged between 8.2 ng/ml to 21.4 ng/ml. Prolactin levels exhibited a mean value of 45 ng/ml at 2 weeks postnatally but this value was increased to 226 ng/ml at the age of 5 weeks.

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Preliminary results on the effects of oestradiol-17β (E₂) treatment in normal rams, wethers and ewe lambs suggests that only the latter group showed a positive response to E₂ treatment, as early as at 2 weeks of age. LH levels were elevated at between 14 to 18 hours after E₂ treatment. In the wethers plasma LH and FSH levels were suppressed by E₂ treatment.

These results confirm our previous findings (Lee et al., 1974) of elevated LH and FSH levels in ram lambs at the age of 5 - 6 weeks; in addition, preliminary results of prolactin levels indicate also a rise during this postnatal period. The findings of elevated LH and FSH levels within 2 weeks in ram lambs castrated on the first neonatal day suggests that an inhibitory feedback relationship of the testis and pituitary had already been established. It is intriguing that despite the absence of testis, there was an increase in FSH levels at 5 weeks of age suggesting a change in the hypothalamic control of FSH independent of testicular secretion.
THE PRESENCE OR ABSENCE OF TESTOSTERONE DURING THE NEONATAL DEVELOPMENT OF RATS HAS BEEN SHOWN TO EFFECT NOT ONLY THE CENTRAL NERVOUS SYSTEM BUT ALSO THE SENSITIVITY OF ANDROGEN DEPENDENT TARGET TISSUES (BEACH, NOBLE AND ORNDOFF, 1969). THE PRESENT STUDY WAS INITIATED TO DETERMINE THE EFFECT OF NEONATALLY ADMINISTERED OESTROGENS ON PUBERTAL HORMONE LEVELS IN BULL CALVES.

FIVE BULL CALVES WERE INJECTED INTRAMUSCULARLY WITH 6 MG OF OESTRADIOL-17β-3-BENZOATE AT BIRTH AND THEN DAILY DOSSES OF 3 MG WERE ADMINISTERED FOR THE NEXT 20 DAYS. TWO OTHER MALE GROUPS CONSISTING OF 5 NORMAL CALVES AND 4 CASTRATES (STEERS) WERE INJECTED WITH THE CARRIER ONLY. THE MALE CALVES WERE FROM 2 FRIESIAN SIRS AND EACH GROUP CONSISTED OF AT LEAST 2 CALVES PER SIRE. ALL THE ANIMALS PLUS 5 JERSEY HEIFER CALVES WERE RUN TOGETHER UNTIL PUBERTY. BLOOD SAMPLES WERE TAKEN AT REGULAR INTERVALS AND PLASMA TESTOSTERONE AND LUTEINISING HORMONE (LH) CONCENTRATIONS WERE MEASURED BY RADIOIMMUNOASSAY.

DURING THE FIRST WEEK OF LIFE TESTOSTERONE LEVELS WERE SIMILAR FOR ALL GROUPS (0.12 ng/ml). THE HEIFERS AND STEERS RETAINED THESE LOW LEVELS THROUGHOUT THE STUDY. NORMAL BULL CALVES SHOWED A GRADUAL RISE IN TESTOSTERONE LEVELS UNTIL 4 MONTHS OF AGE AT WHICH TIME THE PLASMA LEVELS ROSE STEEPLY TO REACH 6.7 NG/ml BY THE 5TH MONTH. THE OESTROGENISED MALES HAD A SIMILAR RISE IN TESTOSTERONE LEVELS EXCEPT THAT THE RISE WAS DELAYED BY 2-3 WEEKS. WHEN 500 IU HCG WERE GIVEN INTRAVENOUSLY TO ALL GROUPS AT 4 MONTHS OF AGE THE PLASMA TESTOSTERONE CONCENTRATIONS OF ALL THE MALES ROSE. THE INCREASE IN THE NORMAL MALES WAS SIGNIFICANTLY GREATER THAN THAT OF THE OESTROGENISED MALES (P < 0.05) BUT THIS DIFFERENCE WAS DECREASED AFTER HCG TREATMENT AT 5 MONTHS AND HAD DISAPPEARED AFTER SIMILAR TREATMENT AT 7 MONTHS OF AGE.

THE PLASMA LH CONCENTRATIONS DECREASED OVER THE FIRST 7 DAYS AFTER BIRTH IN ALL GROUPS AND THEN GRADUALLY RISE AND THERE WERE NO SIGNIFICANT DIFFERENCES BETWEEN THE SEXES EVEN UP TO 4 MONTHS OF AGE. THE STEERS' LH LEVELS, HOWEVER, ROSE RAPIDLY FROM DAY 30 AND BY 4 MONTHS THE LEVELS WERE HIGHER THAN ALL THE OTHER GROUPS. THE OESTROGENISED MALES SHOWED NO RISE IN LH UNTIL THE END OF THE OESTROGEN TREATMENT AND THEN THE LEVELS SLOWLY ROSE, SO THAT BY 4 MONTHS THERE WERE NO REAL DIFFERENCES BETWEEN THEM AND NORMAL MALES.

THESE PRELIMINARY RESULTS INDICATE THAT NEONATALLY ADMINISTERED OESTROGEN ON MALE CALVES DELAYS NORMAL PUBERTAL DEVELOPMENT.

REFERENCE

PITUITARY-TESTICULAR RESPONSES IN RAMS TO PROLONGED INFUSIONS OF LH-RELEASING HORMONE.

W.J. Bremner, J.K. Findlay, D.M. de Kretser, I.A. Cumming and B. Hudson
Medical Research Centre, Prince Henry's Hospital, Melbourne, 3004, Department of Agriculture and Reproduction Research Section, S.S. Cameron Laboratory, University of Melbourne, Werribee, 3030 and Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, 3052.

While the responses of LH and FSH to bolus administration of LH-releasing hormone (LH-RH) have been studied extensively in many species, gonadotrophin responses to prolonged administration of this agent have not been well characterized. Gonadal steroid production in response to the elevated levels of gonadotrophin produced has received relatively little attention, particularly in animals. We have administered constant intravenous infusions of various dosages of LH-RH to rams and studied the patterns of LH, FSH, testosterone (T) and oestradiol (E2) produced.

Seven Corriedale rams aged 2-3 years were studied. Synthetic LH-RH (Hoechst) was administered by infusion pump (4.0 ml/hr) into one jugular vein. Blood for hormone studies was obtained from an indwelling catheter in the other jugular vein. Two rams received each of 4 different dosages of LH-RH (1.0, 0.5, 0.1 and 0.05 μg/min) in 4-hour infusions separated by at least one week. Three basal blood samples at 15-min. intervals were obtained; further sampling was at 15-min. intervals during the infusions and at 30-min. intervals for 2 hours following the infusions. Five other rams received 4-hour infusions of 0.5 μg/min with an identical blood sampling schedule except that blood was obtained at 5-min. intervals during the first hour of the infusion. LH (modification of Goding et al., 1969), FSH (Salamonsen et al., 1973), T (Wang et al., 1974) and E2 (modification of Dufau et al., 1970) were measured by specific double-antibody radioimmunoassays.

During the two higher dosage infusions (1.0 and 0.5 μg/min) given to each of 2 rams, LH increased dramatically within 15 mins. from basal values of 1.0-2.4 ng/ml (NIH-S-8) to levels of 15-50.4 ng/ml, then remained stable or fell slightly until 60 to 75 mins. when a second sharp rise began, reaching peak values of 85-138 ng/ml at 105-150 mins. After this time, LH values fell (23 to 57 ng/ml at 4 hours) in spite of continued LH-RH infusion and decreased further in the 2 hours after the infusion (5.4 to 12.6 ng/ml at 6 hours). The pattern of a rapid early increase in LH until 20 to 30 mins. then a plateau until 60 to 75 mins. followed by a second dramatic increase was confirmed in the 5 rams studied with 5-min. blood sampling during the first hour of the infusions. During the 0.1 μg/min. studies, the early phase of LH release was markedly reduced (3.2-15.1 ng/ml at 15 mins.), but the later phase was similar to that found in the higher dosage infusions (83.3-112 ng/ml at 150 mins.). During administration of the lowest dosage (0.05 μg/min.), no early LH release was detected (1.5-3.6 ng/ml at 15 mins.) and the later phase was also reduced (30-42 ng/ml at 150 mins.). FSH values during infusions of all 4 dosages rose very slightly and very gradually, from basal levels of 10-65 ng/ml (NIH-S-6) to maximal values of 22-82.5 ng/ml at 120-150 mins., after which they decreased to 10-80 at 4 hours and to 10-64 ng/ml at 2 hours after the infusions. No dose-response relationship was apparent. No FSH response was demonstrable during one infusion at the highest dosage.
T levels began a dramatic increase 15-30 mins. following the LH increase. T rose from control levels of 138-962 ng/100 ml to maximal levels of 650-2180 ng/100 ml at 90-180 mins. The maximal increments in T averaged 1180-1250 ng/100 ml during the infusions of 1.0, 0.5 and 0.1 ug/min and 700 ng/100 ml in the 0.05 ug/min. studies. In spite of the marked increases in T, no significant changes could be demonstrated in serum E2 levels (range <15-21 pg/ml) during LH-RH infusions of 0.5 and 0.1 ug/min.

These results demonstrate two phases of LH increase and only one phase of FSH increase during constant LH-RH administration to rams, similar to data we have obtained in humans (Bremner and Paulsen, 1974; de Kretser et al., 1974). In addition, a refractory state of the pituitary to LH-RH stimulation was produced after 105-150 mins. of the infusions where gonadotrophin levels decreased markedly in spite of continued releasing hormone administration. Since much less hormone was released during the low dosage infusions, it seems unlikely that the refractory state is due to simple depletion of pituitary hormone stores. It may be that the high levels of T produced exert an inhibitory feedback effect on the ram pituitary.

REFERENCES

THE EFFECT OF GONADOTROPHIN RELEASING HORMONE (GnRH) ON THE LH RESPONSE TO OESTRADIOL-17B (E2) IN THE SPAVED EWE
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It has been proposed that oestrogens may potentiates the LH response to GnRH (Reeves et al. 1971) and that the positive feedback effect of E2 is directed at the hypothalamus where it stimulates GnRH release (Jonas et al. 1973). This paper examines, (a) effect of GnRH on E2-induced LH release, and (b) effect of E2 on GnRH-induced LH release. Four groups, each of 5 spayed ewes, received i.v. injections of either 40 ug E2, 20 ug GnRH or saline as described in Table 1. Jugular blood samples were collected at 30-60 minute intervals for 29 hours and the plasma assayed for LH. Plasma LH concentrations (LER 1374A) for selected time periods were subjected to an analysis of variance.

TABLE 1
EXPERIMENTAL DESIGN GIVING TREATMENT TIMES AND THE MEAN LH LEVELS (ng/ml) WITHIN SPECIFIED TIME PERIODS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Time (hr)</th>
<th>Time Period (hr)</th>
<th>Mean LH Level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>E2</td>
<td>0 - 5</td>
<td>4.0 (b)*</td>
</tr>
<tr>
<td>II</td>
<td>E2</td>
<td>5.5 - 9</td>
<td>3.6 (b)</td>
</tr>
<tr>
<td>III</td>
<td>E2</td>
<td>9.5 - 13</td>
<td>6.1 (b)</td>
</tr>
<tr>
<td>IV</td>
<td>Saline</td>
<td>14 - 24</td>
<td>8.1 (a)</td>
</tr>
</tbody>
</table>

*Within time periods: a > b > c, (P <0.01)

The results indicate that; E2 treatment lowered LH basal values (Period 0-5 hr); E2 did not potentiate or depress GnRH-induced LH release (Period 5.5-9 hr); in groups II and IV, which received two GnRH injections, E2 did not significantly alter the magnitude of or the ratio between the responses to the first and second GnRH injection (Period 5.5-13 hr); the LH response to E2 in the group II given two GnRH injections was less (P <0.01) than groups I or III given no or only one GnRH injection (Period 14-24 hr).

These results confirm the negative and positive feedback of E2 on LH. E2 had no effect on LH response to GnRH but GnRH overcame the negative feedback effect of E2 on LH and reduced the positive feedback effect of E2 probably because of pituitary depletion of LH. Furthermore the increase in LH responsiveness to GnRH on days 15 and 16 of the oestrous cycle is more likely due to the absence of progesterone (Hooley et al. 1973) than a potentiating effect of E2.

REFERENCES
EFFECTS OF LOCAL HEATING AND RETE TESTIS FLUID OF FSH AND TESTOSTERONE LEVELS IN BLOOD PLASMA OF MALE RATS.

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It is known that selective destruction of the germinal epithelium causes plasma FSH levels to increase, presumably by reducing the production in the testis of a substance ('inhibin') that specifically suppresses the secretion of FSH (Van Thiel et al., 1972). Inhibin is thought to originate in the Sertoli cells or early germinal elements and its activity in rete testis fluid (RTF) has previously been demonstrated (Setchell and Jacks, 1974). In the present study, local application of heat was used to damage germinal elements in rats. Since an apparent, specific elevation in circulating FSH resulted, these heat-treated animals were used as models for testing differences of inhibin-like activity in RTF from rats exhibiting high vs low spermatogenic activity.

The scrotum of each of 27 Charles River CDF inbred male Albino rats, 63 days old, was exposed to heat by immersion in water at 43°C for 15 min. (Chowdhury and Steinberger, 1964). Six other rats, left untreated served as controls. Seven days after exposure, ram RTF or saline was injected i.p. twice daily, 0.66 ml at 09.00 hours and 1.33 ml at 17.00 hours, for 6 consecutive days. On the morning following the last day of injection, the rats were decapitated and blood was collected into heparinized receptacles and centrifuged. Plasma FSH and testosterone (T) levels were determined by radioimmunoassay (Odell, Rayford and Ross, 1967; Bartke et al., 1973). Rete testis fluid, containing >40 X 10^6 spermatozoa/ml (RTF-H) was compared with RTF from the same ram, but with a sperm concentration <0.4 X 10^6 cells/ml (RTF-L). Samples of RTF, collected continuously at 15°C (Voglmayr et al., 1967) from two rams, were tested.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>FSH (ng/ml)</th>
<th>T (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non/heated saline</td>
<td>6</td>
<td>381 ± 19</td>
<td>1.64 ± 0.23</td>
</tr>
<tr>
<td>Heated/saline</td>
<td>9</td>
<td>623 ± 13</td>
<td>1.15 ± 0.14</td>
</tr>
<tr>
<td>Heated/RTF-H</td>
<td>9</td>
<td>527 ± 31</td>
<td>1.45 ± 0.14</td>
</tr>
<tr>
<td>Heated/RTF-L</td>
<td>9</td>
<td>629 ± 39</td>
<td>1.63 ± 0.16</td>
</tr>
</tbody>
</table>

Table 1 shows that local heating of the testes caused a marked elevation (P < 0.01) in plasma FSH levels. The testis weight of the heated rats was less than half that of the non-heated controls, but plasma T levels, presumed to parallel LH levels, were similar. Injection of RTF-H resulted in a significant decrease (P < 0.025) of the elevated plasma FSH levels in the locally heated rats, whereas RTF-L was ineffective. These results suggest that the inhibin-like activity in RTF may be related to sperm production rates.
Melatonin (3-methoxyacetylserotonin) is generally regarded as the putative pineal hormone. However, few reliable and/or practical techniques are available for its assay in blood or tissues other than the pineal.

Our interest is in the function of the pineal in pregnancy in sheep and in this communication we report on attempts to identify melatonin in the blood of pregnant sheep and within the fetal circulation using a sensitive and highly specific bioassay.

Pregnant Merino crossbreed ewes of known gestation (Day 120-147) were comfortably housed for several days in 12:12 light-dark conditions. Prior to sampling they were sedated, without disturbance with sodium pentobarbitone and a 200 ml sample of jugular vein blood taken. A hysterotomy was then rapidly performed and a sample of fetal heart blood taken. Plasma was stored at -10°C until assayed within 7 days. For assay, the plasma was treated with 0.1M NaOH and extracted with chloroform. The extract was then taken to dryness in vacuo and the residue resuspended in isotonic fish buffer so that a thousand fold concentration of the original plasma volume was achieved. This was then injected intraperitoneally (10 μl/fish) into 8-10 pencil fish Nannostomus beckfordi anomalous according to the technique of Reed and Finnin (1973). This unique bioassay depends upon changes in melanosome distribution of the day band of the fish, and the definitive end point is judged visually. The assay is highly specific for melatonin, and related indoles do not significantly cross react (Ruffin, Reed and Finnin, 1969). The useful range of the assay is 50-150 pg per injected sample and 80% recovery was achieved in the extraction procedure employed.

Twenty ewes and their fetuses were examined, eleven were sampled at about 12.00 hr and nine at about 01.00 hr after 6 hr darkness. In daytime samples, melatonin was detected in one ewe sample (Day 146) and in one fetal sample (Day 120). Both results were at the lower limit of sensitivity of the assay i.e. 5 pg ml⁻¹. Melatonin was detectable (5-25 pg ml⁻¹) in 5 of the 9 samples of ewe blood taken at 01.00 hr, but was only found in 1 of the 8 fetal samples tested.

The levels of the hormone detected in sheep blood are such that they lie on the lower limits of the bioassay employed and consequently a confident recognition of a pattern was not possible, but the results did suggest that at least in ewes the hormone is more readily found during the hours of darkness. Preliminary data on melatonin in pineal tissues also indicates a day-night variation.

Early histological (Jordan, 1911) and more recent enzyme studies (Kennaway et al., 1974) indicate that the pineal is functioning in fetal life and has the potential to synthesize methoxyindoles especially towards the end of gestation.

The failure to obtain convincing evidence of melatonin in fetal blood may indicate that other alkylamines are important in mediating pineal endocrine functions prior to birth.

REFERENCES


The migration of stored human spermatozoa through cervical mucus in relation to the preovulatory LH surge

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The ability of cervical mucus to allow the penetration of preserved spermatozoa has been examined by an in vitro test. The probable timing of ovulation has been inferred from gonadotrophin values, basal body temperature and the clinical assessment of changes in cervical function. The relationship between these parameters and sperm migration has been assessed.

Peripheral venous blood was obtained daily between 0800 and 0900 hours from patients about the periovular period attending for artificial insemination. All patients kept a basal body temperature chart.

Luteinizing hormone was estimated by radioimmunoassay using anti hLH and LER 960 as tracer and standard. The day of the highest LH value was designated Day 0.

Cervical function was assessed clinically by the degree of cervical dilatation, the amount of mucus, the presence of ferning, and the length of Spinnbarkeit, each parameter contributing equally to a cervical score (0 - 12).

Cervical mucus was aspirated daily from the endocervical canal, and aspirated into a graduated 50 μl pipette. One end of the pipette was placed within a small reservoir of 1 - 2 drops of thawed semen and incubated for 60 mins at 37°C with humidification. The column of cervical mucus was then examined at centimetre intervals for the numbers of spermatozoa per high power field using light microscopy.

One hundred and twenty one in vitro tests were performed on the mucus of 23 women over 47 menstrual cycles.

Maximum cervical scores appeared on Day -1 and Day 0 with a considerable diminution in score at Day +1. The day of the thermal shift (greater than 0.2°C) occurred in 66% of cases at Day +1 and the remainder at Day +2. Sperm penetration was markedly increased during Days -1 and 0 compared with Days -2, +1 and +2. It is concluded that cervical mucus is most favourable to the penetration of spermatozoa on the day preceding, and the day of the LH surge and thereafter decreases rapidly. The value of such knowledge to the practice of artificial insemination is discussed.
LUTEOLYTIC PROPERTIES OF STALLION SEMEN

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Bielanski et al. (1974) demonstrated that oestrus and ovulation could be produced in anoestrous and dioestrous mares by the introduction of stallion semen into the uterus. The aim of this study was to compare the effects of prostaglandin $\text{F}_2\alpha$ and stallion semen on the CL of rabbits.

Thirty-three pseudopregnant rabbits were assigned to groups A, B and C. Two ml of physiological saline, 200 $\mu$g PGF$_2\alpha$ (Prostin F2 alpha: Upjohn Company), or stallion semen respectively were introduced into the lumen of one uterine horn during laparotomy carried out on two consecutive days after ovulation. The rabbits in the control group (A) were treated with 2 ml physiological saline per day on days 9 and 10 after ovulation. Group B was divided into two sub-groups. The rabbits in B-1, received 200 $\mu$g PGF$_2\alpha$ on days 5 and 6, while those in B-2 received PGF$_2\alpha$ on days 9 and 10. Group C was also sub-divided and the rabbits in C-1 and C-2 received 2 ml stallion semen on the same time schedule as those in the B sub-groups.

The ovaries were removed 48 hr after the second laparotomy. Macroscopically, there were no differences between the ovaries of rabbits in the different experimental groups. The mean luteal weight was 23 ± 16 mg.

Distinct differences were found, however, in the histological appearance of the luteal cells between treatments, irrespective of whether the CL were present in the ovary, ipsilateral or contralateral to the treated uterine horn.

In group A, the luteal cells had normal vesicular nuclei with distinct chromatin structure, cytoplasm with fine granules and fine secretory vacuoles. On an average there were twenty-five blood vessels in a cross section through the centre of the CL. Luteolysis was evident in the groups injected with PGF$_2\alpha$ and stallion semen. In the luteal cells pyknosis, plasmolysis and 'empty' cells with clumps of compact cytoplasm were visible. There was considerable proliferation of interstitial tissue. The average number of visible blood vessels was reduced to 15 in sub-groups B-1 and C-1 and 3 and 8 in B-2 and C-2 respectively.

Further investigation is in progress to determine the nature of the factor in stallion semen which is capable of inducing luteolysis in mares as well as rabbits. It also remains to be shown whether this factor was producing luteolysis by a direct action on the CL, or indirectly by releasing a luteolytic substance from the uterus.

REFERENCES

THE ROLE OF THE MORPHOGENETIC HORMONES ECDYSONE AND JUVENILE HORMONE IN INSECT SPERMATOGENESIS

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During larval life the insect testis develops slowly, never attaining a stage containing mature germ cells. Similarly, development ceases entirely during the long and often variable quiescent period termed diapause. In the final larval or pupal stage leading to the reproductive adult spermatocyte accumulation, meiosis, and spermiation occur. Early experimental work indicated that the testis was responding to the classic morphogenetic hormones of insects, ecdysone and juvenile hormone (Wigglesworth, 1936; Fukada, 1944; Williams, 1951). These findings have led to the hypothesis that juvenile hormone suppresses the meiotic division, resulting in the accumulation of meiocytes during larval instars. The absence of juvenile hormone in the pre-adult instar, together with the secretion of ecdysone results in a high level of meiosis and spermiation immediately prior to production of the adult (Takeuchi, 1969). Paradoxically however, all elements of spermatogenesis occur both in vitro in the absence of insect hormones, (Marks, 1970), and in the adults of many long-lived species (Phillips, 1970) which contain a theoretically unfavourable hormonal balance.

Quantitative histological analyses have recently demonstrated an absence of specific effects on meiosis by juvenile hormone or ecdysone in the bug Rhodnius prolixus. An alternative hypothesis has been put forward which states that the germ cell system is auto-differentiating and tied to a species specific sequence of divisions. Ecdysone and juvenile hormone exert an influence on the rate of passage through the sequence but can neither halt the process nor alter its direction (Dumser and Davey, 1974). A series of experiments investigating the two main aspects of this hypothesis are reported here:

1. Removal of the corpus allatum, source of the juvenile hormone in various larval stages of the blood feeding bug, Rhodnius results in the production of precocious adults. The level of differentiation of the germ cells is always consistent with the number of cells in the cyst, hence to the history of division of the germ cells and not to external morphologic development of the insect. Precocious adults with mature or immature germ cell complements can thus be produced by selection of appropriately aged larvae, arguing against a role of juvenile hormone in direct suppression of meiocyte or spermatid differentiation, and in support of the autodifferential capacity of the testis.

2. Surgical manipulation of endocrine structures and resupply of synthetic hormone analogues permits the demonstration of firstly an endogenous division rate in the absence of morphogenetic hormones, secondly an acceleration of this rate by ecdysone, the moulting hormone and thirdly the suppression of the ecdysone stimulated division activity by juvenile hormone, without effect on the basal activity level in accordance with the aforementioned kinetic hypothesis of germ cell development.
REFERENCES


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***Department of Histology and Embryology, University of Sydney, N.S.W., 2006.

This study reports an unusual structure in the middle piece of epididymal spermatozoa of the brush-tailed possum, Trichosurus vulpecula.

Spermatozoa were collected separately from the caput and cauda epididymis of the possum, and prepared for electron microscopy following the method of Jones (1973).

Cross and longitudinal sections of the middle piece reveal a single layer of evenly spaced, rounded aggregates of granular material around 60 nm in diameter, lying immediately beneath the plasma membrane. Tangential longitudinal sections show that these form a regular helix around the middle piece, wound in the opposite direction to the underlying mitochondrial helix. Beneath this accessory helix, and separating it from the mitochondria, is a layer of unevenly condensed granular material. Interspersed among the strands of the helix are variable numbers of vesicles. These vesicles are similar to those observed in cast off cytoplasmic droplets. Vesicles of endoplasmic reticulum form a characteristic array in the middle piece bead of mature spermatids of the bandicoot, Perameles nasuta (Sapsford, Rae, and Cleland, 1969). The similarity of the structures observed in the possum, to these vesicles in the bandicoot, suggests that they may be a remnant of spermatid endoplasmic reticulum.

To our knowledge, no structure resembling the accessory middle piece helix of the possum has been reported for other mammals. However, freeze-fractures through the plasma membrane of the midpiece of guinea-pig sperm have revealed circumferentially oriented strands of 50-60 A (5-6 nm) particles (Friend and Fawcett, 1973). In spermatozoa of the spiny dogfish, Squalus acanthias, a helical sheath of filaments, the fibrous midpiece sheath, closely surrounds the mitochondrial sheath (Stanley, 1971).

Whilst the function of this accessory middle piece helix in the possum spermatozoon is not clear, its helical nature and potential to counteract possible structural effects of the mitochondrial helix, are suggestive of some role in the dynamics of spermatozoon flagellar movement in this species.

REFERENCES

METABOLIC AND ULTRASTRUCTURAL CHANGES IN EJACULATED SPERMATOZOA INDUCED BY HEATING THE TESTES OF RAMS

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The harmful effects of a rise in temperature of the scrotal testis on spermatozoa are well known and the pachytene primary spermatocytes have been implicated as the main site of damage in the ram. Voglmayr, Setchell and White (1971), however, found that the metabolic activity of testicular spermatozoa is depressed one to five days after heating the testes indicating damage also during the latter stages of spermatogenesis. This paper extends these observations to ejaculated spermatozoa using glycerol as substrate since Mohri, Hasegawa and Masaki (1970) have found a decrease in the glycerol kinase activity of goat spermatozoa during summer.

Local heat (40-40.5°C) was applied to the scrotum of each of 5 rams for 3 hours. Spermatozoa were collected before, one day after and then at weekly intervals for ten weeks. Metabolic activity, motility and morphology of the spermatozoa were examined on each collection; in addition, the total phospholipid-phosphorus per ejaculate and the phospholipid composition of the spermatozoa were determined. For metabolic studies, washed spermatozoa were incubated for three hours in Warburg flasks with 5 mM glycerol.

There was an immediate reduction in oxygen uptake, with no effect on glycerol utilization or glycerol kinase activity, apparently indicating inhibition of endogenous respiration. One to two weeks later, glycerol breakdown and lactic acid production were severely inhibited. Each metabolic parameter returned to normal by nine or ten weeks after heating the testes. Glycerol kinase activity was slightly inhibited after one week; the most marked effect, however, was after three weeks when glycerol utilization was also severely inhibited. The motility of the spermatozoa was also reduced two weeks after heating, and recovery followed a similar pattern to glycerol breakdown.

Striking changes in the morphology of spermatozoa were also evident. From two weeks after heating the number of separate heads and tails increased, and there was also an increase in the number of spermatozoa with abnormal tails. Electron microscope studies have shown that one week after heating, the plasma membrane of the spermatozoa was rolled up; over the next three weeks it broke down, and by five weeks, the membrane was absent from the nucleus, tail and mitochondrial region. Mitochondrial changes, particularly coiling of the cristae, were also evident at this time and considerable cell debris was apparent. The morphology of the spermatozoa was normal again ten weeks after heating.

Local heating of the testis decreased the production of spermatozoa and, therefore, the total amount of phospholipid-phosphorus extracted per ejaculate. However, the phospholipid composition of the ejaculated spermatozoa was unaffected and the amount of phospholipid per cell showed only a slight decrease. This may reflect the loss of plasma membrane, but would not indicate more extensive breakdown of spermatozoal structure.

These experiments show that local heat disrupts metabolic activity of the ejaculated spermatozoa, and the speed of onset suggests that the site of action is in the epididymis.

REFERENCES
A COMPARISON OF SPERMATOZOA NUMBERS RELEASED IN THE URINE AND IN EJACULATES OF A MARSUPIAL, THE BRUSH-TAILED POSSUM

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Seminal plasma can be readily collected from a number of marsupial species by electroejaculation but only in the brush-tailed possum, does the technique offer promise as a means of collecting spermatozoa (Rodger and White, 1975). In view of the continuous release of spermatozoa in the urine of marsupials (10⁶ - 10⁷ spermatozoa/day) and the long copulation time of some marsupials Bolliger (1946) suggested that true ejaculation, as seen in eutherians and lower vertebrates may not occur. Rodger and White's (1975) data were consistent with Bolliger's hypothesis which suggested that spermatozoa present in ejaculates may have been flushed from the urethra by electrically stimulated prostatic secretion, in a manner analogous to the spermatozoa found in urine.

In the present study not all possums produced ejaculates containing spermatozoa, but responsive animals gave ejaculates of consistent volume and total spermatozoal numbers. Total spermatozoa per ejaculate had a mean of 4.6 ± 1.07 x 10⁷ for nineteen ejaculates from the two responsive animals. The mean volume of these ejaculates was 3.3 ± 0.44 ml. Loss of spermatozoa in the urine was of the order of approximately one tenth of that observed in semen, i.e. 4 x 10⁶ spermatozoa per day per possum. However, towards summer following the second breeding period, the number of spermatozoa found in urine fell to around 10⁴ spermatozoa per day. The number of spermatozoa obtained in both ejaculates and in urine were much lower than found in eutherian mammals, but despite suggestions to the contrary in the literature, ejaculation in marsupials appears to be essentially similar to that observed in eutherians.

REFERENCES


THE SUMMER SOLSTICE AND THE ONSET
OF BREEDING IN THE TAMMAR WALLABY

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On Kangaroo Island most tammar wallabies give birth
between mid-January and mid-February (Andrewartha and Barker,
1969) but the offspring produced at this time have been
conceived a year before and have remained in diapause for 11
months (Berger, 1966). Reactivation of the blastocyst and
associated corpus luteum has been calculated to occur between
December 23 and January 1 (Renfree and Tyndale-Biscoe, 1973).
From other studies reactivation involves the loss of a pituitary
inhibitor of the corpus luteum, CL stimulation of the
endometrium and endometrial stimulation of the blastocyst
(Tyndale-Biscoe, Hearn and Renfree, 1974).

In order to test these conclusions in the natural
environment of Kangaroo Island and to determine how closely
the events are related to the summer solstice, samples of
females were shot each night from December 20-29, 1974.
Pineal and pituitary glands were removed to dry ice within 5
min of shooting and dissection of the reproductive tract
during the next half hour. Dr Seamark will report on the
results from the pineals and Dr Moore on the blastocysts.

In all 54 females were shot and processed between
December 20 and 29, 1974. The animals were shot each night
from 2200 hrs until 0400 during the period when they are feed­
ing out from the scrub on the paddocks. Full moon occurred
on December 29, so that much of each night was moonlit, par­
ticularly during the second half of the trip.

Of the total 9 females were less than one year old, 16
were two years old and 29 three or more years old. All 9
young females had nulliparous pouches but 7 had ovulated prev­
ioulsy and had quiescent corpora lutea and 4 of them had a
blastocyst. This confirms the observation that juvenile
females enter their first oestrus late in the year and then
remain quiescent until December.

All the other females had parous pouches and most were
post lactational, only one still carried a pouch young. Of
these 45 females only one did not have a quiescent corpus
luteum and was in true anoestrus. However, five lacked a
blastocyst so that the overall frequency was 87%. This agrees
with previous indirect observations based on the production of
young from isolated females (Berger, 1966).

The fresh weight of the corpora lutea ranged from 6-19
mg, which does not differ from the quiescent size and showed
no trend with time. Microscopically, the corpora lutea of
some animals were hypertrophied, which is one indication of reactivation but this did not correlate with time either. Morphological parameters of change seem to be too variable or crude to be detected at this initial stage of reactivation.

REFERENCES


PIEAL GLAND CHANGES DURING THE PERIOD OF BLASTOCYST ACTIVATION IN THE TAMMAR WALLABY, MACROPS EUGENII.

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The influence of the pineal gland on the seasonal breeding of rodents has been well established (see Reiter, 1973). Decreasing light photoperiod stimulates the pineal gland to produce higher levels of methoxyindoles, resulting in a decrease in sexual activity. The Tammar Wallaby is a precise seasonal breeder. After mating the blastocyst develops to about 80 cells and then begins embryonic diapause. In this report we document changes in the pineal gland of the female Tammar Wallaby during the period in which the quiescent corpus luteum and blastocyst resume development.

Pineal glands from pregnant female Tammar Wallabies were obtained between 23.00 h and 01.00 h over 8 days during late December 1974 by courtesy of Dr. C.H. Tyndale-Biscoe. The pineal glands were kept frozen on solid CO₂ until assayed. Pineal glands were randomised, weighed and homogenised in 0.1 M KCl. Hydroxyindole O-methyltransferase (HIOMT, EC 2.1.1.4) activity was assayed by the method of Axelrod et al. (1965). Monoamine oxidase (MAO, EC 1.4.3.4) was assayed by the method of Wurtman & Axelrod (1963). Soluble protein was measured after centrifugation of the homogenate by the method of Lowry et al. (1951). The coefficient of variation between assays measured on a single sheep pineal gland for HIOMT and MAO were 6.4% and 14.2% respectively. Enzyme results are expressed as units/gland per 60 min. where 1 unit is equivalent to 1 pmol of ¹⁴C labelled product.

TABLE 1

PIEAL GLAND SOLUBLE PROTEIN, HIOMT ACTIVITY AND MAO ACTIVITY IN THE FEMALE TAMMAR WALLABY.

<table>
<thead>
<tr>
<th>Date</th>
<th>Total soluble protein (µg)</th>
<th>HIOMT (units/gland per)</th>
<th>MAO (units/gland per)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5'</td>
<td>60'</td>
</tr>
<tr>
<td>21/22.12.74</td>
<td>275 ± 38 (5)</td>
<td>627 ± 166</td>
<td>2311 ± 164</td>
</tr>
<tr>
<td>22/12.12.74</td>
<td>296 ± 41 (6)</td>
<td>566 ± 98</td>
<td>2515 ± 248</td>
</tr>
<tr>
<td>23/24.12.74</td>
<td>377 ± 105 (5)</td>
<td>799 ± 164</td>
<td>2684 ± 413</td>
</tr>
<tr>
<td>24/25.12.74</td>
<td>315 ± 32 (5)</td>
<td>275 ± 42 *</td>
<td>2839 ± 358</td>
</tr>
<tr>
<td>25/26.12.74</td>
<td>338 ± 35 (3)</td>
<td>603 ± 50</td>
<td>2750 ± 239</td>
</tr>
<tr>
<td>26/27.12.74</td>
<td>310 ± 21 (4)</td>
<td>810 ± 112</td>
<td>3194 ± 334</td>
</tr>
<tr>
<td>27/28.12.74</td>
<td>366 ± 70 (4)</td>
<td>426 ± 117</td>
<td>3057 ± 717</td>
</tr>
<tr>
<td>28/29.12.74</td>
<td>292 ± 16 (4)</td>
<td>673 ± 155</td>
<td>3312 ± 375</td>
</tr>
</tbody>
</table>

* Indicates significant difference (P < 0.05) from preceding group.
The number of animals in each group is shown in parentheses.

Results from other studies on the animals used here have shown that blastocyst activation occurred in the majority of animals around the time when pineal HIOMT activity was at its lowest. Berger (1970) has implicated photoperiod as a factor in the precise resumption of embryonic development in the Tammar Wallaby. These results further implicate the pineal gland and its methoxyindoles in the timing of blastocyst activation.
REFERENCES


REACTIVATION OF RNA SYNTHESIS IN MACROPOD EMBRYOS AFTER DIAPAUSE,

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The activation of the genome of dormant macropod blastocysts has been studied during growth stimulation. Changes in the transcriptional activity of the embryo cells were measured by assay for endogenous, DNA-bound RNA polymerases. During assay, the polymerases catalyze the synthesis of RNA from the ribonucleoside triphosphates: ATP, GTP, CTP and tritiated UTP. The distribution and amount of polymerase activity in the nucleus may be correlated with the activity of different groups of genes. Nucleolar polymerases are involved in the synthesis of ribosomal RNA and nucleoplasmic polymerases are predominantly concerned with the production of messenger RNA.

Embryos of the Tammar Wallaby Macropus eugenii usually enter dormancy as a normal part of the intrauterine phase. Females which are suckling a young animal in the pouch may carry a dormant embryo (lactational quiescence). Removal of the pouch young during lactation in the first half of the year will cause the embryo to resume development. However, the embryo will not reactivate if the pouch young is removed in the latter half of the year (seasonal quiescence). In this situation, reactivation occurs spontaneously at or near the summer solstice.

Resumption of development at the end of seasonal quiescence was investigated. Embryos were collected between the 19th-29th December, 1974, from a wild population of wallabies on Kangaroo Island. Between 19th-23rd all embryo cells showed relatively low levels of nuclear RNA polymerase activity. However, on the night of 23rd-24th December, a marked change occurred. The nucleolus-associated RNA polymerase activity of embryos was greatly enhanced relative to that in the nucleoplasm. The increased nucleolar activity was maintained throughout the remainder of the study period. By 28th December, 4 days after the activation of the nucleolus, the activity of the nucleoplasmic polymerases had also increased in a small number of embryos. The selective stimulation of ribosomal RNA synthesis in the quiescent embryo suggests that specific gene regulatory factors may be involved in the initiation of growth reactivation.
PRELIMINARY OBSERVATIONS ON REPRODUCTION AND EMBRYONIC DEVELOPMENT IN THE MARSUPIAL DEVIL SARCOPHILUS HARRISII.

by R.L. HUGHES

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The Tasmanian devils examined consisted of 15 females and six males. The animals were captured in the wild during either May 1974 or April 1975.

The breeding season is restricted with the majority of pregnancies occurring between March and May. The pre-ovulatory period is characterised by a marked proliferation of the tissues of the Fallopian tube and the uterus.

The ovaries are approximately equally functional with up to 23 eggs being ovulated in one set. As the pouch contains only four teats considerable reproductive wastage is inevitable.

The gametes are large. Sperm have an overall length of about 213μm and the flagellum diameter in the region of the middle piece is about 2.6μm. Ovulated eggs have an overall diameter of about 300μm (this includes the mucoid coat and shell membrane). The diameter of the vitellus of the egg is approximately 210μm.

Cleavage occurs asynchronously with the result that two-celled eggs and well formed unilaminar blastocysts are present in the same uterus.
The unilaminar blastocysts consist of a single-layered, hollow ball of cells without an inner cell mass and these blastocysts are formed directly from cleaving eggs without an intervening morula stage. Both a mucoid coat and an unruptured shell membrane are present at the unilaminar blastocyst stage.

...
DECREASE IN THE GLYCERYLPHOSPHORYLCHOLINE CONTENT OF THE EPIDIDYMIS AFTER INJECTING α-CHLOROHYDRIN INTO RATS

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Alpha-chlorohydrin, which is a simple mono-chlor derivative of glycerol, has been shown to produce temporary sterility in some species including the rat and ram. The metabolism and motility of spermatozoa are inhibited strongly after injecting α-chlorohydrin into the rat and ram (Brown and White, 1973; Brown-Woodman and White, 1975; Mohri et al. 1975). However, as α-chlorohydrin and glycerol are structurally similar, it seemed possible that α-chlorohydrin may interfere also with the synthesis of glyceryl-phosphorylcholine (GPC) in the epididymis.

In initial studies rats were injected with α-chlorohydrin at a dose of 90 mg/kg, causing a lesion in the caput epididymis. GPC was isolated from the four segments of the epididymis (initial segment, caput, corpus and cauda) by paper chromatography and estimated by phosphorus analysis. There was a reduction in total GPC concentration of the epididymis two days after treatment with α-chlorohydrin and a further decrease until 20 days. However, similar results were obtained on ligating the vasa efferentia, suggesting that the reduction in GPC may be due to the lesion in the epididymis blocking the duct rather than to a direct action of α-chlorohydrin on the epididymis.

Rats were then given 6 daily injections of α-chlorohydrin at a non-lesion forming dose of 15 mg/kg. The GPC in the 4 segments of the epididymis was isolated by cellulose TLC up to 8 days after the final injection. The GPC content of all segments was reduced significantly at all times measured when compared with control animals. This finding indicates that α-chlorohydrin can act directly on the epididymis to reduce the GPC content.

To investigate further the mode of action of α-chlorohydrin, its effect on the uptake of $^{32}$P-orthophosphate into epididymal GPC, has been estimated. Rats were injected with $^{32}$P-orthophosphate and six hours later with α-chlorohydrin; control animals received saline instead of α-chlorohydrin. After 48 hours the animals were killed and the radioactivity in the GPC of the caput, corpus and cauda epididymis measured. Alpha-chlorohydrin appeared to have no effect on the uptake of $^{32}$P into the corpus and cauda, but inhibited uptake into the caput to about 50% of the control level. Control values showed that the caput is the major site of GPC synthesis in the epididymis.

The decrease in GPC synthesis with α-chlorohydrin could well reflect a general decrease in synthetic activity of the epididymis, which could result in a significant change in the environment within the duct through which the sperm pass over several days.

REFERENCES


THE GLYCERYLPHOSPHORYLCHOLINE AND PHOSPHOLIPID PATTERN OF THE GENITAL DUCT AND SPERMATOZOA OF THE AFRICAN ELEPHANT LOXODONTA AFRICANA

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The elephant has no anatomically distinct epididymis and the comparable structure has been referred to, throughout its length, as the Wolffian duct (Short, Mann and Hay, 1967). Previous studies by Jones, Rowlands and Skinner (1974) suggest that this duct performs the main functions of the epididymis in scrotal mammals. It was, therefore, of interest to investigate if the duct produces glycerylphosphorylcholine (GPC), a characteristic secretion of the epididymis (White, 1973), and to see if GPC levels through the duct follow a similar pattern to that of other species. The opportunity was also taken to examine the phospholipid pattern of the spermatozoa from the storage end of the duct.

Material was collected in Kruger National Park, South Africa, as described by Jones et al. (1974). Genital ducts for analysis of GPC were frozen in the field within 30 minutes of collection and subsequently thawed, minced and deproteinized. Samples for phospholipid analysis were stored at 30°C and extracted with chloroform:methanol (1:2, v/v) within 80 minutes of collection. Phospholipid and GPC were estimated by thin-layer chromatography, followed by phosphorus analysis.

The GPC content, estimated from the vasa efferentia and along the length of the Wolffian duct, increased from the testis to the terminal segment. The largest increase was between the segments referred to by Glover and Nicander (1971) as the "pre-initial" and initial segments. This seems to indicate increased synthesis of GPC with maturation of the spermatozoa, and further points to the similarity of this duct in the elephant to the epididymis of other species.

The major phospholipids of the spermatozoa were, in order of quantitative importance, phosphatidyl choline, phosphatidyl ethanolamine, ethanolamine plasmalogen, choline plasmalogen, sphingomyelin, cardiolipin and phosphatidyl inositol. Sphingomyelin was quantitatively the most important phospholipid in the plasma, with appreciable levels of ethanolamine plasmalogen, phosphatidyl serine, phosphatidyl choline and phosphatidyl inositol, as well as a small amount of choline plasmalogen.

The phospholipid pattern of elephant spermatozoa obtained from the terminal segment, which is involved primarily in storage of spermatozoa, would seem to resemble most closely that found previously in the ejaculated spermatozoa of the boar (Darin-Bennett, Poulos and White, 1973). This is in contrast to the spermatozoa of the ruminants, ram and bull, on one hand, and rabbit, dog and human on the other.

REFERENCES

POSSIBLE MECHANISMS INVOLVED IN THE REDUCTION IN MOTILITY OF HUMAN SPERMATOZOA BY COPPER, ZINC AND SILVER

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The rise in popularity of the intrauterine device (I.U.D.) as an alternative to oral contraceptives has led to the study of substances which can enhance I.U.D. efficacy and so many heavy metals, particularly copper (Kesserü, Hurtado and Mühe, 1974), have been investigated in this regard.

In the present study human spermatozoa were incubated with copper, silver and zinc wires (surface area 960 mm²) in 1 ml of calcium-free Krebs Ringer Phosphate buffer pH 7.4, containing [U-C14] glucose (3 mM). Oxygen consumption was measured in a micro Warburg apparatus especially suited for the low oxygen consumption of human sperm. Motility was scored by microscope using a points system ranging from 0 to 10.

Copper decreased sperm motility significantly within one hour and totally within three hours. However, oxygen consumption was stimulated in the presence of copper even after the sperm had been rendered immotile. Glucose utilization, oxidation and lactate production were unaffected. It is suggested that the effect on motility is due to formation of cuprous ions which may readily cleave disulphide bonds in spermatozoal proteins (Oster and Oster 1974) and thus severely disrupt the protein's tertiary structure.

Incubation of sperm with zinc significantly reduced motility over three hours, but not to the extent obtained with copper. Oxygen consumption was also stimulated but only to 50% of the level achieved with copper. However, glucose utilization, oxidation and lactate accumulation were unaffected. Thus it is suggested that zinc exerts its effect on spermatozoal motility in a fashion similar to copper.

After 90 minutes of incubation silver significantly reduced sperm motility. However, in contrast to copper and zinc, silver reduced oxygen consumption, glucose utilization (by 50%) and oxidation (by 38%). Lactate accumulation was unaffected. This suggests that silver, unlike copper and zinc, interferes with enzymes of either the T.C.A. cycle or electron transport chain.

All three metals exhibit significant effects on sperm motility after three hours, with copper the most effective. It is suggested that copper and zinc may seriously affect the structural integrity of the cell membrane while silver inhibits normal spermatozoan energy metabolism.

REFERENCES

PRODUCTION OF PROSTAGLANDIN-LIKE SUBSTANCES BY MOUSE SPERMATOZOA IN VITRO

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When mice mate the males ejaculate semen containing substantial quantities of a prostaglandin-like substance (PG-LS) which appears to be synthesized in the vas deferens, epididymis or testis (Marley and Smith, 1974; 1975). The *in vitro* experiments described here test the hypothesis that this synthesis occurs in the vas deferens, due to the presence of spermatozoa within the lumen, and attempt to assess the factors which might control this synthesis *in vivo*.

Sodium arachidonate was used as substrate throughout. Pairs of vasa deferentia were placed in flasks containing 3.5 ml ice-cold oxygenated Tyrode. The various additions were then made and the tissues chopped *in situ*. The flasks were incubated at 37°C for 30 min in a shaking water bath and then the reaction was stopped by acidification to pH 3. Incubates serving as blanks were acidified immediately before the incubation. PG-LS was extracted into ethyl acetate and the residue from the latter was dissolved in water and bioassayed by a bridging assay against authentic PG. In some experiments a further extraction and chromatography procedure was carried out. All values are corrected for recovery.

There was comparatively little PG-LS synthesized in the absence of arachidonate whereas the production increased almost linearly with an increase in the amount of arachidonate added over a range of 2.5 to 50 μg/flask. Conversion rates ranged from 37% to 5% respectively and the ratio of the amount of PGE-like to PGF-like activity synthesized was approximately 4:1. A substrate concentration of 10 μg was chosen for all subsequent experiments. The addition of 100 or 500 μU phospholipase A to the incubates (probably supramaximal amounts) caused a 3 to 5 fold increase in the amount of PG-LS synthesized and the addition of 50 μg noradrenaline bitartrate to the incubate nearly doubled the yield.

The final experiment attempted to determine whether or not the spermatozoa within the lumen of the vas deferens were responsible for the synthesis of the PG-LS. In some cases therefore the contents of the vas deferens was expelled and the lumen was washed with Tyrode prior to transfer to the flask. This caused a 78% loss of synthesizing ability, compared with unwashed tissue. By contrast, the washings contained 93% of the synthesizing ability.

Thus a possible sequence of events leading to the production of PG-LS at the time of mating might be that under the influence of a phospholipase, perhaps activated by increased sympathetic nervous activity, tissue bound arachidonate is made available to prostaglandin synthetase located within the spermatozoa for conversion into PGE₂ and PGF₂α. The significance of this has yet to be established however.

REFERENCES


INHIBITION OF THE OXIDATIVE METABOLISM OF RAM SPERMATOZOA BY α-CHLOROHYDRIN

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Alpha-chlorohydrin is an effective, reversible antifertility agent in the ram (Brown-Woodman, Salamon and White, 1974) and in vivo and in vitro studies in our laboratory have shown it to be a potent inhibitor of glycolysis (Brown and white, 1973; Mohri, et al. 1975). Three enzymes are inhibited by α-chlorohydrin, viz. glyceraldehyde-3-phosphate dehydrogenase, aldolase and triose phosphate isomerase and the following experiments were designed to determine the extent to which the metabolism of various sperm substrates is affected by α-chlorohydrin.

Washed ram spermatozoa were incubated for 3 hours in Warburg flasks in the presence of α-chlorohydrin (0.01, 1.0 and 100 mM) with [U-14C] fructose (5 mM), [U-14C]glycerol (5 mM), [U-14C]lactate (5 mM), [U-14C]pyruvate (10 mM), [U-14C]acetate (10 mM) and [U-14C]palmitate (2 mM) (attached to 3% bovine serum albumin). In addition, endogenous respiration was measured in the presence of α-chlorohydrin.

With fructose as substrate, even at 0.1 mM α-chlorohydrin, oxygen uptake of the spermatozoa was reduced by 40%, while at higher concentrations the inhibition was even more severe (85% at 100 mM); no lactate accumulated in the presence of α-chlorohydrin, even at 0.1 mM. When the spermatozoa metabolised glycerol, 10-100 mM α-chlorohydrin was required to reduce (60-80%) oxygen uptake; lactate production was reduced at 1 mM α-chlorohydrin but 100 mM was required to produce complete inhibition. With lactate as substrate, oxygen uptake was only reduced by 10% at 10 mM α-chlorohydrin and 65% at 100 mM, while the oxygen uptake was slightly more affected using pyruvate (30% at 10 mM and 40% at 100 mM). There was greater inhibition of oxygen uptake when the spermatozoa metabolised acetate (20% inhibition at 1 mM α-chlorohydrin, 50% at 10 and 100 mM), while with palmitate there was less effect (40% at 10 and 100 mM α-chlorohydrin). The endogenous oxygen uptake of spermatozoa was inhibited by 30% at 10 mM and 40% at 100 mM α-chlorohydrin.

The experiments indicate that the α-chlorohydrin-induced block in metabolism mainly occurs high up in the glycolytic pathway since the metabolism of fructose was inhibited to the greatest extent. This view is supported by the fact that lactate and pyruvate oxidation were only inhibited at high concentrations of α-chlorohydrin when it is possibly acting as a less specific alkylating agent. However the considerable inhibition of oxygen uptake with acetate as substrate suggests an additional site of action of α-chlorohydrin in the Krebs tricarboxylic acid cycle, although palmitate, which is normally oxidized via acetate was not affected to such an extent.

Comparatively high oxygen uptake in the presence of α-chlorohydrin with glycerol as substrate may be due to oxidation of α-glycerophosphate to dihydroxyacetone phosphate in the mitochondria, a step which is presumably insensitive to α-chlorohydrin.

REFERENCES

ENDOCYTOSIS IN THE UTERINE EPITHELIUM OF THE MOUSE

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Histological examination of the rat (Warren and Enders, 1964; Nilsson, 1966; Jöf et al., 1968; Psychoyos et Mandon, 1971; Nilsson, 1972) and mouse (Nilsson, 1962; Bergstrom, 1972; Bergstrom and Nilsson, 1972; 1973) uterus during preimplantation stages or delayed implantation has shown that portions of the luminal epithelial cell surface often bulge or project into the uterine lumen. The projections, which have been described as sea-anemone-like formations or fungus-like protrusions, have recently been named pinopods by Enders and Nelson, (1973). In the rat, the pinopods contain vacuoles measuring 0.5 to 3 µm in diameter. Most investigators suggested that the pinopods pinch off from the cells and release cellular materials into the lumen which nourish preimplantation embryos. However, in the rat, recent investigations showed that the pinopods are involved in endocytosis, not apocrine secretion (Enders and Nelson, 1973; Parr and Parr, 1974). Exogenous tracers could be detected in the pinopod vacuoles shortly after their instillation into the uterine lumen and later in multivesicular bodies and dense bodies in the cells.

In this report, we present evidence that the pinopods of the mouse uterine luminal epithelial cells, during luteal stages, rapidly incorporate exogenous tracer from the uterine lumen into pinopod vacuoles and similar vacuoles in the apical portion of the cells.

The apical surface of the uterine luminal epithelium of 15 mice during preimplantation and delayed implantation stages was studied with light and electron microscopy. Some cells had irregular protrusions or pinopods which frequently contained vacuoles up to 3 µm in diameter. Similar vacuoles were also present in the apical cytoplasm of some cells lacking pinopods. The pinopods were found on day 4 of pregnancy and day 10 of delayed implantation, but were absent on days 2, 3, and 5 of pregnancy.

A study of the absorption of ferritin from the uterine lumen in four mice during delayed implantation showed that the pinopods were involved in the formation of endocytic vacuoles. At fifteen minutes after injection of ferritin into the uterine lumen, all of the pinopod vacuoles that were examined and many vacuoles in the apical cytoplasm contained the tracer. In addition to the pinopod vacuoles, endocytosis was infrequently carried out by coated vesicles.

There was no evidence that pinopods of mouse luminal epithelial cells constitute an apocrine secretion which might be involved in the maintenance of preimplantation blastocysts.

The demonstration of ferritin particles in the pinopod vacuoles in both the mouse and the rat uterine epithelial cells clearly shows that they carry on endocytosis at a time when unimplanted embryos are present in the lumen. The process seems to be terminated as implantation begins. Although several suggestions have been put forward for the possible role of endocytosis during this period (Enders and Nelson, 1973; Parr and Parr, 1974), its function remains unknown.

NILSSON, O. (1962). Z. Zellforsch. 56, 803 - 808
WARREN, R.H., and ENDERS, A.C. Anat. Rec. 148, 177 - 195
PRELIMINARY INVESTIGATION INTO UTERINE EPITHELIAL CELL TYPES USING CLUSTER ANALYSIS.

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Differences appear in staining intensity and ultrastructural appearance of the normal cycling diestrous mouse uterine epithelial cells. Furthermore, differences in the method of ferritin uptake from the uterine lumen by these cells has been demonstrated (Stanton & Smith, 1975). Because no distinguishable feature between the cell types was immediately evident, the cell characteristics had to be more objectively analysed. The statistical analysis used on the cells and their characteristics was a Cluster Analysis.

Classification of the different epithelial cells was done using low-power electron micrographs, noting the position of a cell within the epithelium, the relative size and shape and its nuclear and cytoplasmic constituents.

A total of 125 cell morphological properties were used and degrees of similarity or dissimilarity were calculated by comparing two cells at a time. The similarity coefficients obtained were then used in a Cluster Analysis (CLUSTAN package programme on a CYBER 76 computer). A combination of 4 similarity and 5 hierarchical clustering methods was used.

In all of these methods, two cell types could be distinguished. There remained however, cells which did not enter into these clusters and remained relatively distinct from these two groups.

The uniqueness of the groups was cross-checked by clustering the cell characters. Those cell characters which showed a high Binary Frequency Ratio (% occurrence in cluster/% occurrence overall) for each of the cell clusters appeared independent of each other, confirming the presence of at least two distinct groups of cells, each with its own characters.

The appearance of more than one cell type within this epithelium suggested in preliminary morphological studies has been confirmed using a statistical analysis new to qualitative cell morphology.

REFERENCES

THE UPTAKE AND METABOLISM OF \(^{3}H\)PROGESTERONE BY THE OVARIECTOMISED MOUSE UTERUS

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It is well established that the expression of the physiological effect of progesterone in the uterus depends upon its initial exposure to an oestrogenic stimulation (Courrier, 1950). However the reasons for this dependency and the mechanism of action of progesterone in the uterus are not clearly understood.

The experiments to be described have examined the effect of prior oestrogenic stimulation on the uptake of \(^{3}H\)progesterone following local application to the uterine lumen, the subcellular distribution of radioactivity, and the nature of radio metabolites in the uterus.

Subcutaneous pretreatment with oestradiol-17\(\beta\) (0.1 \(\mu \)g/day for 2 days) did not change the initial uptake of radioactivity at 1 minute after injection but slightly decreased the retention of radioactivity at 60 minutes. This decrease was significant \((P < 0.05)\) following the injection of 10\(^{-4}\)\(\mu \)g but not 10\(^{-2}\)\(\mu \)g \(^{3}H\)progesterone. A rapid uptake of radioactivity was indicated by the low levels of radioactivity remaining in saline washings after one minute.

There was a pretreatment effect in the relative levels of radioactivity in the soluble and "nuclear/myofibrillar" fractions of uterine homogenates prepared from tissue 1 and 60 minutes after \(^{3}H\)progesterone administration. At the later time a greater proportion of the tissue radioactivity was found in the "nuclear/myofibrillar" fraction. No such change was found with animals which had received no pretreatment.

The nature of the radioactive compounds in the tissue fractions also changed with pretreatment and with time. In control animals at 1 minute, greater than 80% and at 60 minutes, greater than 40% of the radioactivity in all fractions was associated with \(^{3}H\)progesterone. In treated animals at 1 minute, less than 25% of the radioactivity in all fractions was associated with \(^{3}H\)progesterone while at 60 minutes the percentage of progesterone in all fractions increased, due in part to a declining level of tissue radioactivity. Irrespective of pretreatment or time of killing, little or no radioactivity was associated with 5a-pregnan-3,20-dione.

In controls radioactivity not associated with progesterone was mainly associated with 5a-pregnan-diols and more polar metabolites. In the treated animals at 1 minute in each fraction greater than 50% of the radioactivity was associated with 20a or 20b dihydroprogesterone, with minor levels of the 5a-reduced products. At 60 minutes the proportion of the former was reduced.

REFERENCES

INTERACTIONS BETWEEN STEROIDS AND SPASMGENS ON THE MYOMETRIUM OF THE EWE

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Although the hormonal changes occurring shortly before and during natural and induced parturition in the ewe have been measured extensively, theories concerning the regulation of parturition in the ewe are lacking in detail because there is little information on the direct effects of these hormones on the myometrium. To study these effects in greater detail estradiol benzoate, progesterone, dexamethazone, oxytocin and PGF, were administered to ewes which had been prepared surgically to provide information on the following points:

1) Effects of the steroids and combinations of the steroids on spontaneous activity of the myometrium.
2) Variations in the effects of the steroids with duration of treatment.
3) Modifications of the spontaneous activity of the myometrium by the spasmogens within each of the steroid treatments.
4) Interactions between the above factors on myometrial activity.

Six ewes, each of which were equipped with an intra-uterine pressure balloon, six extracellular electrodes placed in the myometrium, and an intra-uterine infusion catheter were used. The variables measured were mean pressure, maximum pressure, minimum pressure (tonus), rate of contraction, rate of electrical spike production and spike potential.

A preliminary experiment compared the myometrial activity in the castrate with the activity during estrogen treatment, progesterone treatment or dexamethazone treatment. Oestrogen treatment increased spontaneous myometrial activity by 2 to 4-fold, as compared with the castrate condition or following other steroid treatments. Responsiveness to oxytocin paralleled the level of spontaneous activity. The response to PGF, was highest following oestrogen treatment and declined during the other steroid treatments.

The main experiment was of factorial design with factors arranged as a split-split-plot. Factors were sheep, steroids (estradiol benzoate (E), E plus progesterone (E+P), E plus dexamethazone (E+D) or E+P+D), duration of steroid treatment (days 1, 2, 3, 4) and spasmogen treatment (oxytocin or PGF,).

Progesterone (E+P) modified the effects of oestrogen by increasing tonus (18%) and reducing rate of contraction (30%), rate of spike production (29%) and spike potential (47%). Mean pressure was not modified significantly by progesterone. Changes produced by progesterone suggest desynchronization of cellular activity in the myometrium. Sensitivity to oxytocin was decreased by E+P treatment and there was little effect on the response to PGF, . The mean pressure during oestrogen treatment alone (E) rose with increasing duration of treatment whereas during E+P treatment, mean pressure declined slightly (P < 0.05).

Dexamethazone (E+D) inhibited the spontaneous activity (49%) and oxytocin responsiveness (46%) compared to that during treatment with oestrogen only. During the E+D treatment the decline in the response to PGF, was relatively small (25%). The change in spontaneous myometrial activity and
response to spasmogens during E+D treatment occurred mainly on the first day of treatment and there was little change thereafter. The combination of all these steroids (E+P+D) produced results that were similar to those found during E+D treatment.

These experiments revealed that oxytocin and PGF₂α interact with the steroids in producing their effects on the myometrium. Oxytocin sensitivity is highest during treatment with oestrogen alone and declines markedly under the influence of any combination of the steroids studied. PGF₂α sensitivity was highest during E and E+P treatment and declined slightly during treatment with E+D or E+P+D. It appears that the action of glucocorticoids in initiating parturition may occur in spite of an inhibitory effect on myometrial activity.

PROGESTERONE AND INDUCTION OF LACTATION IN EWES

W.J. Fulkerson, R. Hooley, G.H. McDowell and L.R. Fell
University Farms, Camden, 2570; S.S. Cameron Laboratories, Werribee, 3030; Dairy Research Centre, Richmond, 2753.

The non-pregnant ewe induced to lactate artificially appears to be a useful model for defining the mechanisms of initiation of lactation (Fulkerson & McDowell, 1974, 1975; Fulkerson, McDowell & Fell, 1975). The present studies were conducted, using this model, to evaluate the involvement of progesterone in the lactogenic response in the ewe.

Thirty-six nulliparous ewes, ovariectomized 3 weeks previously, received 10 s.c. injections of 240 µg oestradiol benzoate (OB) plus 60 mg progesterone (P) at intervals of 3 days. Commencing 3 days after the last injection, groups of 4 ewes then were treated for 5 days (trigger phase) as follows. Groups 1 and 2 - 40 µg OB s.c. twice daily; groups 3 and 4 - 2.5 mg OB s.c. twice daily; groups 5 and 6 - 10 mg dexamethasone trimethylacetate (DEX) s.c. once daily; groups 7 and 8 - 1 i.u. syntocinon (SYN) i.v. 4 times daily; group 9 - no hormone. Ewes in groups 2, 4, 6 and 8 also received 20 mg P/day. All ewes were milked by hand from the first day of the trigger phase.

Milk yields were recorded and lactose contents determined. Blood samples, collected at frequent intervals before, during and after the trigger phase, were analysed for prolactin (Fell et al., 1972).

After 21 days of milking, milk yields from ewes in groups 3-6 (c 200 ml/day) were significantly higher (P < 0.05) than those from ewes in groups 1, 2 and 7 (c 75 ml/day) which in turn were significantly greater (P < 0.05) than the trivial yields from ewes in groups 8 and 9. Lactose concentrations in milk from ewes in groups 1 and 3-7 (c 5.5%) were substantially higher than those for ewes in groups 2 and 8.

Prior to the trigger phase, levels of prolactin for all ewes were low (c 20 ng/ml). Within 4 hours of injecting OB (groups 1-4) or SYN without P (group 7), levels of prolactin increased to 350-400 ng/ml and 200 ng/ml respectively then fell to low levels (c 40 ng/ml) over the next 24 hours despite further injections. In contrast, levels of prolactin in serum of all other ewes remained low throughout the experiment.

The results show that the restraining influence of P on lactogenesis in the ewe can be overcome. Injections of P inhibited the lactogenic response to SYN, suppressed the response to low doses of OB, but failed to influence the response to DEX or high doses of OB. Moreover, these results are consistent with the earlier conclusions (Fulkerson et al., 1975) that OB and possibly SYN appear to be lactogenic by virtue of their effect on prolactin secretion whereas DEX appears to act independently of prolactin.

REFERENCES

THE EFFECT OF DIFFERING ENVIRONMENTS ON THE OESTROUS CYCLE AND
OVULATION RATE IN SEVERAL SPECIES OF NATIVE AUSTRALIAN RATS.

W.G. Breed
Department of Anatomy, University of Adelaide, S.A., 5001.

Species of rodents living in different environments show
different population dynamics and in Australia there are a number of
species of Rattus occurring in very diverse ecological niches. The aim
of the present study is to determine whether these species have diverged
in their reproductive potential, and especially in age of puberty,
oestrous cycle length, and ovulation rate. Rattus leucopus leucopus,
R. le cooktownensis, and R. fuscipes coracius occur in rain forests,
Rattus villosissimus occurs in central Australia where plague numbers
occur spasmodically, and other species live in swamplands - R. lutreolus;
southern woodlands - R. fuscipes; and cane fields - R. sordidus. To date,
only from incidental observations of Taylor and Horner (1973) only the
reproductive biology of the last two species has been investigated in
any detail (McDougall, 1946; Taylor & Horner, 1972; Warneke, 1971).

In the present study the following parameters have been
investigated (1) onset of oestrus as indicated by first occurrence of
a cornified vaginal smear, (2) length of oestrous cycle as shown by
vaginal smears, and (3) number of healthy corpora lutea determined by
scanning serial sections of ovaries which usually includes two sets
thus representing two successive ovulations.

Data is presented of animals caught in the wild and caged
singly or from progeny of pregnant females caught in the wild and borne
in captivity.

Of the rain forest species, first oestrus occurred at
94 ± 0 (mean ± SEM) days in R. le cooktownensis, 100 ± 20 days in
R.f. coracius whereas that of R. villosissimus was 70 ± 5 days.

All 9 oestrous cycles of R. le cooktownensis were of
either 4 or 5 days, and 13 out of 16 cycles of R. le leucopus were
less than 10 days and had a mean of 4.0 ± 0.5 days (number of animals,
n = 2), whereas 34 out of 37 oestrous cycles recorded from R.f. coracius
were less than 10 days and had a mean of 5.3 ± 0.2 days (n = 5).
Similarly 44 out of 46 cycles of R. villosissimus were less than 10
days and had a mean of 5.2 ± 0.2 days (n = 6). Identical results were
also obtained for the swamp rat (n = 4).

By contrast, the following numbers of corpora lutea were
observed; in rain forest species R. le cooktownensis had 10.0 ± 0,
and R.f. coracius had 9.0 ± 2.4. However, R. villosissimus had
significantly more corpora lutea with a mean of 41.2 ± 2.4 (p < 0.001),
whereas R. lutreolus had 18.5 ± 0.5. These differences no doubt
reflect larger numbers of oocytes being released at each ovulation in
R. villosissimus compared to other species, and thus indicate considerable
variation in ovulation rate between closely related species of rats
living in different habitats even though oestrous cycle length, and age
of puberty remain unchanged.
PRIMORDIAL FOLLICLES IN THE OVARY OF THE NEONATAL EWE LAMB

Carol Worthington & J.P. Kennedy
School of Wool & Pastoral Sciences, University of New South Wales, Kensington, N.S.W. 2033.

Mauleon (1969) stated that oogonial mitoses in the sheep ceased well before birth but that a decrease in numbers of primordial follicles was difficult to demonstrate in the few months after birth. However, Trounson et al. (1974) found that there was an increase in numbers of primordial follicles between 7 days and 5 months of age when paired ovaries of hemispayed lambs were compared. Additional data on changes in numbers of primordial follicles have been obtained from the lambs described by Kennedy, Worthington and Cole (1974).

Merino lambs were slaughtered at 0, 4, 8, 12, 16.5, 20, 24 and 33 weeks after birth. Ovaries were serially sectioned at 8μm. Primordial follicles were counted in every fortieth section and the total count per ovary was estimated by multiplying the count per section by 40. Results are given in Table 1.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16.5</th>
<th>20</th>
<th>24</th>
<th>33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicles (X10^-3)</td>
<td>47.8</td>
<td>65.6</td>
<td>78.3</td>
<td>96.5</td>
<td>42.5</td>
<td>92.6</td>
<td>84.0</td>
<td>39.7</td>
</tr>
<tr>
<td>± SE</td>
<td>9.2</td>
<td>8.2</td>
<td>18.2</td>
<td>6.0</td>
<td>5.3</td>
<td>7.9</td>
<td>34.4</td>
<td>9.7</td>
</tr>
</tbody>
</table>

Because variances were heterogeneous (Bartlett's test), the data were analysed by the method of weighted mean squares (Snedecor, 1956). Age was found to be a significant main effect (P<0.05). The mean of the means of follicle numbers between 4 and 20 weeks was significantly greater than the mean at birth (P<0.05) using the t-test with weighted variances.

These data provide further evidence that primordial follicles increase after birth in lambs. However, the evidence for post-natal oogenesis is inconclusive because this was a cross-sectional study and mitosis was not observed in primordial oocytes.

Follicle counts were made independently in both ovaries of each lamb and the intraclass correlation of follicle numbers was calculated to be 0.92. The small variation between each pair of ovaries gives confidence in the method of estimating follicle numbers and also indicates that in further work it is not necessary to examine both ovaries.

REFERENCES


GONADOTROPHINS IN THE NEONATAL FEMALE LAMB

Robin Tassell*, W.A. Chamley+, J.P. Kennedy*
*School of Wool & Pastoral Sciences, University of New South Wales, Kensington, N.S.W., 2033 and +Department of Physiology, University of Melbourne, S.S. Cameron Laboratory, Werribee, Victoria. 3030.

Kennedy, Worthington & Cole (1974) showed that ovarian weight in lambs increased significantly between birth and 8 weeks of age, due to the growth and proliferation of small growing and vesicular follicles. The endocrine control of these events was investigated in this study.

Merino lambs were randomly allocated to 6 groups, of 4-6 animals, to be slaughtered at birth or at 2, 4, 6, 8 and 10 weeks of age. Blood samples were taken at hourly intervals for 6 hours prior to slaughter. One ovary from each lamb was homogenised and assayed for progesterone and oestradiol-17β by competitive protein binding. The other ovary was serially sectioned and examined microscopically. Peripheral plasma levels and pituitary content of LH and FSH were determined by radio-immunoassay.

The mean ovarian weights at 4 weeks and 8 weeks were approximately 5- and 10- times greater than at birth (121 ± 4 mg). From 4 weeks onwards the ovaries contained numerous follicles of 1-2mm diameter and from 6 weeks each ovary contained at least one follicle > 2mm in diameter.

Plasma FSH levels ranged between 20 and 51 ng/ml at birth and did not change with increasing age. However, pituitary concentration of FSH rose from 161 ± 26 μg/g at birth to 284 ± 70 μg/g at 10 weeks. Mean levels of LH in plasma did not change significantly between birth (1.2 ± 0.4 ng/ml) and 10 weeks (1.4 ± 0.1 ng/ml). Small peaks of LH (>4.5 ng/ml) were observed in 2/6, 1/5, 2/4 and 2/4 lambs at 4, 6, 8 and 10 weeks, respectively. The pituitary concentration of LH at 6 weeks (305 ± 86 μg/g) was greater than at younger ages.

Ovarian concentration of oestradiol-17β fluctuated widely between lambs of the same group and bore no obvious relationship to age, to follicular development or to plasma levels of gonadotrophins. Ovarian concentration of progesterone fell sharply between 2 and 4 weeks of age.

These data do not provide an obvious explanation for the ovarian development which occurs shortly after birth in the young lamb.

REFERENCES

PLASMA FSH IN MERINO LAMBS SELECTED FOR FECUNDITY

J.K. Findlay and B.M. Bindon
Reproduction Research Section, University of Melbourne, Werribee, 3030; C.S.I.R.O. Animal Genetics, P.O. Box 90, Epping, 2121.

Plasma FSH was measured by radioimmunoassay (Salamonsen et al. 1973) in samples collected at 09.00, 12.00, 15.00 and 18.00 hr on the thirtieth day of life from male and female lambs of the C, O, T and B flocks of the CSIRO fecundity selection experiment. These flocks are respectively the control, and flocks selected for low, medium and high litter size (Bindon and Turner, 1974).

**TABLE 1**

<table>
<thead>
<tr>
<th>Flock</th>
<th>Incidence of Multiple Births (%)</th>
<th>Mean Plasma FSH (ng/ml)</th>
<th>Male lambs</th>
<th>Female lambs</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0 - 10</td>
<td>17.8 ± 2.8 (6)*</td>
<td>42.0 ± 16.6 (4)</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>0 - 10</td>
<td>17.0 ± 1.3 (5)</td>
<td>26.8 ± 2.2 (8)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>50 - 60</td>
<td>17.7 ± 1.4 (5)</td>
<td>111.0 ± 73.5 (7)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>70 - 80</td>
<td>22.2 ± 4.8 (4)</td>
<td>159.1 ± 77.6 (7)</td>
<td></td>
</tr>
</tbody>
</table>

*Bracketed values are the number of lambs contributing to each mean

In male lambs FSH values rarely exceeded the lower limit of sensitivity of the assay (15 ng/ml) and there was little variability in sequential samples from the same animal. Between flock differences were not significant. There were substantial differences in the mean FSH values for female lambs although the SE terms indicate large differences between lambs in the same flock, especially in T and B. However, when the results are compared on the basis of the number of lambs with mean FSH greater than 40 ng/ml, the 14 T + B lambs had significantly higher FSH than the 12 C + 0 lambs ($X^2 (1) = 6.68; P <0.01$).

The LH levels in these samples have been described previously (Bindon and Turner, 1974). It is of interest to note that there is a small but significant correlation ($r =+0.62, P <0.05$) between the mean LH and FSH values from the T and B female lambs. Where elevated values of FSH and LH occurred these tended to be in the same animal. More importantly, sequential FSH values from the same lamb were less variable than the corresponding LH levels. For example, in female lambs the mean coefficient of variation for 4 sequential samples was 0.25 for FSH measurements but 0.46 for LH measurements. This means that the interpretation of FSH levels may be less complicated by the episodic release patterns characteristics of LH in such animals.

These preliminary data suggest that FSH, like LH is higher in young female lambs from flocks that have been selected for high litter size. It is not yet known if FSH is a useful early indicator of fecundity.

**REFERENCES**


FSH AND UNILATERAL OVARIECTOMY IN THE EWE

I.A. Cumming and J.K. Findlay
Department of Agriculture, Victoria and Reproduction Research Section, University of Melbourne, S.S. Cameron Laboratory, Werribee, 3030.

We have used the phenomenon of ovarian compensatory hypertrophy (Mallampati and Casida, 1970; Land, 1973) to examine a possible role of FSH in determining ovulation rate in the ewe. Forty-two ewes were allocated at random within live-weight ranges to 4 groups. FSH concentrations were measured in peripheral plasma by radioimmunoassay (Salamonsen et al. 1973) in half the ewes of each group for up to 36 hours after unilateral ovariectomy (leaving the ovary with a corpus luteum) on either Days 14 or 16 (Groups U14 and U16). Control animals for both Day 14 and Day 16 (Groups C14 and C16) underwent a sham operation and were otherwise treated similarly. Blood samples were taken at 30 min intervals for 3 - 5 hr before operation (Period 1) and then after operation every 15 min for 2 hr (Period 2), 30 min from 2-5 hr (Period 3), hourly from 5-12 hr (Period 4) and continued 2 hourly from 12-36 hr (Period 5). Data on FSH was subjected to analysis of variance. All ewes were slaughtered 12-14 days after the post-operative oestrus to determine ovulation rate and ovarian and corpus luteum weights.

TABLE 1
SUMMARY OF RESULTS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C14</td>
</tr>
<tr>
<td>Total number ewes</td>
<td>10</td>
</tr>
<tr>
<td>Number in oestrus after operation</td>
<td>9</td>
</tr>
<tr>
<td>Ovulation rate per ewe</td>
<td>1.70</td>
</tr>
<tr>
<td>Ovarian weight (g)</td>
<td>1.53</td>
</tr>
<tr>
<td>Weight of CL (g)</td>
<td>0.53</td>
</tr>
<tr>
<td>FSH (% change relative to Period 1)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

*Period 1 vs Period 4, p<0.05 and +p<0.10 within treatment groups

Although ovarian weights had fully compensated by slaughter in both treatment groups, there was a suggestion that compensation of ovulation rate in Group U16 was incomplete (U14 vs U16, p<0.10). FSH values showed a significant increase in Group U14 at 5-12 hr (Period 4) after unilateral ovariectomy and then declined during the next 24 hr. A similar trend (p<0.10) occurred in Group U16, whereas no changes occurred up to Period 4 in either control groups. There was a decline in FSH during Period 5 in Group C16 which was probably associated with the fall in FSH prior to oestrus (Salamonsen et al. 1973). These results suggest that an increase in FSH could be involved in the increase in ovulation rate per ovary following unilateral ovariectomy.

REFERENCES

CHANGES IN TOTAL OVARIAN BLOOD FLOW DURING ANAESTHESIA AND CANNULATION OF UTERO-OVARIAN VEINS

P.E. Mattner, B.D. Stacy and B.W. Brown
C.S.I.R.O., Division of Animal Physiology, Prospect, N.S.W. 2149, Australia

In each of two ewes, Doppler ultrasonic flow probes (White et al., 1974) were implanted around both ovarian arteries. They were used to monitor changes in total blood flow to the ovaries during anaesthesia and during the collection of ovarian venous outflow via catheters (Mattner and Thorburn, 1969). During successive oestrous cycles, either sod. pentobarbitone, sod. thiopentone or halothane was administered on Day 1 and Day 9 of the cycle (oestrus = Day 0). Collection of ovarian venous outflow via catheters was performed with the ewes under sod. pentobarbitone anaesthesia on Day 14 or 15 of the cycle.

At Day 9, prolonged anaesthesia (1.5 to 2 hr) with sod. pentobarbitone or sod. thiopentone had no material effect on total ovarian blood flows. At this stage of the cycle, halothane given alone or after prolonged anaesthesia with the above anaesthetics had a marked effect within 15-20 min. The total blood flow rose by 46% (range 20-90%) for the ovary bearing a corpus luteum (C.L.) and by 128% (90-190%) in the contralateral ovary without a C.L., with the result that the initial, large disparity between the flows to the two ovaries was markedly reduced.

At Day 1 when total blood flow to each ovary was low, anaesthesia with halothane elevated the flow to a similar extent in each ovary (mean 60%, range 40-80%).

During collection of ovarian venous outflow, the total ovarian blood flow, as monitored with the ultrasonic probes, increased by 30% in ovaries bearing a C.L. and by 70% in the contralateral ovary without a C.L.

It appears that, in sheep, some anaesthetics (e.g. halothane) and drainage of ovarian venous blood through canulae may interfere with local control of the ovarian vasculature and cause an increase in the blood supply to the ovary. The effects which were most pronounced in ovaries not bearing a C.L. could markedly influence estimates of total ovarian blood flow based on timed collections of ovarian venous outflow.

REFERENCES

EFFECTS OF LUPIN GRAIN SUPPLEMENTATION ON OVULATION RATE AND FERTILITY OF MERINO EWES.

R.J. Lightfoot and T. Marshall,
Department of Agriculture, South Perth, Western Australia, 6151.

Two experiments were conducted to examine the effects of supplementary feeding with sweet lupin grain (L. angustifolius; 33% crude protein) on ovulation rate and fertility of Merino ewes grazing various pastures in South Western Australia.

Experiment 1 (Lightfoot and Marshall, 1974) was of factorial design (2 X 5; n = 50; N = 500) and examined effects of rate of lupin grain supplementation and base pasture type (dry subterranean clover based pasture v wheat stubble; 7.5 ewes/ha). Lupins were fed thrice weekly commencing 35 days before, and continuing for 18 days after, the start of joining. Ovulation rates were determined by laparotomy during the first 21 days of joining.

RESULTS - EXPERIMENT 1

<table>
<thead>
<tr>
<th>Main Effects</th>
<th>Ovulation rate</th>
<th>% ewes lambing</th>
<th>% ewes twinning</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lupin supplement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 g/hd/day</td>
<td>1.17</td>
<td>69</td>
<td>10</td>
</tr>
<tr>
<td>62.5 &quot;</td>
<td>1.09</td>
<td>71</td>
<td>7</td>
</tr>
<tr>
<td>125 &quot;</td>
<td>1.21</td>
<td>81</td>
<td>13</td>
</tr>
<tr>
<td>250 &quot;</td>
<td>1.37</td>
<td>81</td>
<td>21</td>
</tr>
<tr>
<td>500 &quot;</td>
<td>1.55</td>
<td>85</td>
<td>20</td>
</tr>
<tr>
<td>X^2 lin. lupins</td>
<td>&lt;.01</td>
<td>&lt;.05</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

| 2. Base pasture       |                |                |                 |
| Sub. clover           | 1.34           | 79             | 17              |
| Wheat stubble         | 1.22           | 76             | 12              |
| X^2                   | <.05           | ns             | .05 < p < .1    |

The results showed that lupin grain supplements significantly increased ovulation, lambing and twinning rates. Twinning rates in ewes conceiving after day 18 of joining (i.e. after lupin feeding ceased) fell to control levels.

In Experiment 2 (Marshall and Lightfoot, 1974) control ewes grazing dry subterranean clover based pasture were compared with ewes grazing the same pastures but supplemented with lupin grain at 500 g/hd/day for either 14 or 35 days prior to joining and for 18 days after joining started. Ovulation rates were 1.24, 1.48 and 1.48 and percentage ewes twinning 10, 21 and 23 respectively.

The results indicate that ovulation rate responds rapidly to lupin feeding and that the effect is not necessarily related to liveweight changes.

REFERENCES

THE EFFECT OF LUPIN GRAIN SUPPLEMENTATION ON OVULATION RATE IN BORDER LEICESTER X MERINO EWES

D.J. Rizzoli; R. Baxter; J. Reeve and I.A. Cumming
Department of Agriculture, Victoria, S.S. Cameron Laboratory, Werribee, 3030, and Rutherglen Research Station, Rutherglen, 3685.

Recent reports from Western Australia describe marked increases in the number of ovulations occurring in ewes fed a supplement of lupin grain at February matings (Lightfoot and Marshall, 1974; Marshall and Lightfoot, 1974). As most sheep in Victoria are joined from October to March it was decided to explore the value of lupin grain as a supplement over this period. In an attempt to understand more fully the reasons lupin grain in particular, was so effective as a supplement, two iso-energy supplements (0.5 kg/ewe/day) of either lupin grain or wheat (protein content, 32 and 11% of dry matter respectively) were fed for 14 days prior to joining and for the following 20 days of a mating period to 600 ewes aged 7½ years at the Rutherglen Research Station. Ewes were allotted at random to the lupin or wheat treatments and to six mating groups. At monthly intervals from October to March one group from both the lupin and wheat treatments were joined to fertile rams and following a 20 day mating period ovulation rates for all ewes were determined by counting corpora lutea, either at slaughter or by surgical examination. Mean liveweight of ewes did not differ significantly between wheat or lupin treatments. The mean liveweights of the groups mated from October to March were 65, 67, 64, 63, 61 and 58 kg. During all mating periods, ewes declined in liveweight (rate 0.3 - 2.5 kg) even though supplemented, except the ewes in lupin groups of October and March which gained 0.8 kg. There was no significant difference in ovulation rate between the lupin and wheat groups from October to January (Figure 1). However, ovulation rates increased in February and March (P <0.05) and in these months alone lupin grain supplementation resulted in an increase in ovulation rate above that of ewes given wheat supplementation (P <0.01).

This experiment demonstrates the need to recognize the seasonal factor which is so integrally involved in determining ovulation rate in ewes. Why lupin grain failed to stimulate ovulation rates from October to January is unknown but it is interesting to speculate that ewes during this period may already be expressing their maximum ovulation rates and only in February and March were the pastures deficient in some nutritional requirement for ovulation rate potential to be realised. In February-March lupin grain was more effective than the iso-energy supplementation of wheat in increasing ovulation rates.

REFERENCES
EFFECT OF LUPIN GRAIN, WHEAT AND LUCERNE SUPPLEMENTS ON OVULATION RATE IN MAIDEN BORDER LEICESTER X MERINO EWES

J.L. Reeve; P.A. Kenney; R. Baxter and I.A. Cumming
Department of Agriculture, Victoria, Rutherglen Research Station, Rutherglen, 3685, and 5.S. Cameron Laboratory, Werribee, 3030.

Low ovulation rate is a major factor limiting fecundity of young ewes in the commercial sheep flocks of Australia (Cahill and Blockey, 1974). Recent publications report considerable increases in ovulation rate in mature ewes mated in February and March fed lupin grain at 0.5 kg/ewe/day for 2 weeks prior to joining with continued feeding through the mating period (Rizzoli et al. 1975). No such dramatic increase in ovulation rate has been reported for ewes fed other supplements for such restricted periods. The present study was designed to define the effect of lupins and other supplements on ovulation rate in young ewes joined in February and March. Twelve hundred Border Leicester x Merino ewes, aged 18 months, were allotted at random within liveweight ranges to one of five groups and ewes of each group to three subgroups. The first group was not fed a supplement. The remaining four groups were fed one of the supplements listed. Supplements were fed for 14 days prior to joining and throughout the mating period. Supplements were fed to the three subgroups at 0.17, 0.34 or 0.51 kg/ewe/day. Ovulation rate was determined in all ewes by laparoscopy at which the ovaries were examined and the number of corpora lutea counted. Throughout the experiment ewes grazed pastures where available dry matter was greater than 6,000 kg/ha and crude protein ranged from 6 to 16 per cent of the dry matter. As feed quantity was not limiting, no assessment can be made of the effect of diet selection on crude protein intake from pasture.

TABLE 1 : MEAN OVULATION RATE OF EWES WITHIN SUBGROUPS

<table>
<thead>
<tr>
<th>Supplement Type</th>
<th>Crude Protein (Percent of Dry Matter)</th>
<th>Supplement feeding level* (kg/ewe/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>Green Lucerne</td>
<td>15</td>
<td>1.13 (41)*</td>
</tr>
<tr>
<td>Wheat</td>
<td>11</td>
<td>1.14 (43)</td>
</tr>
<tr>
<td>Wheat and 3% Urea</td>
<td>-</td>
<td>1.16 (43)</td>
</tr>
<tr>
<td>Lupin Grain</td>
<td>28</td>
<td>1.23 (43)</td>
</tr>
</tbody>
</table>

LSD  \( p = 0.05 \) 0.16 0.16 0.16

*Mean ovulation rate of 3 subgroups of unsupplement ewes was 1.13 (42)
+ Figures in parentheses indicate liveweight (kg) 13 days after introduction of rams

At the highest level of supplementation lupin grain resulted in a dramatic increase in ovulation rate (Table 1). The relatively low ovulation rate in the green lucerne subgroup supplemented at 0.51 kg/ewe/day suggests that the increase in protein intake supplied by the lucerne was not sufficient to increase ovulation rates markedly. The low mean ovulation rate of the wheat and wheat and urea subgroup fed 0.51 kg/ewe/day suggests that energy and energy and nitrogen respectively do not in themselves increase ovulation rate. The increase in ovulation rates in ewes fed the lupin grain above that of other diets suggests that either the lupins contain a compound/s of considerable physiological significance or the lupin grain supplies a highly digestible and balanced diet, this being reflected in liveweight and ovulation rate increases.

REFERENCES

CAMP FORMATION IN THE SHEEP OVARIAN FOLLICLE IN RESPONSE TO GONADOTROPHINS

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Adelaide, S.A. 5000, Australia, and ARC Unit of Reproductive Physiology
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Cyclic adenosine-3',5'-monophosphate (cAMP) has been shown to have a role in the initiation of steroidogenesis following the exposure of the ovarian follicle to luteinizing hormone (LH). In this study an examination has been made of the cAMP levels in the ovarian follicle in vitro, following stimulation by the gonadotrophins LH and follicle stimulating hormone (FSH). In addition, the level of cAMP released into the culture medium was determined.

Ovaries were removed from sheep of mixed breed within 40 min. of slaughter. Follicles between 4 and 6 mm in diameter obtained from sheep within Days 4 and 14 of the cycle were dissected from the ovaries and established individually in organ culture as described by Moor (1973). In the first experiment whole follicles were incubated for different times, (0-180 min.), in the presence of 50 mIU ml⁻¹ ovine LH (NIH-LH-S18) and 8 mM-theophylline, homogenized in 50 mM-Tris-HCl buffer, pH7.4, containing 8mM-theophylline and 0.1M-2-mercaptoethanol. cAMP in the tissue and medium was determined using a competitive protein binding assay modified from Brown et al. (1971). Under these conditions the tissue content (mean ± S.E.M.) of cAMP rose from 1.2 ± 0.2 pmol mg⁻¹ at 0 min. to 157 ± 18.8 pmol mg⁻¹ at 180 min. The cAMP released into the medium over the same period rose from 0.4 ± 0.05 pmol mg⁻¹ to 102.6 ± 6.5 pmol mg⁻¹. A similar pattern of cAMP formation in the tissue and release into the medium was observed following exposure to 50 mIU ml⁻¹ FSH (NIH-FSH-S10).

The second experiment was designed to identify the cellular component responsible for the production of cAMP in gonadotrophin-treated follicles. One group of follicles was incubated with LH, 50 mIU ml⁻¹ for 40 min., and then separated into their major cellular components, the theca and granulosa, and assayed separately for cAMP. The second group was separated into theca and granulosa prior to the 40 min. treatment with LH. The results revealed that 94% of the cAMP formed was associated with the thecal layer when the follicle was separated before treatment, compared with 25% associated with the theca when the follicle was separated after treatment.

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>sep. before treatment</th>
<th>sep. after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theca</td>
<td>5.4 ± 1.1 (5)</td>
<td>43.2 ± 2.3 (4)</td>
<td>13.9 ± 3.0 (4)</td>
</tr>
<tr>
<td>Granulosa</td>
<td>1.2 ± 0.2 (3)</td>
<td>3.0 ± 0.6 (4)</td>
<td>27.8 ± 4.5 (4)</td>
</tr>
<tr>
<td>Medium</td>
<td>1.1 ± 0.1 (4)</td>
<td>-</td>
<td>11.9 ± 1.1 (4)</td>
</tr>
</tbody>
</table>

The large release of cAMP into the medium and the localisation of cAMP production in the theca suggests a role for this cyclic nucleotide as an extracellular mediator of gonadotrophin action.
It has been postulated that ovarian cholesteryl esters act both as an important reserve of energy and of the hormone precursors required for steroidogenesis (Armstrong, 1968). The availability of this reserve is under the control of gonadotrophins which act by regulating the enzymes of cholesterol ester metabolism. This study concerns an investigation of sterol metabolism in intact sheep ovarian follicles in culture following exposure to human chorionic gonadotrophin (HCG). Intact ovarian follicles 4-6 mm in diameter, obtained from ewes on Days 4-14 of the oestrous cycle, were dissected free of stromal tissue and established in tissue culture as described by Moor (1973). Theca and granulosa tissues were separated for analysis by microdissection. Lipids in follicular tissue and culture medium were analysed by gas liquid chromatography (glc) as trimethyl silyl or methyl esters following initial isolation of the major lipid groups by thin layer chromatography. Identifications were validated by glc-mass spectrometric techniques. Steroid hormones were assayed by radio ligand binding procedures (Seamark, Moor and McIntosh, 1974).

Oestrogen secretion by untreated follicles remained at about 0.1 nmol mg⁻¹ per day throughout the period of culture: no progesterone was detected. Treatment with HCG (20 iu ml⁻¹) did not affect oestrogen secretion, but caused a dramatic rise in progesterone production to about 0.2 nmol mg⁻¹ per day by Day 3.

The sterol content of theca and granulosa cells separated from follicles cultured in the presence and absence of HCG is shown in Table 1. A progressive diminution of both the cholesterol and cholesterol ester pools occurred during the period of culture but no effects of HCG were evident.

<table>
<thead>
<tr>
<th>Days in culture</th>
<th>Cholesteryl nmoi mg⁻¹</th>
<th>Cholesterol Ester nmoi mg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>theca</td>
<td>granulosa</td>
</tr>
<tr>
<td>1</td>
<td>15.7 ± 1.0 (19)</td>
<td>3.0 ± 0.2 (16)</td>
</tr>
<tr>
<td>2</td>
<td>9.6 ± 2.0 (4)</td>
<td>4.6 ± 0.5 (4)</td>
</tr>
<tr>
<td>+HCG (24hr)</td>
<td>7.2 ± 0.5 (4)</td>
<td>4.1 ± 0.5 (4)</td>
</tr>
<tr>
<td>3</td>
<td>5.4 ± 0.5 (5)</td>
<td>6.4 ± 0.5 (5)</td>
</tr>
<tr>
<td>+HCG (48hr)</td>
<td>5.4 ± 1.0 (5)</td>
<td>4.9 ± 0.5 (5)</td>
</tr>
</tbody>
</table>

The fatty acid composition of the cholesteryl ester fraction (relative molar ratios, C₁₆=1) was C₁₄, 0.42; C₁₆, 1; C₁₆:1, 0.6; C₁₈:1, 1.89; C₁₈:2, 0.14; C₁₈:3, 0.77. No evidence of selective de-esterification with HCG treatment was found. Neither was there evidence of cholesteryl ester synthesis or exchange found in experiments in which follicles were exposed to isotopically labelled cholesterol, arachidonic acid or cholesteryl olate for prolonged periods, with or without HCG. In contrast, a portion of the non-esterified cholesterol pool in the theca and, to a lesser extent in the granulosa,
was shown to exchange with cholesterol in the medium. However, this exchange was not facilitated by the addition of HCG. It was concluded that HCG has little demonstrable effect on cholesterol and cholesteryl ester metabolism in isolated ovarian follicles in culture.

REFERENCES


FSH AND LH ACTIVITIES OF PMSG FRACTIONS ISOLATED FROM SERUM AND FROM FETAL TROPHOBLAST CELLS MAINTAINED IN CULTURE

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Equine gonadotrophin (PMSG) is a sialic acid-containing glycoprotein exhibiting variable proportions of FSH and LH activities. It is widely used in animal production and veterinary medicine for increasing ovulation rate and for the treatment of infertility in domestic animals. Unfortunately, the wide variation in response to injections of PMSG limits its usefulness.

As a first step in clarifying the relationship between the composition of PMSG and its biological activity, with the aim of attempting to standardize and improve its gonadotrophic quality, we have purified it from the sera of individual pregnant mares by an improved method. This product was compared with PMSG which had not been released into the maternal circulation but was isolated from medium in which had been cultured specialized fetal trophoblast cells recovered from pregnant mares (Hamilton, Allen and Moor, 1973). PMSG was determined using the haemagglutination inhibition assay (Allen, 1969).

Isolation from serum was achieved either by the method of Gospodarowicz and Papkoff (1967) or, under milder conditions taking advantage of the low isoelectric point of PMSG, by dialysis against 50 mM-ammonium acetate, pH 4.5, followed by centrifugation. The supernatant or dialyzed foetal culture medium was adsorbed onto a column of Sephadex A-25 (50 mM-ammonium acetate, pH 4.5) and PMSG was eluted with a gradient of NaCl (0-200 mM). After gel filtration (Sephadex G-100; 50 mM-ammonium acetate, pH 4.5) tubes containing activity were loaded onto a column of Sephadex SP-25 equilibrated with the same buffer. The pH was increased by gradient to 8.5 and then the ionic strength was varied to 300 mM in a gradient of ammonium acetate, pH 8.5.

Between one and four PMSG fractions were revealed by the two gradients, or by isoelectric focusing of material from the A-25 step. The isoelectric points of the major serum fractions were 2.8 and 3.2. The chief component produced in culture had an isoelectric point of 3.4. Multiple forms of PMSG have not been reported before and would not have been detected by the isolation method used prior to this.

Rat testis receptor preparations were used to determine the ratio of FSH-like to LH-like activity in each fraction in terms of 125I-labelled FSH and LH (Leidenberger and Reichert, 1972; Reichert and Bhalla, 1974). The FSH/LH ratios of different fractions varied between 0.3 and 2.1, those from culture being low. Furthermore, in whole serum from individual mares, this ratio varied with gestational stage.

The origin and biological significance of the different forms have yet to be determined. In structural terms, they may reflect variations in sialic acid content or perhaps different combinations of the subunits reported by Gospodarowicz (1972) and Papkoff (1974).
The Role of Progesterone in Regulating the Length of Oestrous Cycles in Sheep

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Ginther (1968) and Thwaites (1971) have shown that exogenous progesterone injected into ewes may effect the length of the oestrous cycle. We investigated this finding in more detail. In these experiments the progesterone, dissolved in peanut oil, was injected intra-muscularly into ewes at the dosages and times shown. All treatment groups contained six ewes. Oestrus was detected using vasectomised or entire rams carrying marking harnesses.

In the first experiment the effect of time of progesterone treatment was studied. Progesterone was administered at 40 mg per day for a period of three days and the following results were obtained: Treatments started on day 0 produced a mean cycle length of 12.9 days, day 1 14.0, day 2 15.2, day 3 16.2, day 4 17.3, day 5 18.0, day 8 17.3 and day 11 18.3. The mean cycle length of a control group of ewes treated with peanut oil only was 17.4 days. Shortening of cycle length occurred in all groups treated before day 4 with the mean interval from first injection to oestrus of 13 days.

The minimum dosage of progesterone required to cause shortening of the cycle was investigated in a second experiment. In this experiment ewes were treated with varying progesterone dosages from day 0 for a period of 4 days with the following results:

<table>
<thead>
<tr>
<th>Dosage (mg)</th>
<th>Mean inter-oestrus interval (days)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>17.7</td>
</tr>
<tr>
<td>2.5</td>
<td>17.2</td>
</tr>
<tr>
<td>5</td>
<td>16.5</td>
</tr>
<tr>
<td>10</td>
<td>15.0</td>
</tr>
<tr>
<td>20</td>
<td>12.0</td>
</tr>
<tr>
<td>40</td>
<td>11.7</td>
</tr>
</tbody>
</table>

Luteolysis has been shown to start four days before oestrus (Deane, Hay, Moor, Rowson and Short, 1966). On the basis of this finding and the data reported we postulate that the ability of the uterus to effect luteolysis is dependent on prior exposure to a finite amount of progesterone. In the untreated nonpregnant ewe this priming period extends from day 4 to about day 13.

References


THE EFFECT OF OESTRADIOL-17B, PROGESTERONE AND PROSTAGLANDIN F.2.X ANTIPLASMA ON LUTEAL FUNCTION IN THE EWE

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In an attempt to elucidate the role of progesterone (PGST) oestradiol-17B (E2) and prostaglandin F.2.X (PGF2.X) in the regulation of the oestrous cycle we passively immunized ewes with E2, PGST and PGF2.X antiplasma during the late luteal phase of the cycle. Twenty Romney ewes were treated as follows.

Group 1. E2 antiplasma given on days (D) 9 to 17 of the cycle (5 ewes)
Group 2. PGST antiplasma given on D 9 to 12 of the cycle (2 ewes)
Group 3. PGST and E2 antiplasma given on D 9 to 17 of the cycle (2 ewes)
Group 4. PGST and PGF2.X antiplasma given on D 9 to 12 of the cycle (1 ewe)
Group 5. PGF2.X antiplasma given on D 12 to 14 of the cycle (1 ewe)
Group 6. Ovarioctomised ewe plasma given on D 9 to 17 of the cycle (6 ewes)
Group 7. Three untreated control ewes

The ewes were run with a harnessed vasectomised ram to detect behavioural oestrus. Blood was collected from the ewes throughout the trial and the total levels of E2, PGST and PGF2.X were determined by radioimmunoassay. The free and bound fractions were estimated by equilibrium dialysis at 37°C. Twelve days after the start of treatment the ewes were laparotomised and the ovaries examined. Results of the hormone levels during and following treatment and luteal function at laparotomy are as follows.

Group 1. (i) Free E2 levels were reduced by around 95%.
   (ii) The ewes failed to exhibit oestrus and did not ovulate.
   (iii) Changes in PGST concentrations indicated that luteolysis occurred at the expected time.
   (iv) Increased follicular activity was noted at laparotomy.

Group 2. (i) Total PGST concentrations rose for 4 days, then decreased sharply.
   (ii) Free PGST concentrations were reduced by 50-75%.
   (iii) The ewes ovulated, exhibited oestrus and the corpus luteum (CL) regressed at the normal time.

Group 3. (i) Results were similar to those found for Group 1. However one ewe exhibited oestrus on D 16 of cycle but failed to ovulate.

Group 4. (i) Plasma PGST levels did not fall at the time of expected luteolysis, indicating that the life of the corpus luteum was prolonged. However the ewe receiving anti PGF2.X and anti PGST showed a rise in total PGST levels as found for the Group 2 ewes.

Group 6. (i) These ewes exhibited oestrus, ovulated and the CL regressed at the normal time.
   (ii) Hormone levels were similar to those previously reported (Thorburn et al., 1969; Obst et al., 1971).
We conclude that
1. Substantial reductions in free E₂ concentrations from Days 9-17 of the cycle block ovulation and behavioural oestrus but do not appear to prevent luteolysis.
2. Reduced levels of free plasma PGST concentrations over the latter half of the oestrous cycle do not appear to affect luteolysis or the length of the cycle.
3. Passive immunization against PGE₂ blocked corpus luteum regression, thus providing direct evidence that PGE₂ is the ovine luteolytic factor.

REFERENCES
PREDICTING THE NUMBER OF CORPORA LUTEA IN MERINO EWES

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An attempt has been made to predict the number of corpora lutea from the mating activity of two different groups of Merino ewes. One group (high) consisted of the heavier, 47.7 ± 0.35 kg, and genetically more fertile and fecund ewes and the other (low) consisted of the lighter, 38.9 ± 0.23 kg, and genetically less fertile and fecund ewes. The prediction equation used was:

\[ \text{number of corpora lutea} = 19.64 \left( \frac{T}{N} \right) - 243.9; \quad r = 0.81 \]

The theoretical basis leading to the derivation of this equation has been described (Fowler, 1975).

Each group of 153 ewes was joined with 3 rams for 34 days in autumn 1975. All sheep were individually identified and rams were interchanged between groups regularly. During the first 17 days of joining, each group was observed on six separate occasions for 9 hours each time. The ram and ewe involved in each mount and service was identified and the time was recorded. The ewes were always in view of at least two observers who were located in a mobile observation tower from which a clear and uninterrupted view of the flock could be maintained.

The following measurements were obtained:

1. \( T; \) the sum of times from first to last observed mount for each ewe.
2. \( N; \) the total number of mounts observed during 9 hours.

The ewes were slaughtered at a local abattoir soon after rams were removed and the total number of corpora lutea were counted. For the high group, \( T/N, \) was 24.80 ± 2.09 and for the low group, 23.07 ± 2.11. In the high group, 145 ewes were slaughtered from which 230 corpora lutea were expected and 226 were found. In the low group, 149 ewes were slaughtered from which 211 corpora lutea were expected and 199 were found.

The ratio \( T/N \) has been suggested to be directly related to fertility and fecundity in Merinos (Fowler, 1975).

Ovulation rates in Merinos is influenced by genotype, age and live weight (Braden and Baker, 1973). The groups which provided the prediction equation and those used in the present study were consciously selected to differ in these aspects. Accurate prediction of the number of corpora lutea is possible only if the total effect of all interacting influences that can operate in any flock, can be described objectively and the ratio \( T/N \) offers this possibility. However, the work is at a very early stage and more successful predictions are necessary before acceptance could be considered.

The new prediction equation is:

\[ \text{number of corpora lutea} = 256.9 - 2.64 \left( \frac{T}{N} \right) \]

This equation describes the relationship as it has been found for 5 different flocks in which genotype, age, live weight and year of joining vary.
EFFECTS OF COLCHICINE ON THE STRUCTURE AND FUNCTION OF THE CORPUS LUTEUM IN THE SHEEP

R.T. Gemmell and B.D. Stacy
CSIRO Division of Animal Physiology, Prospect, N.S.W. 2149, Australia

A previous ultrastructural study has drawn attention to the presence of secretory granules in the corpus luteum (CL) (Gemmell, Stacy and Thorburn, 1974); the formation and secretion of granules was related to the known pattern of progesterone secretion in the cycling ewe. To gain further information on the role of the granules in the mechanism of progesterone secretion, the effects of colchicine on the CL have been investigated. Insulin is secreted in granule form and colchicine inhibits this process by disrupting the microtubules that transfer granules from the interior to the periphery of the pancreatic β cell (Malaisse, 1973). Microtubules are also present in the luteal cell and it was of interest to know whether secretory granules and hormone secretion in the two different types of endocrine cell would exhibit comparable responses to colchicine.

Ewes close to day 10 of the cycle were given an intravenous injection (1 mg/kg) of colchicine; at intervals up to 4 hr after treatment animals were anaesthetized and the ovaries fixed by vascular perfusion with glutaraldehyde. Samples of luteal tissue were prepared for examination in the electron microscope; blood levels of progesterone were taken from the jugular vein.

The morphology of the luteal cells was found to have changed 45 min. after the injection, the number of densely staining granules in the cytoplasm and in the intercellular space had decreased. By 2 hr there was no sign of the granules being secreted nor were they readily detectable within the cell. With the disappearance of the granules, densely staining vesicles began to appear near the Golgi complexes, and these abnormal organelles became more prominent 4 hr after injection of colchicine. Absence of microtubules and proliferation of agranular endoplasmic reticulum were also features of the response to the drug. The concentration of progesterone in peripheral plasma fell to 86% of control values at 1 hr, 62% at 2 hr, 51% at 3 hr and 48% at 4 hr after treatment.

These results indicate that colchicine disrupts the microtubular system in the luteal cell and thereby inhibits the intracellular transport of granules. The decline in progesterone values accompanying the interference to granule transfer and secretion provides further evidence that progesterone is secreted by the luteal cell in granule form (Gemmell, Stacy and Thorburn, 1974).

The increase in agranular endoplasmic reticulum indicated that the cells were still able to synthesize steroids after colchicine treatment. Evidence of this was also seen in tissue levels of progesterone; 4 hr after colchicine luteal tissue contained twice the normal amount of progesterone. As in the pancreas, the action of colchicine appeared to be directed towards hormone secretion and not biosynthesis.

REFERENCES

MORPHOLOGICAL AND FUNCTIONAL CHANGES IN THE CORPUS LUTEUM AFTER RESTRICTION OF OVARIAN BLOOD FLOW

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C.S.I.R.O., Division of Animal Physiology, Prospect, N.S.W. 2149, Australia

Pharriss and Wyngarden (1969) originally suggested that the demise of the corpus luteum (CL) is brought about by the veno-constrictive action of prostaglandin (PGF$_{2a}$) on the ovarian vasculature. Subsequently little supporting evidence has emerged and some results have tended to discount the suggestion (Labhsetwar, 1974). Certain experiments in sheep have, however, given a definite indication of selective reduction in blood flow to the CL following administration of PGF$_{2a}$ via a uterine vein (Thorburn and Hales, 1972). The morphological effects of PGF$_{2a}$ on the ovine CL have been reported (Stacy, Gemmell and Thorburn, 1975); speculation arose that it might be possible to induce these regressive changes artificially by reducing the flow of blood to the CL.

To test this possibility a cuff was placed surgically round the ovarian artery of ewes near the mid point of the luteal phase of the cycle. The cuff was adjusted so that blood flow to the CL was visibly reduced as judged by blanching of the superficial tissue. The animals were allowed to recover and 48 hr later were re-anaesthetized and the ovaries fixed by vascular perfusion with glutaraldehyde. Luteal tissue was taken for electron microscopic examination; blood samples for the determination of progesterone were taken from the jugular vein.

In several animals the treatment resulted in a marked fall in plasma progesterone concentration and this was accompanied by morphological changes in the corpus luteum. The luteal cells bore unmistakable signs of degeneration typical of the changes seen in normal or in PGF$_{2a}$ - induced regression (Stacy, Gemmell and Thorburn, 1975). Granule secretion had decreased, autophagocytic bodies were present and lipid droplets were profusely distributed throughout the cytoplasm.

These experiments were crude because there was no quantitative assessment of the impairment to blood flow following partial occlusion of the ovarian artery. Nevertheless the results are consistent with the concept that the luteolytic action of PGF$_{2a}$ is mediated by its effects on the ovarian vasculature.

REFERENCES

PLASMA PROGESTERONE LEVELS IN NON PREGNANT SOWS SHOWING DELAYED RETURN TO OESTRUS FOLLOWING MATING

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An analysis of breeding records from several commercial piggeries revealed that more than 10 per cent of breeding sows and gilts showed a delayed return to oestrus (>42 days) following an unsuccessful mating. A method of early pregnancy diagnosis was needed to identify those sows affected. Vaginal biopsies, taken 20-22 days after mating are inaccurate for diagnosing non pregnant sows showing this delayed return to oestrus (Williamson and Hennessy, 1975). The vaginal epithelium of such sows was similar to that of pregnant sows, indicating that at the time biopsies were taken the sows were either pregnant and later suffered embryonic or fetal death or that an endocrine imbalance due to cystic or quiescent ovaries was present.

The present study was undertaken to assess the accuracy of sows plasma progesterone concentration 18 days after mating as a method of pregnancy diagnosis in commercial piggeries suffering this delayed return to oestrus.

Sows and gilts were bled from a marginal ear vein 18 days after mating. Plasma progesterone was assayed using a modification of the method described by Cain et al (1972). Free $^3$H progesterone was separated from that bound to progesterone binding protein obtained from guinea pig plasma, by incubating with 1 ml of 10 mg per cent charcoal (Norit A) for 15 min at 4°C and then centrifuging for 10 min at 2,000 g.

Sows with plasma progesterone concentrations lower than 5 ng per ml were classified non pregnant. Farrowing records for all sows sampled were used to assess the accuracy of pregnancy diagnosis.

| TABLE 1 | ACCURACY OF PREGNANCY DIAGNOSIS BASED ON PLASMA PROGESTERONE CONCENTRATION |
| --- | --- | --- |
| Number of sows | Number correctly diagnosed | % correct |
| Pregnant sows | 66 | 66 | 100 |
| Non Pregnant sows | 19 | 10 | 53 |
| Delayed return to oestrus (>42 days) | 15 | 7 | 47 |
| Normal return to oestrus (18-24 days) | 4 | 3 | 75 |
| Overall | 85 | 76 | 89 |
Approximately half of the non pregnant sows showing a delayed return to oestrus were classified as being pregnant on the basis of their plasma progesterone concentrations. These results indicate that, as in the vaginal biopsy trial, these sows suffered embryonic or fetal death after sampling, or an endocrine imbalance after mating.

Estrogen concentrations are presently being measured in the plasma samples to establish if a hormone imbalance is present in this delayed return to oestrus.

REFERENCES
INDUCTION OF PARTURITION IN SHEEP
WITH A SINGLE DOSE OF OESTRADIOL-BENZOATE

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The rise in the level of oestrogen in maternal ewe plasma is well
documented (Challis, 1971; Obst and Seamark, 1972). This study investigated
whether a single dose of oestradiol-benzoate (ODB) administered to ewes during
the last week of gestation could induce parturition.

One hundred and eighty-eight Merino ewes which had been mated and had not
subsequently returned to oestrus were separated into weekly mating groups. Within
groups the ewes were allocated at random to either a treatment (84 ewes) or a
control (104 ewes) sub-group. The treated ewes received a single intra-muscular
injection of 20 μg ODB in 2 ml of peanut oil, when the ewes in the group were
at the 142 - 148 days of gestation. The control group ewes were allowed to
lamb normally under the same conditions as the treated ewes.

By 48 hr and 96 hr after treatment 65% and 84% respectively of the treated
group had lambed whilst only 12.5% and 34.6% of the control group had lambed by
this time. The incidence of dystocia and the birth-weight, survival and growth
rate of lambs born was similar in the control group and in the treated ewes which
lambed within 96 hr of ODB treatment. However the 16% of treated ewes which
did not lamb until 5 - 8 days after treatment showed a significantly higher incidence
of dystocia (P<0.05) than both the control group or remaining treated ewes,
and significantly more of the resultant lambs (16/18) were either still born or
died soon after birth.

A single dose of exogenous ODB administered after 142 days of gestation can
cause parturition to be initiated within 96 hr in the majority of ewes. The
malfunction at parturition in the 16% of treated ewes suggests that in these
ewes a premature or insufficient increase in the level of circulating oestrogen
may disrupt the normal physiology of parturition. The high incidence of lamb
losses in ewes which did not respond to treatment would not permit the use
of ODB to control parturition in commercial flocks at this stage.

REFERENCES


COMPARISON OF DEXAMETHASONE TRIMETHYLACETATE (DTMA)
AND PROSTAGLANDIN (PG) F₂₅ AS ABORTIFACIENTS IN THE COW

C.A. Sloan
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A reliable technique for aborting cows in the second and third trimesters was required to obtain non-pregnant cows for training technicians in Artificial Insemination. The technique also has application in cases of mismating. Forty-two cows were used in experiments designed to assess the abortifacient potential and safety of several drugs. DTMA (Opticortenol - Ciba Geigy) was used alone by single or repeated intra-muscular (I/M) injections, or in association with stilboestrol dipropionate (Stilboestrol - May and Baker) or oxytocin (Oxytocin - Intervet) (Table 1). Abortion occurred in cows given DTMA at a six-day interval, but the treatment-abortion interval was longer than with a four-day treatment interval or the injection of 25 mg I/M over multiple sites (5 x 5 mg). By comparison the stilboestrol dipropionate and oxytocin produced a considerable delaying effect on DTMA induced abortion (Table 1). Studies with PGF₂₅ (PGF₂₅ in ethanol - Fuji) are reported for a further 10 cows, where dosages administered directly into the foetal fluids caused rapid expulsion of the foetus with no complications to the cow (Table 1). The time-sequence of jugular plasma progesterone from pre-treatment to post-abortion was monitored. In general, the mean progesterone concentrations in venous blood had decreased within two days after DTMA was administered, and showed less variation in cows treated at four rather than six-day intervals.

TABLE 1
EXPERIMENTAL DETAILS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Subsequent Compound</th>
<th>Dose</th>
<th>Days*</th>
<th>Treated</th>
<th>Aborted</th>
<th>Number of cows</th>
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<tr>
<td>DTMA</td>
<td>25 mg I/M</td>
<td>25 mg I/M</td>
<td>4</td>
<td>16</td>
<td>12+</td>
<td>5</td>
<td>4 (1)+</td>
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</table>

* Days between initial and subsequent treatments
+ Figures in parentheses represent number of cows with an unexpelled dead foetus
† Foetal membranes retained in two cows

DTMA was found to be a safe and efficient abortifacient in the second and third trimesters, provided the time taken to induce abortion is not critical. The initial results with PGF₂₅ administered to the foetal fluids suggest this procedure may be more satisfactory if rapid abortion or removal of foetal debris is required.
HORMONAL CHANGES IN COW AND CALF ABOUT NORMAL AND INDUCED CALVING

J.P. Drinan, M.S.F. Wong and R.I. Cox

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School of Biological Sciences, Macquarie University, North Ryde, N.S.W. 2113

The use of synthetic corticosteroids to induce calving suggested the desirability of comparing the hormonal events about normal and induced calving. It was also considered that this induced parturition might provide a further insight into the sequence of hormonal changes preceding birth.

The hormonal changes around parturition were studied in 11 cows and 3 of their calves. With two of these cows, Hereford - Friesian crosses, it was possible to contrast the data from consecutive calvings, normal in 1972 and induced in 1973. Flumethasone (10 mg) was given i.m. on Days 267 and 272 of gestation; calving occurred 58 and 45 hr later. Jugular blood samples were taken from the cows with increasing frequency as parturition approached. During the 24 hr after birth, frequent concurrent samples were taken from cows and calves. Competitive protein binding assays were used to measure oestrone (E\textsubscript{1}), oestradiol (E\textsubscript{2}), Progestins (P) and corticosteroids (C). Oestrogen sulphates (ES) were measured as free oestrogens after solvolysis.

Many hormonal changes observed at normal parturition confirmed and extended earlier observations. Thus P declined over the last 30 days of gestation, over the last 15 days E rose and an E\textsubscript{1} peak was observed in the last 10 days and prior to the peak at parturition. Of particular interest was the observation that E\textsubscript{1}S reached a maximum concentration of about 10 ng/ml some 10 days before the unconjugated E\textsubscript{1} peak. Post partum P concentrations remained close to baseline until after onset of oestrus for 3 cows studied.

The sequence of events following flumethasone administration included an immediate drop in C in cow plasma and a decline in P, 14 and 12 hr later. The other endocrine events preceding induced parturitions were similar to normal: E\textsubscript{1} and E\textsubscript{2} rose to peaks immediately about birth and P remained at low levels over at least 30 hr before birth. The E\textsubscript{1} peak occurring 2-5 days before the normal birth was absent.

After induced birth, C remained low in cow plasma, but as high as normal in calf plasma, but as high as normal in calf plasma, remaining high for at least as long a time. Maternal E\textsubscript{1} concentrations (400 and 1600 pg/ml) and E\textsubscript{2} (40 and 200 pg/ml), E\textsubscript{1}S (14 and 42 ng/ml) and E\textsubscript{2}S (0.5 and 1.0 ng/ml) were initially similar to those for cows with normal births. The clearance of these oestrogens was, however, slower in the cows with induced parturition. Instead of falling to baseline within 48 hr of calving, E\textsubscript{1} remained about 50 pg/ml until placental expulsion about 100 and 115 hr later.

Immediately after the induced births, calf E\textsubscript{1} (30 and 80 pg/ml) and E\textsubscript{2} (40 and 60 pg/ml) were at similar concentrations to those observed with normal birth, but the sulphates were at higher concentrations. (Normal: 31 and 5; induced: 50 and 26 ng/ml E\textsubscript{1}S) (Normal: 14 and 6; induced: 45 and 22 ng/ml E\textsubscript{2}S). The clearance of these oestrogens was slower in the induced calves.

Apart from the expected suppression of maternal corticosteroid concentrations, hormonal patterns before parturition were similar for normal and induced parturition; however, post-partum differences were evident.
EFFECTS OF MEDROXY-PROGESTERONE ACETATE AND OESTRADIOL 17β ON PREGNANCY MAINTENANCE IN OVARIECTOMIZED RATS

N. W. Bruce and R. K. Bartholomeusz
Department of Anatomy and Human Biology, University of Western Australia, Nedlands, W.A. 6009

Rabbits, ovariectomized early in pregnancy and supplemented only with medroxy-progesterone acetate (MPA) produce heavier fetuses and placentas than normal (Bruce, 1973). However, in preliminary trials with rats, ovariectomy with MPA supplementation alone failed to maintain pregnancy. These trials were extended to provide a firmer basis for comparison.

Eighteen rats were injected with 1 mg of medroxy-progesterone acetate (Depoprovera, Upjohn) intramuscularly on Day 11 and were ovariectomized on Day 12 of gestation (Day 1 is the day that spermatozoa are first found in a vaginal smear). In addition, 8 of the 18 rats were injected daily with 0.5 or 1.0 μg of oestradiol 17β starting on Day 12. On Day 22, all rats were killed and their uterine contents were examined and compared with the number of implantation sites at the time of ovariectomy.

Of the 10 ovariectomized rats supplemented with MPA alone, only 2 live foetuses were found at term; one in each of 2 rats. Both foetuses appeared to be malformed due to high intra-uterine pressures. About 30% of the original implantation sites were still represented by almost normal sized placentas at Day 22. However, these placentas had a characteristic conical rather than hemispherical shape.

Seven of the 8 ovariectomized rats supplemented with MPA and oestradiol 17β had live foetuses at Day 22. The mean number of foetuses surviving was 5.2 out of 12.0 implantation sites. Foetal and placental weights were slightly lower than in control rats. However, no other abnormalities were evident.

Pregnancy can be maintained in ovariectomized rabbits supplemented with MPA only. However, ovariectomized rats appear to require oestrogen supplementation in addition.

REFERENCES

THE UPTAKE AND INCORPORATION OF [U-14C]GLUCOSE DURING CULTURE OF MOUSE BLASTOCYSTS PREVIOUSLY UNDERGOING NORMAL IMPLANTATION AND INDUCED DELAY OF IMPLANTATION

I. L. Pike* and R. G. Wales†
* Department of Veterinary Physiology, The University of Sydney, N.S.W. 2006 and
† School of Veterinary Studies, Murdoch University, W. A. 6153.

Implantation may be delayed in the mouse during lactation or following ovariectomy early in pregnancy. Few experiments have examined the metabolism of energy substrates by the blastocyst at this time and in the present studies, the uptake and utilization of glucose by mouse blastocysts at implantation and during the delay of implantation resulting from ovariectomy or administration of antiserum raised against luteinizing hormone (anti-LH) have been investigated.

Embryos were obtained from random-bred mice on (i) Day 5 of pregnancy where Day 1 is the day of vaginal plug ("implanting" blastocysts) (ii) Day 8 of pregnancy following ovariectomy on Day 3 ("ovariectomy delayed" blastocysts) and (iii) Day 8 of pregnancy following subcutaneous injection of 0.2 ml of rabbit anti-LH serum at 0900 h on either Day 2 or Day 3 ("anti-LH delayed" blastocysts). The embryos were washed and cultured in medium containing 5.56 mM [U-14C]glucose (specific activity of 3 μCi/mole) as sole energy substrate for either 0.5 or 5 hr. Following culture, the embryos were recovered, washed free from exogenous radioactivity and the total uptake of glucose and incorporation of glucose into various biochemical components were estimated using standard techniques.

During incubation for 0.5 hr in radioactive glucose, no significant difference was found in the uptake of label by "implanting" and "ovariectomy delayed" blastocysts but the uptake by "anti-LH delayed" blastocysts was less than 50% of that of the other blastocysts. In a further experiment, the short term (0.5 hr) uptake of glucose by "implanting" blastocysts decreased rapidly during in vitro culture in non-radioactive glucose medium but the uptake by "anti-LH delayed" embryos remained unaltered.

The level of label accumulated by "ovariectomy delayed" blastocysts during culture for 5 hr was markedly reduced when compared with "implanting" blastocysts. More than 70% of the glucose carbon incorporated by these blastocysts over this time was extracted in the acid-soluble fraction. Using the "implanting" blastocysts as a control, less label was found in both the non-glycogen and glycogen portions of the acid-soluble fraction of the delayed blastocysts. The amount of label in the protein fraction was also reduced during the delay. Little difference was observed in the incorporation of label into the remaining fraction (lipid and nucleic acids) of the blastocysts.

Previous studies have suggested that the mouse embryo enters a state of relative metabolic quiescence during the delay of implantation. The results of the present experiments are in agreement with this hypothesis. Furthermore, the administration of anti-LH during early pregnancy inhibits the uptake of glucose by the mouse embryo perhaps due to hindrance to glucose entry by the binding of the antibody to LH-like antigenic sites around the surface of the blastocyst as uptake by these embryos was low from the time of embryo collection.
PROTEINS OF THE UTERINE SECRETION IN SHEEP

L.D. Staples, I.A. Cumming, R.A.S. Lawson and F.J. Morgan
St. Vincent's School of Medical Research, Fitzroy, 3065, and
Department of Agriculture, S.S. Cameron Laboratory, Werribee, 3030.

The uterine environment is considered to be an important factor affecting blastocyst development before implantation. In the rabbit a uterine-specific protein (blastokinin or uteroglobin) which can account for up to half of the secreted protein has been demonstrated to influence blastocyst development in vitro (Gulyas, Daniel and Krishnan, 1969; El-Banna and Daniel, 1972).

Secretion of this protein is influenced by progesterone. For embryo survival in the ewe synchronous development of the blastocyst with the uterine environment is required (Moore and Shelton, 1964). This relationship is influenced by the level and duration of progesterone influence (Lawson, unpublished). Together, these findings suggest that specific uterine secretions may influence blastocyst development in the ewe.

Uterine protein secretions were examined throughout the oestrous cycle in Border Leicester x Merino ewes, and in spayed ewes injected with 7 or 15 mg progesterone/day for periods 0 to 16 days. Uterine protein samples were obtained by flushing the lumen of the uterus with 5 ml of 0.15M NaCl. After dialysis of flushings against water the secretions were examined by total protein analysis (ninhydrin method), polyacrylamide gel electrophoresis (7% gels, Tris-glycine buffer pH 8.28) and for ability to specifically bind progesterone (competitive binding assay). Total protein recoveries ranged between 2 - 9 mg with no differences between early or late phases of the oestrous cycle or between entire ewes and progesterone-treated spays.

The pattern of protein secretion separated as described, was dominated by albumin with fewer bands in the macroglobulin area than serum. In some animals an additional protein band, not seen in serum, was observed in the post-albumin region from day 10 to 16 of the oestrous cycle. At no time was this a major component of the secretion. This fraction was not apparent in flushings from spayed or progesterone-treated spayed ewes, and although the constancy of its appearance remains to be confirmed, it has similar electrophoretic mobility to a uterine specific protein recently reported to appear after day 14 in the sheep (Menezo, 1973). No significant differences in progesterone binding by uterine secretions could be observed between the groups of animals.

We have observed no dominant uterine secretory protein analogous to rabbit blastokinin in sheep under the conditions described above, and it seems that any cyclical changes in uterine protein secretion may be more subtle in this species.

REFERENCES

AN IMMUNOLOGICAL TEST FOR PREGNANCY IN THE EWE

R.A.S. Lawson, Mildred Cerini and J.K. Findlay
Department of Agriculture, Victoria and Reproduction Research Section, University of Melbourne, S.S. Cameron Laboratory, Werribee, 3030.

Antigen(s) specific to pregnancy have been detected by immunofluorescence in the embryo, uterus, corpus luteum and maternal circulation of pregnant ewes from as early as Day 6 after mating (Cerini, Findlay and Lawson, 1975). Apart from any active role played by these antigens in the establishment of pregnancy, their presence offers the opportunity to develop immunological tests for pregnancy in sheep and other domestic animals.

Simple haemagglutination, using one drop of a 1/16 dilution of rabbit anti-sheep embryo serum added to a 5% (v/v) dilution of whole blood, was used to detect antigen adsorbed onto maternal erythrocytes. The diagnoses, confirmed by slaughter, on 330 ewes which had been mated up to 55 days before testing, and on an unmated control flock tested at monthly intervals for 3 months are shown in Table 1. The accuracy of pregnancy diagnosis relative to the stage of gestation at testing in ewes subsequently confirmed pregnant is shown in Table 2.

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
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<tr>
<td>THE ACCURACY OF PREGNANCY DIAGNOSIS BY HAEMAGGLUTINATION IN MATED AND UNMATED EWES</td>
</tr>
<tr>
<td>Flock</td>
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<tr>
<td></td>
</tr>
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<tr>
<td></td>
</tr>
<tr>
<td>Unmated control</td>
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</table>

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>THE ACCURACY OF PREGNANCY DIAGNOSIS RELATIVE TO STAGE OF GESTATION</td>
</tr>
<tr>
<td>Stage of gestation (days)</td>
</tr>
<tr>
<td>No. of pregnant ewes</td>
</tr>
<tr>
<td>Correct diagnoses (%)</td>
</tr>
</tbody>
</table>

Fifty-five percent of false positive tests were attributed to ewes in which embryo death and resorption was considered to have occurred. The remaining 45% of false positive tests may be accounted for by operator error in embryo recovery from slaughter material and reading the immunological test. The incidence of false positive tests due to non-specific haemagglutination was low in the unmated control flock. The high incidence of false negatives which increased in the 41-50 day period in the mated flock suggested that the presence of excess unbound antigen in the plasma may have interfered with haemagglutination. This was confirmed using haemagglutination inhibition and passive haemagglutination tests. It was concluded that immunological methods could be used to diagnose pregnancy from an early stage in ewes. The inability of the simple haemagglutination test to distinguish between high and low titres of antigen indicated the necessity to use an alternative haemagglutination procedure to improve the accuracy of diagnosis.

REFERENCE

THE DISTRIBUTION OF PROSTAGLANDIN F, PROGESTERONE AND PREGNANCY-SPECIFIC ANTIGEN IN UNILATERALLY PREGNANT EWES

J.C. Cerini, Mildred Cerini, J.K. Findlay and R.A.S. Lawson
Reproduction Research Section, University of Melbourne and
Department of Agriculture, Victoria, S.S. Cameron Laboratory, Werribee, 3030.

Pregnancy-specific antigen(s) has been shown by immunofluorescent protein tracing to be present from Day 8 in the uteri, corpora lutea, embryonic membranes and circulation of pregnant ewes (Cerini, Findlay and Lawson, 1975). The antigen(s) was not detected in non-pregnant ewes nor in other tissues of pregnant ewes.

The distribution of this antigen(s) relative to progesterone and prostaglandin F in pregnant and non-pregnant ewes has been investigated. Unilaterally pregnant ewes (18) were prepared by surgically dividing the uterine horns of ewes with bilateral ovulations and transferring a blastocyst into one horn. At laparotomy on Day 14 or 17, jugular, uterine and ovarian venous blood samples were taken. The uteri were flushed with saline to recover embryos which had developed. Tissue samples from each uterine horn and both ovaries were fixed in phosphate buffered 10% formalin for subsequent immunofluorescence studies (Cerini et al. 1975).

Immunofluorescence studies revealed that in the non-pregnant uterine horns PGF was distributed on the surface of the columnar epithelium, in cells of the endometrium and in the walls of blood vessels in the myometrium. By contrast, in pregnant uterine horns PGF was detected only in the lamina propria and myometrium. PGF was also localised in the walls of blood vessels in corpora lutea of both ovaries. In both non-pregnant and pregnant uterine horns, progesterone was distributed throughout the endometrium and myometrium. Progesterone was present in about 90% of luteal cells in corpora lutea ipsilateral to the pregnant uterine horn but only in about 20% of cells in regressing contralateral corpora lutea. The pregnancy-specific antigen(s) was present in the lumens of pregnant uterine horns, in the myometrium and blood vessels of both the pregnant and non-pregnant horns, in the ipsi- and contralateral corpora lutea and in the peripheral circulation. Using a haemagglutination inhibition assay, the concentrations of antigen in peripheral and venous blood from both uterine horns was found to be identical. This study shows; (a) a different distribution of PGF in the tissues of pregnant and non-pregnant uterine horns, (b) the intrauterine distribution of progesterone was not influenced by pregnancy, whereas there was less in the regressing CL and (c) pregnancy-specific antigen(s) was found in both regressing and maintained corpora lutea.

REFERENCE

(In press).
PRELIMINARY CHARACTERIZATION OF PREGNANCY SPECIFIC ANTIGEN(S) IN THE EWE

Mildred Cerini, J.C. Cerini, J.K. Findlay and R.A.S. Lawson
Reproduction Research Section, University of Melbourne and
Department of Agriculture, Victoria, S.S. Cameron Laboratory, Werribee, 3030.

Antigen(s) specific to pregnancy have been detected by haemagglutination in
the plasma and on the erythrocytes of pregnant ewes as early as Day 6 after
mating (Cerini, Findlay and Lawson, 1975). Preliminary characterization of the
antigen(s) has been carried out using uterine vein plasma of Day 10 pregnant
ewes as a source of antigen and a passive haemagglutination test to monitor
antigen titres.

The activity in plasma is stable at 4°C for at least 1 week and at -15°C
for at least 12 weeks. The activity was slightly reduced after heating at 56°C
for 30 min and was destroyed after heating at 80°C at 30 min. Following dialysis
(MW cut off 6000-8000) against phosphate-buffered saline (pH 7.2) overnight at
4°C, the titre of antigen in dialysed plasma remained unchanged. Ammonium
sulphate precipitation revealed that 45% saturation was the lowest effective
concentration which removed the antigen from solution. Electrophoresis on
cellulose acetate membranes at pH 9.5 indicated that the antigen(s) had an
electrophoretic mobility in the post-albumin region, similar to α-globulins and
acid-glycoproteins. Treatment of pregnancy plasma with N-trichloroacetic acid
completely removed the antigen(s) from solution, indicating a protein component
is necessary for expression of antigenicity. Sodium meta-periodate at 0.20 M
to 0.05 M was effective in destroying the activity of the pregnancy-specific
antigen in plasma whereas after treatment with 0.25 M metaperiodate, the activity
was unchanged. This suggests that the antigen contains a carbohydrate component.
Filtration of pregnancy plasma on Sephadex G-25 (fractionation range 100-5000 MW)
showed that the antigen was present in the main protein fraction collected in
the exclusion volume of the column.

These preliminary studies of pregnancy-specific antigen(s) detected
immunologically in the plasma of pregnant ewes indicate that they are of a
molecular weight greater than 8000 and have protein and carbohydrate components.

REFERENCE

CERINI, Mildred, FINDLAY, J.K. and LAWSON, R.A.S. (1975)
59

THE FUNCTION OF ANTERIOR PITUITARY HORMONES IN THE INITIATION
OF IMPLANTATION IN MICE

A. A. Gidley-Baird and C. W. Emmens
Department of Veterinary Physiology, University of Sydney, N.S.W. 2006

The work reported here is an extension of that presented last year
(Gidley-Baird and Emmens, 1975), which demonstrated the capacity of various
combinations of anterior pituitary hormones to initiate implantation in
hypophysectomised mice. The aim of this work was to determine the function of
these hormones in achieving their effects.

Four groups each of 15 female QS mice hypophysectomised at 11.00 hr
day 2 of pregnancy (day 1 being the day on which the copulatory plug is found),
were injected subcutaneously at 14.00 hr day 2 with the following:- Group 1,
0.1 ml of 15% gelatin vehicle; group 2, 50 \( \mu \)g of NIAMD Rat Prolactin RP-1
(Rat PRO); group 3, 50 \( \mu \)g of NIH LH S-16; group 4, 50 \( \mu \)g of Rat PRO plus
50 \( \mu \)g of LH. Five mice from each group were killed at 8, 16 and 32 hr after
their injection. The plasma was collected and stored at -20°C until assayed for
progesterone content by a protein binding method.

Female QS mice were hypophysectomised on day 2 of pregnancy and given
progesterone replacement therapy in conjunction with either NIH LH S-16, Rat PRO
or NIAMD Rat FSH B-1 until day 8, when they were killed and examined for implanted
embryos.

Female QS mice were hypophysectomised on day 2 of pregnancy and the
capacity of NIH Prolactin S-11 and Oestradiol-17\( \beta \) (E\( \beta \)) to initiate implantation
was tested in a 2 x 3 factorially designed experiment. Mice were killed and
examined for implanted embryos on day 8.

Mice which showed post-partum copulatory plugs and each suckling
8 pups were divided into 4 groups of 7 and on day 5 of pregnancy had their litters
removed and received the following treatments:- Group 1, 0.2 ml normal rabbit
serum (NRS) at 06.00 hr day 5; group 2, 0.2 ml NRS at 12.00 hr day 5; group 3,
0.2 ml anti-NIH LH S-16 serum (ALH) at 06.00 hr day 5; group 4, 0.2 ml ALH at
12.00 hr day 5.

Both LH and prolactin individually and in combination were capable of
significantly increasing plasma progestagen levels.

100% of hypophysectomised mice receiving progesterone plus FSH showed
implanted embryos. Groups receiving progesterone plus LH or prolactin showed no
implantation.

Only hypophysectomised mice receiving prolactin + E\( \beta \) had implanted
embryos. The group injected with prolactin plus the low dose of E\( \beta \) had 8 out of
9 mice with implants.

ALH failed to block implantation in mice after litter removal.

The results of the first three experiments together with the work of
Gidley-Baird and Emmens (1975) suggest that LH and prolactin are responsible for
the progesterone production while FSH is primarily responsible for the oestrogen
production required for implantation in the mouse. The result of experiment (4)
together with the fact that implantation can occur in the absence of pituitary LH
suggests that ALH may also block implantation at a site other than that concerned
with the inhibition of progesterone production. It is possible that this site
might involve cross reaction with an LH-like substance found on the surface of
pre-implantation mouse embryos (Wiley, 1974).

EFFECTS OF PROGESTERONE AND OESTRADIOL ON ENDOMETRIAL METABOLISM IN THE OVARIECTOMIZED EWE.

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

The hormonal regulation of the environment in the uterus in which early embryonic development occurs is poorly understood. The effects of progesterone secreted before oestrus and oestradiol secreted at about the time of oestrus on endometrial metabolism during early pregnancy were investigated, using a model system employing ovariectomized ewes. In another concurrent experiment the effects of these two components of ovarian secretion on the survival and development of transferred embryos were examined (Moore & Miller, 1975). At the start of the experiment (Day 0) all ewes received 25 μg oestradiol, and they were then placed under one of five oestradiol/progesterone (E/P) regimes (Table 1). The regimes have been fully described (Moore & Miller, 1975).

Three ewes from each of the 5 treatment regimes were killed around 08.00 h on each of Days 18, 21 and 24; 1, 4 and 7 days after induced oestrus in ewes which received oestrous E. Promptly after slaughter slices of endometrium were prepared from dissected caruncles. RNA and DNA were extracted from endometrial homogenates by incubation at 90° for 20 min. in 0.5 M HClO₄, and assayed colorimetrically (Miller & Baggett, 1972). Rates of protein synthesis were determined from the in vitro incorporation of [methyl-³H]methionine into protein during 2 h incubations of slices in Eagle's HeLa medium (Munns & Katzman, 1971).

### TABLE 1 - TREATMENT REGIMES

<table>
<thead>
<tr>
<th>Group</th>
<th>Base E</th>
<th>Priming P</th>
<th>Oestrous E</th>
<th>Maintenance P</th>
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</tbody>
</table>

Three ewes from each of the 5 treatment regimes were killed around 08.00 h on each of Days 18, 21 and 24; 1, 4 and 7 days after induced oestrus in ewes which received oestrous E. Promptly after slaughter slices of endometrium were prepared from dissected caruncles. RNA and DNA were extracted from endometrial homogenates by incubation at 90° for 20 min. in 0.5 M HClO₄, and assayed colorimetrically (Miller & Baggett, 1972). Rates of protein synthesis were determined from the in vitro incorporation of [methyl-³H]methionine into protein during 2 h incubations of slices in Eagle's HeLa medium (Munns & Katzman, 1971).

### TABLE 2 - RNA/DNA RATIOS IN THE ENDOMETRIUM

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Days after oestrus (in ewes which received oestrous E)</th>
<th>18</th>
<th>21</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>E.P.E.P.</td>
<td>.913</td>
<td>.786</td>
<td>.649</td>
</tr>
<tr>
<td>2</td>
<td>E.P.E.-</td>
<td>.865</td>
<td>.676</td>
<td>.530</td>
</tr>
<tr>
<td>3</td>
<td>E.P.-.P.</td>
<td>.585</td>
<td>.512</td>
<td>.558</td>
</tr>
<tr>
<td>4</td>
<td>E.-.E.P.</td>
<td>.742</td>
<td>.667</td>
<td>.723</td>
</tr>
<tr>
<td>5</td>
<td>E.-.-.P.</td>
<td>.410</td>
<td>.423</td>
<td>.544</td>
</tr>
</tbody>
</table>
When maintenance P was omitted (Group 2 vs. Group 1) there was a small decrease (P<0.005) in RNA/DNA ratios on Days 21 and 24 (Table 2). In ewes which received maintenance P the omission of oestrous E (Groups 3 and 5 vs. Groups 1 and 4) markedly decreased RNA/DNA ratios and rates of synthesis of protein (P<0.005). By Day 24 the effect on protein synthesis had disappeared, and that on RNA/DNA was reduced. Omitting priming P had no effect on rates of protein synthesis, but did reduce RNA/DNA ratios on Days 18 and 21 (P<0.005). There were no interactions between oestrous E and priming P on RNA/DNA ratios. In ewes which received maintenance P the effects of priming P and of oestrous E on RNA/DNA ratios in the endometrium followed the same pattern as their effects on survival of embryos (Moore & Miller, 1975). It is suggested that changes in the RNA/DNA ratio reflected the effects of priming P and of oestrous E on the potential of the endometrium for glandular secretion under the subsequent influence of progesterone (maintenance P).

REFERENCES


PROGESTERONE AND OESTRADIOL REQUIREMENTS FOR THE SURVIVAL OF EMBRYOS IN THE OVARIECTOMIZED EWE

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

It has been shown that progesterone will maintain pregnancy in the ovariectomized ewe. However, studies have been confined to cyclic animals ovariectomized within a few days after oestrus, or to ovariectomized ewes to which embryos were transferred following oestrus induced by oestrogen given after a period of progesterone treatment. Thus, little is known of any role that progesterone secreted before oestrus, or oestrogen secreted at about the time of oestrus, might play in the subsequent survival and development of embryos.

In the present experiment a single embryo collected from donor ewes four days after oestrus was transferred to each of 59 recipient ewes that had been ovariectomized at least two months prior to the experiment. At the start of the experiment (Day 0) all ewes received 25 µg oestradiol (Base E2) and they were then placed under one of five oestradiol/progesterone (E2/P) regimes (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Base E2</th>
<th>Priming P</th>
<th>Oestrous E2</th>
<th>Maintenance P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E</td>
<td>P</td>
<td>E</td>
<td>P</td>
</tr>
<tr>
<td>2</td>
<td>E</td>
<td>P</td>
<td>E</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>E</td>
<td>P</td>
<td>-</td>
<td>P</td>
</tr>
<tr>
<td>4</td>
<td>E</td>
<td>-</td>
<td>E</td>
<td>P</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Base E2 - 25 µg on Day 0
Priming P - 5 mg twice daily on Days 3 - 14 (incl.)
Oestrous E2 - Total of 35 µg given during Days 15 and 16

<table>
<thead>
<tr>
<th>Day and dose E2 (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 15</td>
</tr>
<tr>
<td>1600 hr 3.5</td>
</tr>
<tr>
<td>2400 hr 7.0</td>
</tr>
<tr>
<td>Day 16</td>
</tr>
<tr>
<td>0800 hr 14.0</td>
</tr>
<tr>
<td>1600 hr 7.0</td>
</tr>
<tr>
<td>2400 hr 3.5</td>
</tr>
</tbody>
</table>

Maintenance P Day and dose (mg) 
18 19 20 21 22 23 24-25 26-42
a.m. 0.5 a.m. a.m. a.m. a.m. a.m. a.m. a.m. a.m. a.m.
0.5 0.625 0.75 1.0 1.25 1.5 2.0 2.5 3.0 4.0 5.0 6.0 8.0 8.0 12.0

P and E2 were given by intramuscular injection and each component of the treatment regimes was an attempt to simulate a phase of ovarian steroid secretion during the oestrous cycle in the intact ewe. All ewes received one embryo on Day 21, four days after induced oestrus in ewes which received oestrous E2. Treatment with maintenance P continued to Day 42 (21 days after transfer) when all animals were autopsied. At autopsy, normal and apparently viable embryos were recovered from 11 ewes (Table 2).
TABLE 2
PROPORTION OF EWES WITH NORMAL EMBRYOS

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.P.E.P.</td>
<td>E.P.-P.</td>
<td>E.-E.P.</td>
<td>E.-.P.</td>
<td>E.-..P.</td>
</tr>
<tr>
<td>8/13</td>
<td>0/11</td>
<td>1/13</td>
<td>2/11</td>
<td>0/11</td>
</tr>
</tbody>
</table>

Clearly progesterone given after oestrus was necessary for survival of embryos, and equally apparent is the need for both oestrogen and progesterone given prior to oestrus. When either priming P or oestrous E, were omitted from the treatment schedule (Groups 3 and 4), very few embryos survived. When both were omitted (Group 5) no embryos survived.

The results suggest that in the entire cyclic ewe progesterone secreted prior to oestrus and oestradiol secreted around the time of oestrus are involved in the subsequent survival and development of embryos.

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

The storage of cattle embryos has been an area of continued investigation, but to date reliable methods for frozen storage have been reported for only one species - the mouse (Whittingham, Leibo and Mazur, 1972; Wilmut, 1972), although there is a report of a single pregnancy following the transfer of some 22 frozen cattle embryos to recipient cows (Wilmut and Rowson, 1973).

In an initial experiment in the present study, 21 cattle embryos collected as morulae and early blastocysts from donors seven and eight days after oestrus were cooled to 6°C (0.7°C/min.), held at 6°C for 1, 2 or 4 days, and then warmed to 37°C (0.7°C/min.) and cultured in vitro for 36 hours. Thirteen embryos developed during culture to expanded or hatched blastocysts and 11 of the 13 were transferred to nine recipients, of which three were diagnosed as pregnant, by rectal palpation, eight weeks after transfer. Duration of storage had no effect upon development in culture, but none of four recipients which received embryos which had been stored for four days were diagnosed as pregnant.

Subsequently, 39 embryos of similar age were cooled and stored in liquid nitrogen (-196°C) for two to seven weeks. They were then warmed and cultured for 12 hours. Cooling, storage, warming and culture were carried out in pyrex glass tubes containing Dulbecco phosphate buffer enriched with 25% heterologous bovine serum (DB + 25% S). Before cooling dimethylsulphoxide (DMSO) in DB + 25% S was added slowly over 20 min. to give a final concentration of 1.5 M DMSO. The tubes were then cooled to 0°C at a rate of 0.7°C/min. Further cooling to -55°C (0.13 - 0.15°C/min.) was carried out in a dry ice/ethanol bath. The tubes were then transferred to a liquid nitrogen container and slowly lowered through the vapour into storage in liquid nitrogen.

Following storage the tubes were warmed to 0°C at rates of 1.2 or 2.2°C/min. (measured over the range -50 to 0°C) and from 0°C to 30°C at a rate of 0.7°C/min. DMSO was removed by washing in DB + 25% S containing decreasing concentrations of DMSO and the washed embryos were then cultured for 12 hours at 37°C. During culture 16 of the 39 embryos developed to expanded or hatched blastocysts while the remaining 23 showed no, or limited development. Eleven of the 16 and 12 of the 23 were transferred to 17 recipients. Recipients received either one embryo that had developed, or two which had shown no, or limited development in culture. Rectal palpation eight weeks after transfer showed that six recipients were pregnant and all had received embryos which developed during culture.

The results show that cattle embryos can be stored in either the liquid or frozen state, but liquid storage is probably limited to a few days.

Acknowledgement is made to Australian Transplant Breeders for use of facilities and for financial assistance.
There seems to be no information on fertility of boar semen frozen-stored over a prolonged period. To examine the effect of long-term storage on fertility, a semen bank has been laid down, and this communication presents the results for the first stage of the fertility test in which boar semen stored for 2½ years was used for insemination.

The semen was collected by the manual method, and the sperm-rich fraction of ejaculates was diluted at 30°C (1:2) with a tris-based diluent [250 mM tris-111 mM fructose-15 mM EDTA (disodium salt)-79.5 mM citric acid]. The diluted semen containing 15% (v/v) egg yolk and 2% (v/v) glycerol was cooled to 5°C in 1.5 hour, then frozen in pellet form (0.25 ml) on dry ice and subsequently stored in liquid nitrogen.

Oestrus was detected by teaser boars twice daily, and surgical inseminations were performed 19-28 hours after detection. For insemination, 2-3 pellets were thawed in a water-bath at 37°C. The inseminating doses were 0.10 and 0.20 ml, containing $6.7 \times 10^6$ and $13.4 \times 10^6$ motile spermatozoa. After exposing the reproductive tract by mid-ventral laparotomy, the thawed semen was introduced into the oviduct via the utero-tubal junction.

A total of 22 animals were inseminated, of which 20 (90.9%) farrowed 122 pigs. The average litter size was 6.1. Farrowing for 0.10 and 0.20 ml inseminate volumes were 10/11 and 10/11. All 14 animals inseminated before ovulation and 6 of 8 animals inseminated after ovulation farrowed.

Surgical insemination with semen frozen-stored for 2½ years yielded high fertility. Semen stored for longer than this period will be used in future fertility tests.
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<td>LAUNCESTON</td>
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